BIODIVERSITY CHALLENGES AND THREATS; CURRENT SCENARIO

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P G and Research Department of Botany
Sree Narayana College, Kollam

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Sree Narayana College, Kollam
2023

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ISBN 978-81-958369-2-5
Biodiversity Challenges and Threats; Current Scenario

Published by
PG and Research Department of Botany
Sree Narayana College, Kollam
Kerala, India-691001
Phone : +914742998877
Mobile : +914742998877
Email : snckollam@gmail.com
Web : snckollam.ac.in

Typesetting and Cover
Dr. Vilash Viswambharan
PG and Research Department of Botany
Sree Narayana College, Kollam

Printing
Pournami Graphics
S. N Press Compound
Near S.N College, Kollam

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10.04.2023

FOREWORD

I am delighted to write the foreword for the book "**Biodiversity Challenges and Threats: Current Scenario**," which is published by the Post Graduate and Research Department of Botany at Sree Narayana College in Kollam, Kerala, where I am an alumnus. This book is edited by Dr. Vilash Viswambharan, Dr. Nadarajan Ratheesh and Dr. Latha Sadandandan. The book is divided into twenty-seven chapters, providing a comprehensive overview of emergent discourses within the field, such as the conservation of endangered species, the protection of traditional knowledge, conserving biodiversity through *in situ*, *ex situ*, and *in vitro* methods, and ethnobotany, among others. I am hopeful that all the chapters of this book will serve as a useful reference for researchers, academicians, scientists, environmentalists, and the general public, helping to cultivate vibrant perspectives on the threats and challenges to biodiversity in the contemporary world.

The Convention on Biological Diversity (CBD) defines biodiversity as "the variability among living organisms from all sources, including terrestrial, marine, and aquatic ecosystems, as well as the ecological complexes of which they are part. This includes diversity within species, as well as between species and ecosystems." The 2019 IPBES Global Assessment Report estimated that 75% of the terrestrial environment and 40% of the marine environment exhibit "significant signs of degradation." Consequently, around 1 million species, which accounts for 25% of the animal and plant groups assessed, are presently threatened with extinction. Habitat destruction, habitat fragmentation, habitat degradation, overexploitation of species for human use, the introduction of exotic species, and the increased spread of diseases are among the significant threats to biodiversity caused by human activities. To fully assess the current status of biodiversity, it is essential to understand the past loss and gain of species. By reflecting on the past, we can identify failures and successes in present biodiversity dynamics within the context of changing global scenarios. Therefore, let us prioritize protecting existing biodiversity to ensure a sustainable and environmentally friendly future.

I congratulate the editors, **Dr. Vilash Viswambharan**, **Dr. Nadarajan Ratheesh** and **Dr. Latha Sadanandan** for their effort in bringing out this book. I hope that this book will be well-received by all individuals interested in biodiversity conservation.

Pushpangadan

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CONTENTS

Chapter 1

FOLIAR MYCOBIONTS ON MEDICINAL PLANTS FROM SACRED GROVES OF ARUVAPPULAM AND PRAMADOM PANCHAYAT OF PATHANAMTHITTA DISTRICT OF KERALA STATE

Soumya Prasad, Archana G R

Abstract Fungal diversity has a great contribution to the biodiversity on earth. Black mildews are a group of fungi that are thought to be host–specific which produce black colonies on the host surface and infect mostly leaves, soft stems and petioles. The infection requires a humid environment and the development of infection produce various vegetative and reproductive structures. The present study involves the collection, identification and documentation of foliar mycobionts on medicinal plants collected from sacred groves of Aruvappulam and Pramadom Panchayat of Pathanamthitta district of Kerala State. In this study, the detailed microscopic examination of the fungi namely; *Asterina congesta* Cooke on *Santalum album* L., *Asterina jambolana* Kar & Maity on *Syzygium* species, *Meliola ichnocarpi-volubili* Hansf. on *Ichnocarpus frutescens* (L.) R.Br., *Meliola strychni- multiflorae* Hansf. on *Strychnos nux- vomica* L., *Meliola strychni* Mibey on *Strychnos nux- vomica* L. and *Questieriella strychni* Hosag. on *Strychnos nux- vomica* L. The collections were identified and deposited in the fungal herbarium of S. G. College, Kottarakara.

Keywords: *Black mildews, mycobionts, Asterina, Meliola, Questieriella*

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Introduction

Sacred groves are blessed with many native plants and other life forms along with conserved geographical features protected by human societies on religious grounds. They are considered as a huge reservoir of biodiversity including many valuable medicinal plants used in various systems of medicine (Sreeja and Unni, 2016). Biodiversity - rich sacred groves have immense ecological significance as they conserve valuable resources including medicinal plants (Praveenkumar, 2018). According to Boraiah *et al.*, (2003), about 60% of the regenerating species in them provide suitable medicines and nearly 40% of medicinal plants are unique. These sacred groves provide an ideal habitat for the proper growth of many fungal species thereby playing a major role in the conservation of fungi (Brown *et al.*, 2006). The fungi inhabiting the leaves are referred to as phylloplane or foliicolous fungi. Foliicolous fungi constitute a major part of microbial diversity which includes rusts, smuts, powdery mildews, black mildews, *etc.* (Jayashankara, 2012). Inhabiting on the surface of the leaves, they produce special organs, attain special adaptations and act as necrotrophs or biotrophs. Black mildews can maintain a parasitic symbiosis (Hosagoudar, 2012) with their host plants. They do not cause any serious symptomatic appearance on their host. Black mildews belongs to different taxonomic groups namely; Meliolaceous fungi, Asterinaceous fungi, Schiffnerulaceous fungi, and Hyphomycetous fungi (Hosagoudar, 2011). The present study aims to investigate the foliar fungal diversity on medicinal plants of the sacred groves of Aruvappulam and

Pramadom panchayat of Pathanamthitta district, Kerala. Foliar microfungi such as *Asterina jambolana* Kar & Maity on *Syzygium* species, *Asterina congesta* Cooke on *Santalum album* L., *Meliola ichnocarpi-volubili* Hansf. on *Ichnocarpus frutescens* (L.) R.Br., *Meliola strychni- multiflorae* Hansf., *Meliola strychni* Mibey, and *Questieriella strychni* Hosag. on *Strychnos nux-vomica* L. (Plate I, II & III) were obtained from the study area. *Strychnos nux-vomica* L. exhibits a wide variety of pharmacological properties. It can be used for the treatment of various ailments such as bronchitis, gonorrhea and diabetes in Ayurvedic and Unani systems of medicines (Razzaq *et al.*, 2020). *Ichnocarpus frutescens* (L.) R.Br. is a precious medicinal plant that has been used for the treatment of various disorders such as fevers, diabetes, nephrolithiasis, liver disorders *etc.* (Meher *et al.*, 2018). Sandalwood and the essential oil derived from Sandal heartwood have been used in Ayurveda, Siddha and Unani systems of medicines for the treatment and prevention of a number of ailments (Kumar *et al.*, 2015). Different species of *Syzygium* possess a wide range of medicinal properties and has anti–fungal, anti – bacterial activity (Nigam *et al.*, 2012).

Materials and methods

Field exploration trips were conducted to various sacred groves of Aruvappulam and Pramadom panchayat of Pathanamthitta district of Kerala for the collection of foliar mycobionts. The collected plants were placed individually in polythene bags and field notes regarding the pathogenicity of fungi, nature of colonies, nature of the infection, locality, altitude, *etc.* were prepared. These infected plant parts were pressed and dried in-between blotting papers. After ensuring their dryness, they were kept in manifold or butter paper folders. Such materials were later used for the microscopic study. In the case of ectophytic or superficial fungi, scrapes were made directly from the infected host parts and were mounted in Lactophenol (Rangaswamy, 1975). Dematiaceous fungi were first mounted in 10% KOH solution and later transferred to Lactophenol in order to make the septa visible. Fungal colonies on the leaves were analysed with the help of hand lenses for morphological studies. In order to study the colonies *in situ*, nail polish technique was used (Hosagoudar and Kapoor, 1985). Permanent slides were prepared using DPX as mountant and these slides were used for future studies. For the innate fungi, free hand sections were made, mounted in lactophenol, a tinge of cotton blue stain was used to stain the hyaline fungal parts. Observations were made under the compound microscope. Measurement was taken with the help of an ocular micrometer on a calibrated microscope. Previous collections were observed. Recent nomenclature was verified and experts were consulted. A description of the fungal species along with photomicrographs was done. All the fungal specimens are documented and deposited in the St. Gregorios College Herbarium, Kottarakara (SGCH).

Results

Description to the species

1. *Asterina jambolana* Kar & Maity, Trans. Brit. Mycol. Soc. 54: 438, 1970; Hosag., Balakr. & Goos, Mycotaxon 59: 180, 1996; Hosag. & Abraham, J. Econ. Taxon. Bot. 4: 576, 2000; Hosag.,C.K. Biju & Abraham, J. Econ. Taxon. Bot. 25: 306, 2001; J. Mycopathol. Res. 40:195, 2002; Hosag., Zoos' Print J. 18: 1283, 2003; Hosag., Zoos' Print J. 21: 2327, 2006; Hosag., Chandraprabha & Agarwal, Asterinales of Kerala, p. 96, 2011.

Colonies epiphyllous, dense, up to 8 mm in diameter, scattered to confluent. Hyphae flexuous, undulate to curved, branching irregular, alternate to rarely unilateral at acute angle, loosely to closely reticulate, cells 19 -34 ×3.8-5.7 µm. Appressoria two celled, unilateral, sometimes alternate to opposite, antrorse, subantrorse to rarely retrorse, curved, 9.5-17 µm long; stalk cells cuneate 3.8-7.6 µm; head cells oblong, globose, ovate, entire to rarely sublobate, 5.7 -9.5 ×3.8-7.6 µm long. Thyriothecia grouped to scattered, orbicular to elliptical, margin fimbriate, fringed hyphae compactly arranged, stellately dehiscing at the centre, up to 510 µm in diam; asci globose, octosporous, up to 76 µm in diameter; ascospores light brown, uniseptate, conglobate, one cell larger than the other, slightly constricted at the septum 26.6 -34 × 10.6 -17 µm.

Pycnothyriospores pyriform, brown, wall smooth, 9.5 -11.4 ×3.8-5.7 µm.

Material examined: On leaves of *Syzygium* sp. (Myrtaceae), Valiyathottathil Sri Bhagavathy temple Kavu, Kerala, October 31, 2020; Soumya Prasad SGCH 66.

2. *Asterina congesta* Cooke, Grevillea 8: 95, 1879; Hansf. & Thirum., Farlowia 3: 305, 1948; Hosag., Balakr. & Goos, Mycotaxon 59: 172, 1996; Hosag. Krishnan & Abraham, New Botanist 24: 28, 1997; Hosag., Chandraprabha & Agarwal, Asterinales of Kerala, p.57, 2011.

Colonies amphigenous, thin to dense, up to 9mm in diameter, scattered to confluent. Hyphae flexuous, undulate to curved, branching opposite, unilateral to irregular at acute angle, loosely to closely reticulate, cells 7.6 -22.8 × 3.8 -5.7 µm. Appressoria unicellular, alternate to unilateral, oblong to slightly globose, entire to rarely sublobate, antrorse to retrorse, 7.6 -11.4 × 3.8 -7.6 µm long. Thyriothecia grouped to scattered, orbicular, up to 114 µm in diameter, margin crenate to fimbriate, fringed hyphae loosely arranged, stellately dehiscing at the centre; ascospores brown, uniseptate, conglobate, one cell larger than the other, wall smooth, 22.8 -26.6 × 11.4 -13 µm. Pycnothyriospores brown, pyriform, wall smooth, 10.64 -16 × 4.5 -7.6 µm.

Material examined: On leaves of *Santalum album* L. (Santalaceae),Sree Bhuvaneshwary temple kavu, Kerala, October 31, 2020; Soumya Prasad SGCH 64.

3. *Meliola strychni* Mibey in Mibey & Hawksworth, Mycol. Pap.174: 75, 1997.

Colonies amphigenous, dense, up to 6mm in diameter, confluent. Hyphae flexuous to curved, branching opposite at acute angle, loosely to closely reticulate, cells 24.7 - 41.8 × 3.8 - 5.7 µm. Appressoria two celled, alternate, unilateral to irregular, straight to curved, antrorse, subantrorse to retrorse, 19 -27µm long; stalk cells cylindrical to cuneate, 3.8 - 11.4 µm long; head cells ovate, entire,rarely angulose to sublobate, 9.5 -15.9 × 7.6 -15 µm. Phialides mixed with appresoria, opposite, alternate to unilateral, ampulliform, 11.4 – 26.6 × 5.7 -7.6 µm. Mycelial setae scattered to grouped around perithecia, straight to curved, acute to obtuse at the apex, up to 420 µm long. Perithecia scattered to grouped, globose, verrucose up to 190 µm in diameter; ascospores 4- septate, obovoidal, slightly constricted at the septum, 32 - 36 × 11 -13 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae), Sree Mahadeva temple Kavu, Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 55; Karavelil Padinjare madom Kavu Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 62.

4. *Questieriella strychni* Hosag., J. Econ. Taxon. Bot. 28:196, 2004. Hosag., The genus Schiffnerula in India. Plant Pathology & Quarantine 1(2), 140, 2011.

Colonies amphigenous, dense, up to 6mm in diameter, confluent. Hyphae flexuous to undulate, branching opposite, alternate at acute angle, loosely toclosely reticulate, cells 11.4 - 26.6 × 3.8 - 5.7 µm. Appressoria opposite, alternate, unilateral, globose, entire, 5.7 - 11.4× 7.6- 9.5µm. Questieriella type of conidia produced from the pore of hyphal cells, scattered, fusiform, pale brown, distal cells were much smaller and paler than the central two cells, acute to obtusely rounded at the apices, 3- septate, constricted at the septum, 45.6 - 57 × 11.4 - 12 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae), Sree Mahadeva temple Kavu, Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 55;Karavelil Padinjare madom Kavu, Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 62.

5. *Meliola strychni-multiflorae* Hansf., Sydowia 11 (1-6): 59, 1958.

Colonies hypophyllous, thin, up to 5 mm in diam., scattered. Hyphae substraight, flexuous, undulate to few curved, branching opposite at acute angle, loosely to closely reticulate, cells 9.5 - 38 × 3.8 - 7.6 µm. Appressoria two celled, alternate, unilateral, straight to curved, antrorse, subantrorse to rarely reflexed, 15 - 38 µm long; stalk cells cuneate to cylindrical, 3.8 - 19 µm long; head cells, ovate, oblong, globose, entire, rarely sublobate to angulose, 11.4 - 26.6 × 7.6 – 9.5 µm. Phialides mixed with appresoria, opposite, alternate to

unilateral, ampulliform, 7.6 - 22.8 ×3.8 - 7.6 µm. Mycelial setae grouped around perithecia to scattered, simple, straight to curved, acute to obtuse at the apex, up to 510 µm long. Perithecia scattered to few grouped, globose to ovate, verrucose, up to 129 µm in diam; ascospores 4-septate, cylindrical to obovoidal, slightly constricted at the septum, 30.4 - 38×11 - 13µm.

Material examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae), Sree Mahadeva temple Kavu, Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 56.

6. *Meliola ichnocarpi-volubili* Hansf., Sydowia 16: 320, 1963.

Colonies epiphyllous, thin, up to 2 mm in diam., scattered. Hyphae substraight to flexuous, branching opposite at acute angle, loosely to closely reticulate, cells 11.4 - 38 × 3.8 - 5.7 µm. Appressoria two celled, alternate to unilateral, straight to rarely curved, antrorse to rarely retrorse, 11- 17 µm long; stalk cells cuneate, 3.8 - 7.6 µm long; head cells globose, ovate, entire to rarely angulose, 7.6- 11.4× 5.7 - 7.9 µm. Phialides mixed with appresoria to sseparate opposite to irregular, ampulliform, 11.4 - 19 × 3.8 - 7.6 µm. Mycelial setae scattered to grouped around perithecia, acute to obtuse at the apex, simple, straight to curved, up to 750 µm long. Perithecia scattered, globose, up to 140.6 µm in diam; ascospores 4– septate, obovoidal, slightly constricted at the septum, 30.4- 38 ×8.36- 15.9 µm.

Material examined: On leaves of *Ichnocarpus frutescens* (L.) R.Br.(Apocynaceae), Karavelil Padinjare madom Kavu, Pramadom, Kerala, October 29, 2020, Soumya Prasad SGCH 61.

PLATE I Host plants from the study area

Santalum album

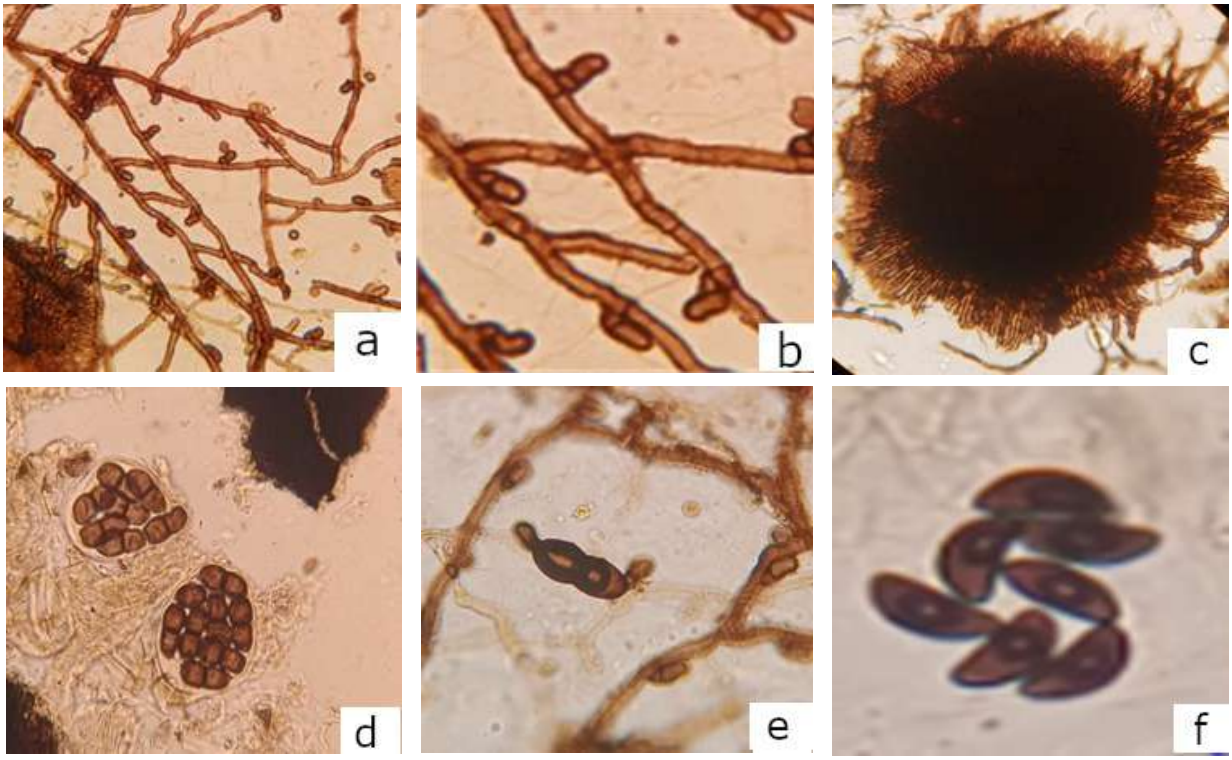
Syzygium sp.

Strychnos nux- vomica

Ichnocarpus frutescens

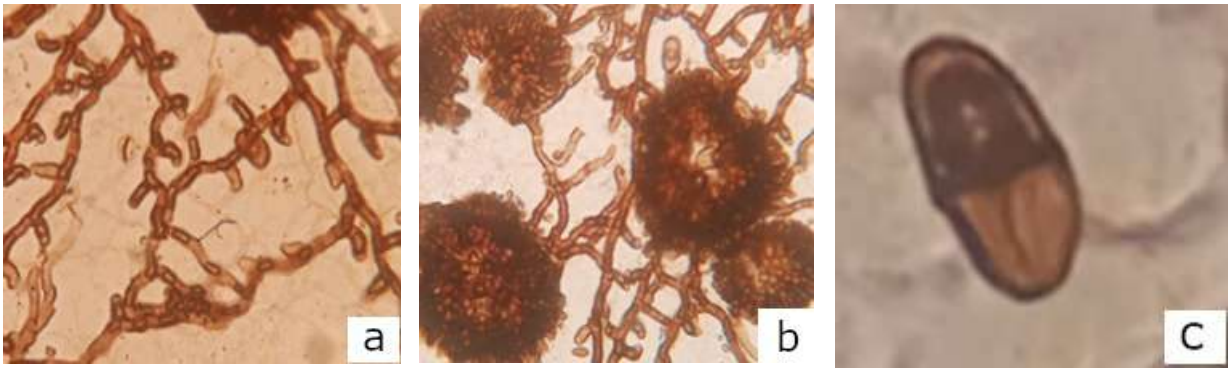
PLATE II Photomicrographs of fungal colonies

Fig 1: *Asterina jambolana* Kar & Maity



a & b. Appressoriate mycelium c. thyriothecium d. ascus e. ascospore f. pycnothyriospores

Fig 2: *Asterina congesta* Cookea.



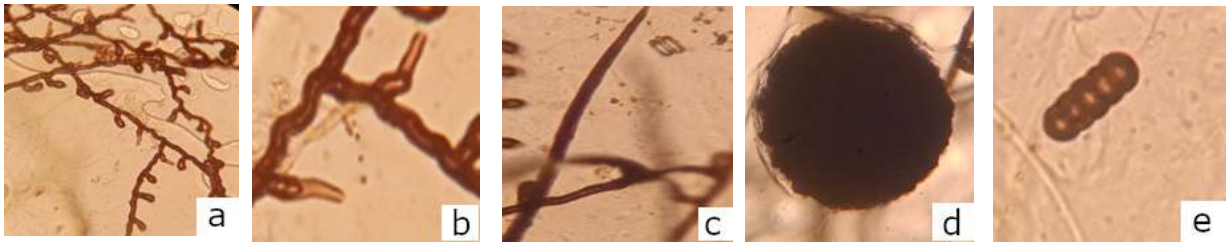
a. Appressoriate mycelium b.thyriothecium c.ascospore

Fig 3: *Questieriella strychni* Hosag.

a & b. Appressoriate mycelium c. conidia

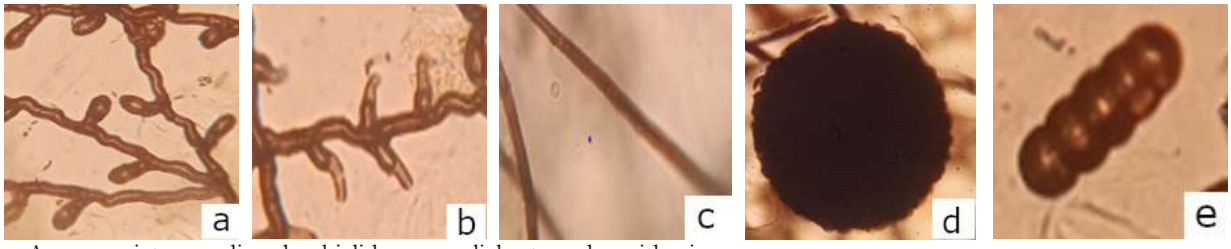
PLATE III Photomicrographs of fungal colonies

Fig 4: *Meliola strychni* Mibey



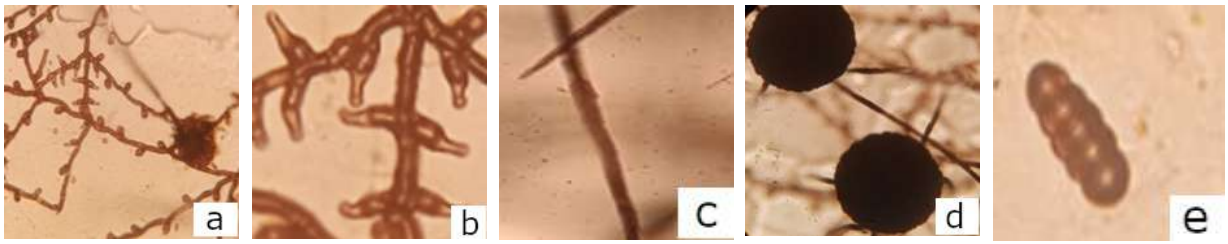
a. Appressoriate mycelium b. phialide c. mycelial setaed. perithecium e. ascospore

Fig 5: *Meliola strychni-multiflorae* Hansf



a.Appressoriate mycelium b. phialide c. mycelial setae d. perithecium e. ascospore

Fig 6: *Meliola ichnocarpi-volubili* Hansf.



a. Appressoriate mycelium b. phialide c. mycelial setae d. perithecium e. ascospore

Discussion

The present study aimed to study the foliar mycobionts on medicinal plants from sacred groves of Aruvappulam and Pramadom panchayat of Pathanamthitta district of Kerala State. The vegetations in these sacred groves were luxuriant and comprises several trees mixed with shrubs, lianas and herbs. The flora of this grove is represented by *Saraca asoka* (Roxb.) de Wilde, *Santalum album* L., *Strychnos nux - vomica* L., *Ichnocarpus frutescens* (L.) R.Br., *Alangium salvifolium* Wang., *Syzygium* species, *Mussaenda* species, *Azadirachta indica A. Juss.*, *Tectona grandis* Linn.f., *Mallotus* species, *Terminalia catappa* L., *Sarcostigma kleinii* Wight & Arn. etc. Foliar fungi on *Strychnos nux-vomica* L., *Ichnocarpus frutescens* (L.) R.Br., *Santalum album* L. and *Syzygium* species were recorded here.

Conclusion

During the study, sacred groves of varying sizes were identified in these panchayats. The present study identified and documented six foliar mycobionts on *Strychnos nux-vomica* L., *Ichnocarpus frutescens* (L.) R.Br., *Santalum album* L. and *Syzygium* species namely; *Asterina congesta* Cooke, *Asterina jambolana* Kar & Maity, *Meliola ichnocarpi-volubili* Hansf., *Meliola strychni-multiflorae* Hansf., *Meliola strychni* Mibey, *Questieriella strychni* Hosag. The present work will serve both as a reference and stimulus for further work aimed at disclosing the diversity of foliar fungi of the Pathanamthitta district of Kerala state. It will also be helpful for identifying the diversity of foliar mycobionts on various medicinal plants. Sacred groves are well explored by researchers for their floristic diversity. But the foliar fungal diversity was scantily studied in

these groves. More research is needed to assess the foliar fungal diversity in these groves. But nowadays, sacred groves are on the verge of degradation due to growing urbanization and industrialization and the steady erosion of religious and cultural belief systems. Even a small sacred grove can support long lasting services, local biota, may provide medicinal plants, economically important plants, etc. Therefore necessary actions and measures are needed for the protection of these unique forest patches.

Acknowledgment

We express our sincere gratitude to the Council of Scientific and Industrial Research (CSIR) for providing financial assistance. Grateful thanks to the Principal of Mahatma Gandhi College, Thiruvananthapuram and St. Gregorios College, Kottarakara for providing the necessary facilities.

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Chapter 2

ASSESSMENT OF FLORAL DIVERSITY IN THE HERBAL AND SPICES GARDEN AT RAJAGIRI COLLEGE OF SOCIAL SCIENCES-VALLEY CAMPUS, KAKKANAD, ERNAKULAM DISTRICT

Krishnakumar N M, Antony Ceasar S

Abstract The present study was undertaken to document the diversity of flora in the herbal and spices garden of Rajagiri College of Social Sciences-Valley Campus, Kakkanad, Ernakulam district, Kerala. The documentation of floral diversity helps to recognize the overall ecological conditions and understanding of the economic, medicinal and traditional importance of plant diversity. Documentation of floral diversity is the first step ahead before the next step of the conservation of these natural resources. The floristic survey and documentation of a particular area provide information on the distribution of plant species. It also plays an important role in creating public awareness regarding the conservation and sustainable utilization of natural resources. The flora of the herbal and spices garden of Rajagiri College of Social Sciences-Valley Campus is not yet documented. The present investigation identified a total of 117 plant species including herbs and spices represented by 110 genera belonging to 47 different families. Among the herbs and spices in Rajagiri Valley Campus, the Apocynaceae family ranks first (10 plant species) followed by Leguminosae (8 plant species), Lamiaceae (8 plant species), Asteraceae (Compositae) (7 plant species) and Zingiberaceae (6 plant species). Other families represent a small share of the total number. There are four plant species coming under Rare, Endemic, Endangered and Threatened (RET) category namely *Aporosa cardiosperma* (Gaertn.) Merr. (Family: Phyllanthaceae), *Coscinium fenestratum* (Goetgh.) Colebr. (Family: Menispermaceae), *Holostemma ada-kodien* Schult. (Family: Apocynaceae) and *Pterocarpus santalinus* L. f. (Family: Leguminosae)

Keywords: *Rajagiri College of Social Sciences, Kakkanad, Floral diversity*

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Introduction

Indian biodiversity encompasses a variety of climatic conditions such as tropical, subtropical, temperate, alpine etc., due to wide variations in temperature and precipitation. These climatic variations make the biodiversity rich in flora and fauna. India has about 2.4% of the total land area of the world but it accounts for approximately 8% in terms of total number of species found over the world. The majority of the species are found in certain biologically rich zones of tropical forests. Increased rate in clearing of tropical forest areas and decline in their plant diversity across the world has necessitated identifying biodiversity hotspot locations and *in situ* conservation of biodiversity by mapping the distribution of vegetation diversity across different habitats and landscapes and monitoring rates of their change over time. Hotspots are identified based on the number of endemic species and the degree of threat to the ecosystem for in situ conservation of biodiversity. Out of 35 hotspots identified, 4 occur in India namely, Western Ghats, Himalaya, Indo-Burma and Sundaland (including Nicobar group of Islands). The critical biodiversity assessment and its conservation strategies is an important task ahead among ecologists and environmentalists due to the rich biodiversity wealth of the country (Tripathi *et al.*, 2015).

The floristic survey of a particular area helps in

understanding the overall ecological and geographical conditions which can be deciphered by classifying the recorded plants into various biological life forms. It gives a profound understanding and appreciation of plants and trees in a particular area and their medicinal and economic values. The survey also provides the authorities concerned in formulating and implementing various policies and strategies for the sustainable management and conservation of natural resources. Moreover, floristic investigations provide reliable information about the taxonomic classification, distribution, ecology and uses of various plant species. Floristic surveys help in explaining the plant biodiversity providing information regarding the current status, new invasion, revision of the flora, ecosystem function and its conservation in a particular geographical area (Krishnakumar and Ramesh, 2022). There are 4,679 taxa of flowering plants in Kerala, belonging to 1,360 genera in 212 families (Sasidharan, 2004). The floral diversity documentation studies of various forest, mangrove and campuses help in exploring the whole plant diversity of the particular study area. The vegetation analysis is conducted by using the quantitative characteristics of the plants for estimating the importance of various plant species (Trivedi *et al.*, 1998). The information regarding species diversity, ecosystem structure and composition helps in the conservation of endemic plant species and hence the documentation of biodiversity is very important and it is an urgent need.

Rajagiri College of Social Sciences (Autonomous) (RCSS) owes its existence to the CMI (Carmelites of Mary Immaculate) fathers and it was established in 1955, as the Department of Social Work at Sacred Heart College, Thevara and shifted to Rajagiri, Kalamassery in 1967. Now, it is located on two picturesque campuses- the Hill Campus at Kalamassery and the Valley campus at Kakkanad in Kochi, Kerala. There are no previous studies conducted on plant diversity of the Kakkanad campus. In the present study, we report the floral diversity of the herbal and spices garden at Rajagiri College of Social Sciences-Valley Campus, Kakkanad, Kerala.

Materials and Methods

Study area

The present floral study was conducted at the herbal and spices garden at Rajagiri College of Social Sciences Campus and is located at Valley Campus, Kakkanad in Ernakulam district, Kerala, at an elevation of 15 m altitude above mean sea level (Fig.1 and Fig.2). The area is geographically located at 9.96433° N latitude and 76.32049° E longitude.

Floristic analysis

The study was conducted between the period of 2019-2020. Weekly field observations were made for the collection and identification of different species and details such as habit, botanical name, family and uses were noted according to Bentham and Hooker's classification. Digital photographs of freshly collected plants were also taken. The flowered twigs were collected for identifying the plant species. The voucher Herbarium specimens were prepared following Bridson and Forman (1998) and deposited at the Bio-tech Research Lab of Biosciences department, RCSS for future reference. Identification of plants was carried out with the help of available Flora and other standard publications [(Gamble and Fischer, 1936); (Rao,1914); (Manilal and Sivarajan, 1982); (Sasidharan, 2004); (Sasidharan and Sivarajan, 1996); (Sreekumar and Nair, 1991); (Nayar *et al.*, 2006)]. Further, their identification was confirmed by matching with authentic specimens in Kerala Forest Research Institute (KFRI), Peechi and Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE-JNTBGRI), Thiruvananthapuram.

Results and Discussion

The main reasons for habitat loss and biodiversity destruction are environmental pollution, increasing population, climate change and the introduction of exotic species. The natural vegetation has been considerably altered and modified by human activities.

The present investigation identified 117 species of plants including herbs and spices belonging to 110 genera under 47 families. Out of 117 species, 98 species are dicots and 19 species are monocots.

Out of 110 genera, 91 genera are dicots and 19 genera are monocots. The herbs and spices of Rajagiri Valley Campus represented 36 families of dicotyledons and 11 families of monocotyledons (Table 1). There are 100 medicinal plant species and 17 spices plant species are present in the Campus. The plants consisting of herbs and spices, has 50 species of herbs, 25 species of shrubs, 19 species of trees and 23 species of climbers. The following plants species of Herbs and Spices present in Rajagiri Valley Campus coming under RET (Rare, Endemic, Endangered and Threatened) category (Fig. 3).

Fig. 1 Study area Rajagiri Valley Campus, Kakkanad

Fig. 2 Study area Rajagiri Valley Campus, Kakkanad

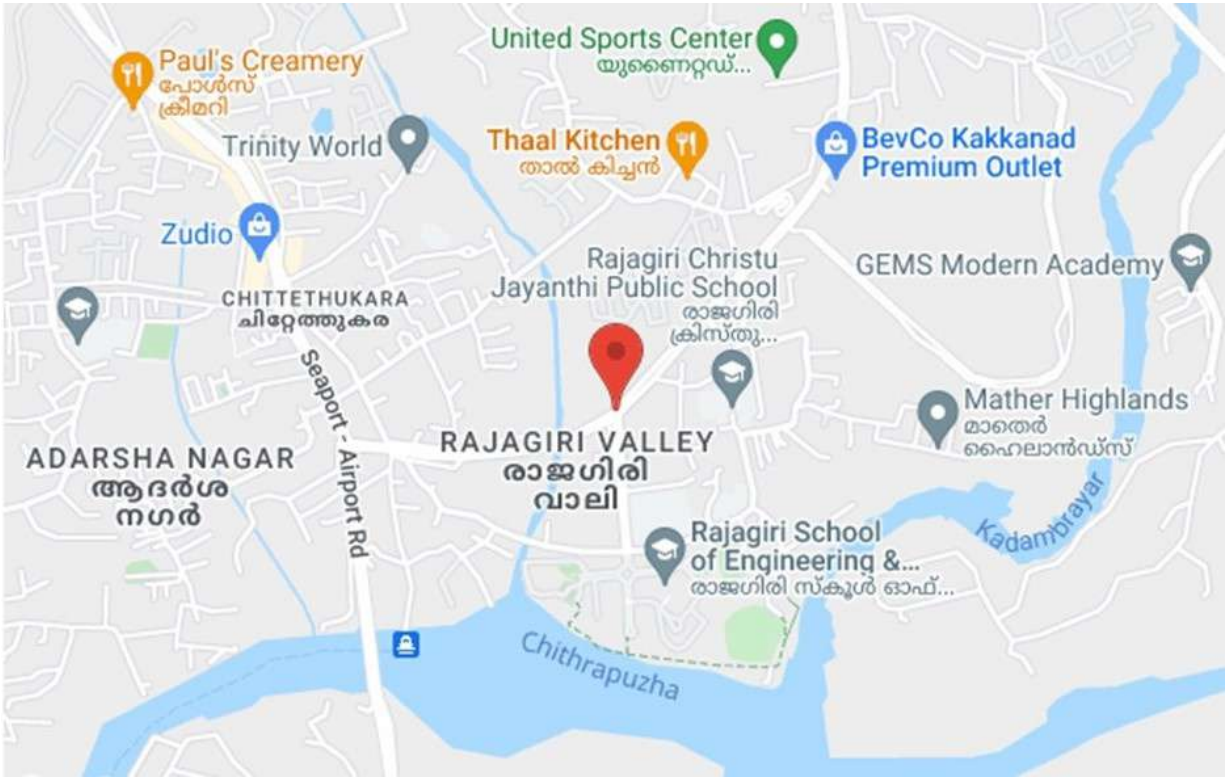
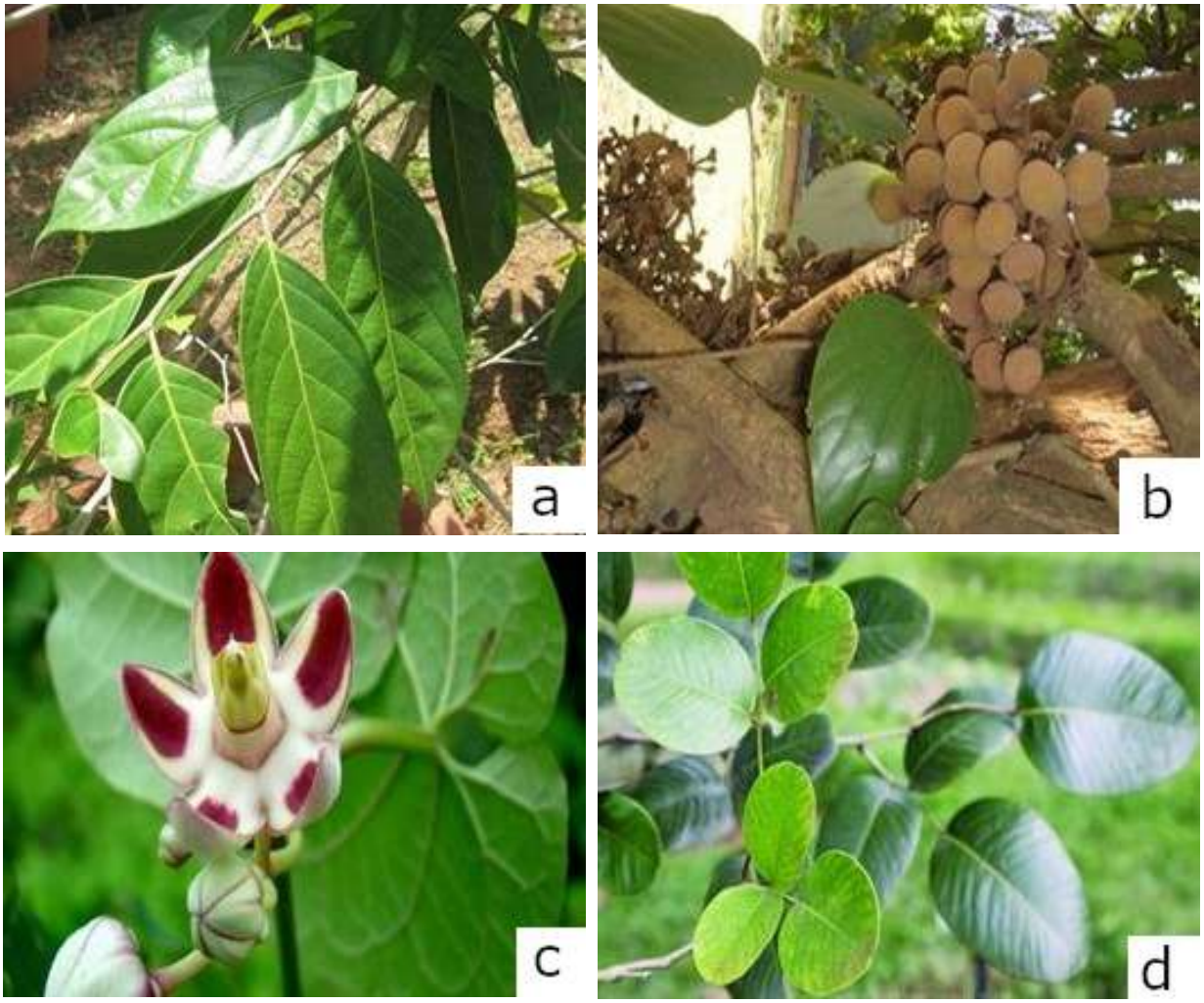


Fig. 3 Herbs and spices present in Rajagiri Valley Campus Campas under RET



a. *Aporosa cardiosperma* b. *Coscinium fenestratum* c. *Holostemma ada-kodien* d. *Pterocarpus santalinus*

	Family	Genera	Species
Dicots	36	91	98
Monocots	11	19	19
Gymnosperms	0	0	0
Total	47	110	117

1.*Aporosa cardiosperma* (Gaertn.) Merr. (Family: Phyllanthaceae): Vulnerable

2.*Coscinium fenestratum* (Goetgh.) Colebr. (Family: Menispermaceae): Decreasing species

3.*Holostemma ada-kodien* Schult. (Family: Apocynaceae): Vulnerable

4.*Pterocarpus santalinus* L. f. (Family: Leguminosae): Endangered

Among the herbs and spices in Rajagiri Valley Campus, Apocynaceae family rank first (10 plant species) followed by Leguminosae (8 plant species), Lamiaceae (8 plant species), Asteraceae (Compositae) (7 plant species) and Zingiberaceae (6 plant species). Other families represent a small share of the total number (Table 2).

Table 2: List of herbs and spices plant species placed in different plant families

Family	No. of herbs/ spices species	Family	No. of herbs/ spices species
Acanthaceae	5	Apiaceae	1
Acoraceae	1	Apocynaceae	10
Amaranthaceae	2	Araceae	1
Aristolochiaceae	1	Asparagaceae	1
Asphodelaceae	1	Asteraceae	7
Bixaceae	1	Caricaceae	1
Celastraceae	1	Clusiaceae	1
Convolvulaceae	2	Costaceae	1
Cyperaceae	1	Dioscoreaceae	1
Euphorbiaceae	4	Hypoxidaceae	1
Lamiaceae	8	Leguminosae	8
Lythraceae	3	Malvaceae	3
Meliaceae	1	Menispermaceae	5
Moraceae	1	Myristicaceae	1
Myrtaceae	2	Nyctaginaceae	1
Lauraceae	1	Oleaceae	2
Orchidaceae	1	Oxalidaceae	2
Rubiaceae	4	Rutaceae	2
Phyllanthaceae	3	Piperaceae	3
Plantaginaceae	1	Plumbaginaceae	1
Poaceae	4	Ranunculaceae	1
Sapindaceae	1	Simaroubaceae	1
Solanaceae	5	Vitaceae	2
Zingiberaceae	6		

The plant species documented in the herbal and spices garden at the Valley Campus of Rajagiri College of Social Sciences (Autonomous) (RCSS), Kakkanad is enlisted in Table 3.

Conclusion

The survival and well-being of the present day human population depends on the environment and the human life directly and indirectly depends on the flora and fauna of the specific geographical area. The various plant species have economic, nutritional, medicinal, fuel and aesthetic values and these plants preserves ecological balance. From the present study, we found that the RCSS Campus herbal and spices garden consists of highly diversified flora and it is rich in the plants of economic and medicinal importance. It is very important to know the significance of the plant diversity so that the conservation and multiplication of such plant species become quite imperative, especially in the context of plants which are on the verge of extinction. The documentation of campus floral diversity is very important as it is vital that native and endemic species of plants are conserved. Lack of awareness among the common people about various plant species in their locality which have economic, medicinal and cultural importance is one of the problems constraining the conservation efforts of the authorities concerned. The documentation of the floral diversity of the campuses and its publication will give an insight to the student community, the need for conservation and sustainable utilization of natural resources including these plant species.

Table 3: Plant species in the herbal and spices garden at the Valley Campus of RCSS, Kakkanad

Sl. No.	Botanical Name	Family	Common Name	Local Name	Habit
1	*Achyranthes bidentata* Blume	Amaranthaceae	Ox Knee	'Cherukadaladi'	H
2	*Acorus calamus* L.	Acoraceae	Sweet Flag	'Vayambu'	H
3	*Adhatoda vasica* Nees.	Acanthaceae	Malabar Nut	'Aadalodakam'	S
4	*Aerva lanata* (L.) Juss.	Amaranthaceae	Mountain Knotgrass	'Cheroola'	H
5	*Aloe vera* (L.) Burm. f.	Asphodelaceae	Aloe Vera	'KattarVazha'	H
6	*Alpinia calcarata* Roscoe	Zingiberaceae	Greater Galangal	'Chittaratha'	H
7	*Andrographis paniculata* (Burm. f.) Nees	Acanthaceae	Green Chireta	'Kiriyath'	H
8	*Aporosa cardiosperma* (Gaertn.) Merr.	Phyllanthaceae	Lindley's Aporosa	'Vetti'	T
9	*Aristolochia indica* L.	Aristolochiaceae	Indian Birthwort	'Garudakkodi'	C
10	*Asparagus racemosus* Willd.	Asparagaceae	Satavar	'Shatavari'	C
11	*Ayapana triplinervis* (Vahl) R. M. King & H. Rob.	Compositae	Ayapana	'Ayyappana'	S
12	*Azadirachta indica* A. Juss.	Meliaceae	Neem Tree	Aryaveppu	T
13	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Water hyssop	'Brahmi'	H
14	*Biophytum sensitivum* (L.) DC.	Oxalidaceae	Little Tree Plant	'Mukkutti'	H
15	*Bixa orellana* L.	Bixaceae	Achiote	'KuranguManjal'	S
16	*Boerhavia diffusa* L.	Nyctaginaceae	Punarnava	'Thazhuthama'	H
17	*Caesalpinia pulcherrima* (L.) Sw.	Leguminosae	Peacock Flower Pride of Barbados	'Rajamalli'	S
18	*Calotropis gigantea* (L.) Dryand.	Apocynaceae	Crown Flower	'Erukku'	S
19	*Cardiospermum halicacabum* L.	Sapindaceae	Balloon Plant or Love in a Puff	'Uzhinja'	C
20	*Carica papaya* L.	Caricaceae	Papaya	'Papaya'	T
21	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Madagascar Periwinkle	'Ushamalari'	H
22	*Centella asiatica* (L.) Urb.	Apiaceae	Indian Pennywort	'Kudangal'	H
23	*Chamaecostus cuspidatus* (Nees& Mart.) C. D. Specht & D. W. Stev.	Costaceae	Insulin plant	'InsulinChedi'	H
24	*Chonemorpha grandiflora* G. Don	Apocynaceae	Frangipani Vine	'Perumkurumba'	C
25	*Chromolaena odorata* (L.) R. M. King & H. Rob.	Compositae	Siam Weed	'Communist Pacha'	S
26	*Chrysopogon zizanioides* (L.) Roberty	Poaceae	Vetiver	'Ramacham'	H
27	*Cinnamomum verum* J. Pres	Lauracea	Cinnamon Tree	'Karuvappatta'	T
28	*Cissampelos pareira* L.	Menispermaceae	Velvet Leaf	'Malathangi'	C
29	*Cissus quadrangularis* L.	Vitaceae	Veldt Grape	'Changalamparanda'	C
30	*Citrus limon* (L.) Osbeck	Rutaceae	Lemon Tree	'Cherunarakam'	T

Sl. No.	Botanical Name	Family	Common Name	Local Name	Habit
31	*Coleus zeylanicus* (Benth.) L. H. Cramer	Lamiaceae	Hrivera	'Iruveli'	H
32	*Coscinium fenestratum* (Goetgh.) Colebr.	Menispermaceae	Tree Turmeric	'Maramanjal'	C
33	*Croton oblongifolius* Roxb.	Euphorbiaceae	Naga Danti	'Naga Danti'	T
34	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Golden Eye Grass'	Nilappana'	H
35	*Curcuma longa* L.	Zingiberaceae	Turmeric	'Manjal' H	
36	*Cyclea peltata* (Lam.) Hook. f. & Thomson	Menispermaceae	Indian Moon-Seed	'Padakkizhangu'	C
37	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Lemon Grass	'Injippullu'	H
38	*Cynodon dactylon* (L.) Pers.	Poaceae	Bermuda Grass	'Karuka'	H
39	*Cyperus rotundus* L.	Cyperaceae	Nut grass	'Muthanga'	H
40	*Datura metel* L.	Solanaceae	Devil's Trumpet and Metel	'NeelaUmmam'	S
41	*Desmodium gangeticum* (L.) DC.	Leguminosae	Sal Leaved Desmodium	'Orila'	H
42	*Desmodium triflorum* (L.) DC.	Leguminosae	Creeping Tick Trefoil,	'Nilamparanda'	H
43	*Desmostachya bipinnata* (L.) Stapf	Poaceae	Halfa Grass	'Darbha'	H
44	*Eclipta prostrata* (L.) L.	Asteraceae	False Daisy	'Kayyonni'	H
45	*Emilia sonchifolia* (L.) DC. ex DC.	Compositae	Lilac Tassel Flower	'Muyalchevi'	H
46	*Euphorbia hirta* L.	Euphorbiaceae	Common Spurge	'Nilappala'	H
47	*Evolvulus alsinoides* (L.) L.	Convolvulaceae	Slender Dwarf Morning Glory	'Vishnukranthi'	H
48	*Garcinia gummi-gutta* (L.) Roxb.	Clusiaceae	Malabar Tamarind	'Kudampuli'	T
49	*Gardenia jasminoides* J. Ellis	Rubiaceae	Cape Jasmine	'Gandharajan'	S
50	*Gymnema sylvestre* (Retz.) R. Br. ex Sm.	Apocynaceae	Australian Cow Plant	'Chakkarakkolli'	C
51	*Hedychium coronarium* J. Koenig	Zingiberaceae	White Butterfly Ginger Lily	'Kalyana Sougandhikam'	H
52	*Hemidesmus indicus* (L.) R. Br. ex Schult.	Apocynaceae	Indian Sasaparilla	'Naruneendi'	C
53	*Hemigraphis colorata* (Blume) Hallier f.	Acanthaceae	Red Flame Ivy	'Murikooti'	H
54	*Hibiscus rosa-sinensis* L.	Malvaceae	China Rose	'Chemparathi'	S
55	*Holostemma ada-kodien* Schult.	Apocynaceae	Holostemma Creeper	'Adapathiyan'	C
56	*Hygrophila auriculata* (Schumach.) Heine	Acanthaceae	Marsh Barbel	'Vayalchulli'	H
57	*Indigofera tinctoria* L.	Leguminosae	True Indigo	'Neela Amari'	S
58	*Ipomoea sepiaria* Koenig ex Roxb.	Convolvulaceae	Morning Glory	'Thiruthali'	C
59	*Ixora coccinea* L.	Rubiaceae	Jungle Geranium	'Chethi'	S
60	*Jasminum grandiflorum* L.	Oleaceae	Royal Jasmine	'Kaattu Pichakam'	C
61	*Kaempferia galanga* L.	Zingiberaceae	Aromatic Ginger	'Kacholam'	H
62	*Kaempferia rotunda* L.	Zingiberaceae	Indian Crocus	'Chengazhineer Kizhangu'	H

Sl. No.	Botanical Name	Family	Common Name	Local Name	Habit
63	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	Pride of India	'Manimaruthu'	T
64	*Lawsonia inermis* L.	Lythraceae	Henna	'Mayilanji'	S
65	*Leucas aspera* (Willd.) Link	Lamiaceae	Thumbai	'Thumba'	H
66	*Mentha piperita* subsp. *citrata* (Ehrh.) Briq.	Lamiaceae	Peppermint	'Peppermint'	H
67	*Mimosa pudica* L.	Leguminosae	Touch-me-not	'Thottavaadi'	H
68	*Morus alba* L.	Moraceae	Mulberry	' Mulberry'	T
69	*Murraya koenigii* (L.) Spreng.	Rutaceae	Curry Leaf Tree	'Kariveppu'	T
70	*Myristica fragrans* Houtt.	Myristicaceae	Nutmeg	'Jaathi Maram'	T
71	*Myxopyrum smilacifolium* (Wall.) Blume	Oleaceae	Myxopyrum	'Chathura Mulla'	C
72	*Naravelia zeylanica* (L.) DC.	Ranunculaceae	Ceylon Naravelia	'Vaathakkodi'	S
73	*Nerium oleander* L.	Apocynaceae	Oleander	'Arali'	S
74	*Ocimum americanum* L.	Lamiaceae	American Basil	'KattuThulasi'	H
75	*Ocimum tenuiflorum* L.	Lamiaceae	Holy Basil	'Krishna Thulasi'	H
76	*Oldenlandia corymbosa* L.	Rubiaceae	Diamond Flower	'Parpatakappullu'	H
77	*Oxalis corniculata* L.	Oxalidaceae	Creeping Wood Sorrel	'Puliyarila'	H
78	*Phyllanthus emblica* L.	Phyllanthaceae	Indian Gooseberry	'Nelli'	T
79	*Phyllanthus niruri* L.	Phyllanthaceae	Gale of the Wind	'KeezharNelli'	H
80	*Physalis minima* L.	Solanaceae	Native Gooseberry	'Njottanjodiyan'	H
81	*Pimenta dioica* (L.) Merr.	Myrtaceae	Allspice	'Sarvasugandhi'	T
82	*Piper betle* L.	Piperaceae	Betel	'Vettila', 'Nagavalli'	C
83	*Piper longum* L.	Piperaceae	Long Pepper	'Thippali'	C
84	*Piper nigrum* L.	Piperaceae	Pepper	'Kurumulaku'	C
85	*Plectranthus amboinicus* (Lour.) Spreng.	Lamiaceae	Mexican Mint	'Panikoorka'	H
86	*Plumbago indica* L.	Plumbaginaceae	Indian Leadwort	'Chethikoduveli'	S
87	*Premna serratifolia* L.	Lamiaceae	Headache Tree	'Munja'	S
88	*Psilanthus travancorensis* (Wight &Arn.) J.-F. Leroy	Rubiaceae	Psilanthus	'Pushkara Mulla'	S
89	*Pterocarpus santalinus* L. f.	Leguminosae	Red Sandalwood	'RaktaChandanam'	T
90	*Punica granatum* L.	Lythraceae	Pomegranate	'Mathalam'	S
91	*Ricinus communis* L.	Euphorbiaceae	Castor Oil Plant	'Aavanakku'	S
92	*Rotheca serrata* (L.) Steane&Mabb.	Lamiaceae	Blue Fountain Bush	'CheruThekku'	S
93	*Salacia reticulata* Wight	Celastraceae	Salacia	'Ekanayakam'	S
94	*Scindapsus officinalis* (Roxb.) Schott	Araceae	GajaPippali	'Gajapippali'	C
95	*Senna occidentalis* (L.) Link	Leguminosae	Coffee Senna	'Ponnaveeram'	S
96	*Sida cordifolia* L.	Malvaceae	Flannel Weed	'Kurumthotti'	H
97	*Simarouba glauca* DC.	Simaroubaceae	Paradise Tree	'Lakshmi Tharu'	T
98	*Solanum trilobatum* L.	Solanaceae	Purple Fruited Pea, Egg plant	'Putharichunda'	S

Sl. No.	Botanical Name	Family	Common Name	Local Name	Habit
99	*Solanum xanthocarpum* Schrad. & H. Wendl.	Solanaceae	Surattense	'Kantakari Chunda'	H
100	*Stevia rebaudiana* (Bertoni) Bertoni	Compositae	Stevia plant	'Stevia'	H
101	*Strobilanthes ciliata* Nees	Acanthaceae	Lesser Kurinji	'Karim Kurinji'	H
102	*Syzygium aromaticum* (L.) Merr. & L. M. Perry.	Myrtaceae	Cloves	'Grampu'	T
103	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	Apocynaceae	Crape Jasmine	'Nandya-rvattom'	S
104	*Tamarindus indica* L.	Leguminosae	Tamarind	'Valanpuli'	T
105	*Theobroma cacao* L.	Malvaceae	Coco Plant	'Coco'	T
106	*Tilia coraracemosa* Colebr.	Menispermaceae	Tiliacora Plant	'Valli Kanjiram'	C
107	*Tinospora cordifolia* (Willd.) Miers	Menispermaceae	Heart-leaved Moonseed	'Chittamruthu'	C
108	*Tragia involucrata* L.	Euphorbiaceae	The Indian Stinging Nettle	'Kodithoova'	H
109	*Trichopus zeylanicus* Gaertn.	Dioscoreaceae	Trichopus Plant	'Arogyappacha'	H
110	*Tylophora indica* (Burm. f.) Merr.	Apocynaceae	Indian Ipecac	'Vallippala'	C
111	*Vanilla* sp.	Orchidaceae	Vanilla	'Vanilla'	C
112	*Vernonia cinerea* (L.) Less.	Asteraceae	Little Ironweed	'Poovamk-urunnal'	H
113	*Vitis vinifera* L.	Vitaceae	Common Grape Vine	'Munthiri'	C
114	*Wedelia chinensis* (Osbeck) Merr.	Asteraceae	Chinese Wedelia	'Manjakanjunni'	H
115	*Withania somnifera* (L.) Dunal	Solanaceae	Indian Ginseng	'Amukkuram'	S
116	*Wrightia tinctoria* R. Br.	Apocynaceae	Dyer's Oleander	'Danthappala'	T
117	*Zingiber officinale* Roscoe	Zingiberaceae	Ginger	'Inchi'	H

H – Herb; S – Shrub; C – Climber; T - Tree

Acknowledgement

We acknowledge the Management of Rajagiri College of Social Sciences (RCSS) (Autonomous), Kalamassery, Kochi, Kerala for providing facilities for the successful completion of the Research Project. The funding from Rajagiri College of Social Sciences (RCSS) (Autonomous), Kalamassery for Minor Research Project under Faculty Research Programme of RCSS to the first author (NMK) is gratefully acknowledged.

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Chapter 3

AVIAN DIVERSITY AND ITS CONSERVATION CONCERNS IN A RAMSAR SITE, ASHTAMUDI WETLAND, KERALA, INDIA

Dilraj C P, Jisha S, Hari B

Abstract Diversity and abundance of the avifauna of Ashtamudi wetland (Ramsar site No. 1204) situated in the Kollam district of Kerala State were assessed from September 2018 to August 2019. A direct observation method was followed to record bird species in the sampling sites. 92 bird species belonging to 74 genera, 40 families, and 16 orders were recorded during the study. Of these, 19 species (20%) were winter migrants and eight species (9%) were local migrants, and 65 species (71%) were resident birds. Based on the International Union for Conservation of Nature Red List of Threatened Species, 87 of 92 species (95%) fall under the Least Concern (LC) category and 5 species (5%) belonged to Near Threatened (NT) category. Birds belonging to the Near Threatened category were *Anhinga melanogaster*, *Mycteria leucocephala*, *Limosalimosa*, *Threskiornis melanocephalus*, and *Numenius arquata*. The most dominant birds were, *Egretta garzetta* (19%), *Haliastur indus* (17%), *Corvus splendens* (16%); *Microcarbo niger* (8%), and *Ardeola grayii* (3%). The highest number of species was recorded during February (70) followed by March (68), lowest was in June (48) and July (49). Two bird species came under the abundant category, and 16 species and nine species came under the Rare and Occasional categories, respectively. The major threats identified for the avian fauna were anthropogenic activities such as the reclamation of the wetland for residential purposes, other constructions, aquaculture, unscientific fishing, and solid waste disposal. The importance of Ashtamudi wetland as one of the habitats in the Central Asian Flyway (CAF) of migratory birds along the Kerala coast was also discussed.

Keywords: *Bird population, Near Threatened, Habitat Deterioration, Near Threatened, Anhinga melanogaster, Ardeidae*

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Introduction

Wetlands are temporarily or permanently covered water bodies and one of the most productive ecosystems on the earth, intermediate between terrene and marine ecosystems, which play a significant role in the ecological sustainability of an area (Orimoloye *et al.*, 2020). The physical nature of wetlands, such as shallow depth, large surface area, and high shoreline complexity, contributes to their biodiversity richness and the capacity for retaining nitrogen (Larsanders *et al.*, 2005). The wetland habitat relies on seasonal variabilities, such as rainfall and temperature variations, for its proper establishment. The boundary between a wetland and the upland forms a transition zone of varying water regimes and plant communities (Weller, 1999). The ability to survive in a unique hydric soil is a distinguishing adaptive feature of wetland flora (Cowardin *et al.*,1979). In India 67,420 wetlands, cover an area of about 4.1 million ha., out of these, 2,167 are natural, whereas 65,253 are man-made (Anonymous, 1990). Gopal and Sah (1995) classified wetlands in India based on their salinity, physiognomy, flood duration, and location. Out of the 37 Ramsar sites from India, three are from Kerala, viz, Vembanad-Kole wetland, Ashtamudi Lake and Sasthamkotta Lake. The Ashtamudi Lake, one of the important Ramsar sites (No. 1204, Designation date 19/08/2002, area 61,400 ha) in India, is the second largest and the deepest wetland

ecosystem in Kerala, situated in the Kollam district (MoEFCC, 2020). The Kottuli and Kadalundi wetlands in Kerala also have national importance. All these five areas are identified under the National Wetland Conservation Programme (Rachana and Azeez, 2010). Nayana and Saikat (2021) have analysed the 11 important ecosystem services provided by the Ashtamudi wetland. Inland navigation, fishery, and recreation are some of the direct use benefits of the Ashtamudi wetland. Indirect use benefits include sequestration of carbon and shrimp larvae protection by the mangrove vegetation (Anoopa, 2012).

Avian fauna act as an indicator of threatened habitats and ecosystems. They depend on wetland areas for various activities such as feeding, breeding, roosting, etc. (Howes and Bakewell, 1989). Out of 9,993 recognized bird species within the world (Walter *et al*., 2014), 1,340 bird species were found within the Indian landmass (Manakandan and Pittie, 2001) and 310 species are known to be dependent on wetlands (Kumar *et al*., 2005). Water birds generally include a large group of species such as *Anseriforms*, *Charadriiformes*, *Cioniiformes*, *Graviiformes*, *Gruiformes*, *Pelecaniformes*, *Podicipediformes*, *Procellariiformes*, etc., which display various wetland adaptations (Francois and Alan, 2008).

The type of vegetation, depth of water column, and water quality are the major factors responsible for distributing birds in wetlands (Weller, 1999; Caziani and Derlindati, 2000; Jayson, 2001; Seymour and Simmons, 2008). Heterogeneous wetlands exhibit higher avifauna diversity than other types (Datta, 2011). An area that regularly holds 1% or more of a waterbird population qualifies as a wetland of international importance under the Ramsar Convention of Wetlands (Anonymous, 2010). The South Asian wetland systems are facing immense anthropogenic pressure, which can adversely affect the structure of avian communities (Anonymous, 2018). Indian wetlands act as one of the major wintering grounds and stop-over sites for migratory birds in the Asian Flyway (Sandilyan *et al*., 2010). The wetlands of Kerala have prime importance in the sustenance of numerous birds (Neelakantan, 1982; Neelakantan *et al*., 1993; Sivaperuman and Jayson, 2000; Aarif and Basheer, 2012). Sitaram (2014) recorded 57 species of birds from the Ashtamudi wetland. Hari *et al*. (2018) studied the diversity of avifauna of Kandachira wetland, the southernmost part of the Ashtamudi wetland, and recorded 81 species. Birds serve as a flagship species for imperilled habitats and ecosystems, and as early warning systems for environmental changes and habitat alterations. Conservation efforts focused on birds often help preserve many other less conspicuous organisms and their habitat.

In the current scenario, Ashtamudi Lake is on the verge of immense deterioration due to various anthropogenic activities such as solid waste disposal, unscientific fishing activities, and reclamation of the wetland area (George and Jayakumar, 2011). Several studies have been conducted in this area related to deteriorating water quality, especially in the Neendakara estuary region, and excessive salinity intrusion mainly due to a reduction in summer flow (George and Jayakumar, 2011; Mophin and Prasad, 2017). One of the most important elements in bird conservation is habitat protection. Birds that are breeding in poor-quality habitats, will not contribute to a sustainable population throughout the year (Pulliam and Danielson, 1991). Ashtamudi backwater is declared a Ramsar site (No. 1204, 19.8.2002), but no efforts were taken yet for documenting the conservation aspects in relation to avian fauna. In this context, this study focused on avian diversity, annual species composition, abundance, resident and migratory status, and International Union for Conservation of Nature (IUCN) status. Moreover, the study aimed to investigate the threat faced by the avian fauna in the study area.

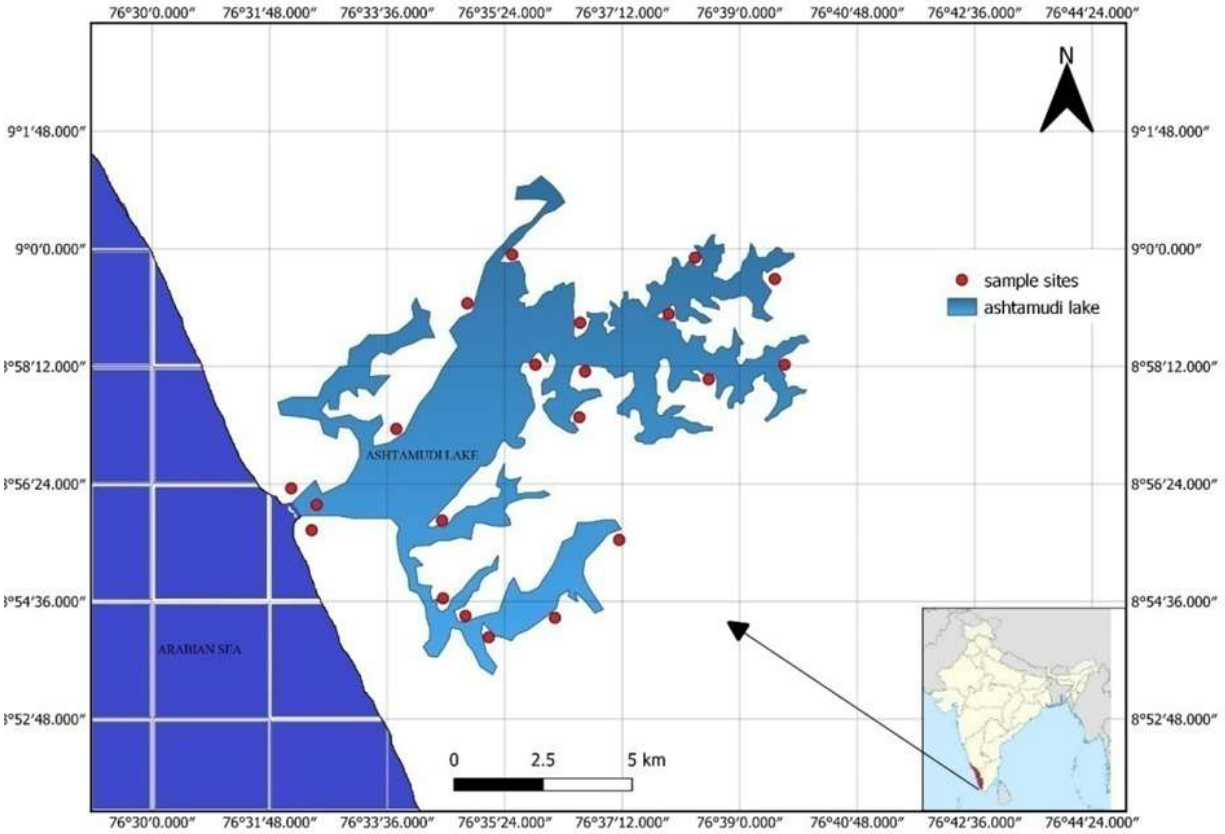
Materials and methods

Study area: The Ashtamudi wetland (Kollam district of Kerala, India) has a total geographical area of 61.4 km2 with geographical coordinates of 8o56'46.18"N and 76o33'16.33"E. The Lake has a permanent connection with the Arabian Sea and water is exchanged by daily tides between the two. The Kallada river contributed to the inflow of water into the Ashtamudi wetland. Based on the habitat

type, 21 sampling sites were identified in this area for the study (Fig. 1).

Sampling surveys: The sampling surveys were carried out from September 2018 to August 2019. The direct observation method (Altman 1974) and line transect method (Gregory *et al*., 2004) were used to estimate the bird population and diversity in each site. The birds were recorded from a 25-100m distance on either side of the transect line (Sutherland, 2006). The number of transects was based on the relative extent of the study area. Observations were carried out both in the mornings and evenings when the birds were most active (between 06:00 am 10.00 am and 16:00 pm to18.00 pm). A survey was conducted on foot, identified and counted with the help of binoculars (10x50), DSLR camera (AF-S 55-200mm Nikkor lens), Spotting scope (80mm Celestron), and a field guide (Grimmet *et al*., 2016; Neelakantan, 2017). Birds were identified up to the species level. The abundance status of the birds was categorised into A: Abundant > 5000, C: Common–100≤5000, UC: Uncommon–10≤99, RA: Rare–2≤9, OC: Occasional – sighted only once (Francis, 2015). IUCN Red List of Threatened Species for global conservation (Anonymous, 2021) was used to evaluate the conservation status of the avian fauna recorded in the present study. Resident and Migratory status of birds were recorded as per Grimmet *et al.* (2016) and Jayson and Sivaperuman, 2005. Standardized common and scientific names (Praveen, 2015) were used. Threats to the avifauna of Ashtamudi Lake were identified, and reported during each field survey, and photographs were also taken.

Fig 1. Map showing avian survey study sites in Ashtamudi Wetland, Kerala, India



Results and discussion

The study recorded 92 bird species belonging to 74 genera, 40 families, and 16 orders from Ashtamudi wetland area during the sampling period (Table 1). High avifauna diversity in the Ashtamudi wetlands showed the importance of their habitat and its conservation. Similar observations and results were made by Hari *et al.* (2018) who reported 81 species of birds from Kandachira, the southern part of the Ashtamudi wetland. Narayanan *et al.* (2011) recorded 225 bird species that belong to 15 orders and 59 families in Kuttanad wetland, Kerala, which is a part of Vembanadu Kole. A total of 82 species under 36 families belonging to 13 orders were reported in the Munderikadavu wetland of the Kannur district (Roshnath and Sruthi, 2015). 131 avian species belonging to 45 families were identified from the estuarine wetland area of Bhaynder and Naigon, Maharashtra (Lad and Patil, 2015). This indicates the suitability of the habitat for birds to successfully perform their life activities like feeding and breeding.

Out of 16 orders observed from the Ashtamudi wetland, the Order Passeriformes dominated the list (17 families with 32 species) followed by Charadriiformes (4 families with 17 species), Pelecaniformes (2 families with 15 species), Coraciiformes (2 families with 5 species), Suliformes (2 families with three species), Piciformes (2 families with 2 species), Gruiformes, Accipitriformes (1 family with 3 species each), Ciconiiformes, Columbiformes, Cuculiformes, Strigiformes (1 family with 2 species each) Anseriformes, Caprimulgiformes, Psittaciformes, Bucerotiformes (1 family with 1 species each) (Fig. 2). Hari *et al.* (2018) reported similar observations in Kandachira wetland area, where the Order Passeriformes was the dominant, and it was followed by the Order Charadriiformes. Patel and Ravel (2019) from Prashanvada wetland, Gujrat, India reported that Passeriformes (24 families and 55 species) was the most dominant order and followed by Charadriiformes (7 families and 24 species), and a minimum of one family and one species reported from Order Strigiformes, Apodiformes, and Bucerotiformes. Roy *et al.* (2016) reported that 75% of bird species from the Chupi-Char wetland of West Bengal, India comes under the Order Passeriformes.

Among the 40 families recorded from the sampling stations, the dominant family was Ardeidae, represented by 13 species, followed by Scolopacidae with 5 species. The families having the lowest representations, with one species each were Anatidae, Upupidae, Apodidae, Recurvirostridae, Coraciidae, Hirudinidae, and Dicruridae. Vyas *et al.*

Fig 2. No. of species and families of birds recorded in each avian order at Ashtamudi wetland during Sept. 2018-Aug. 2019.

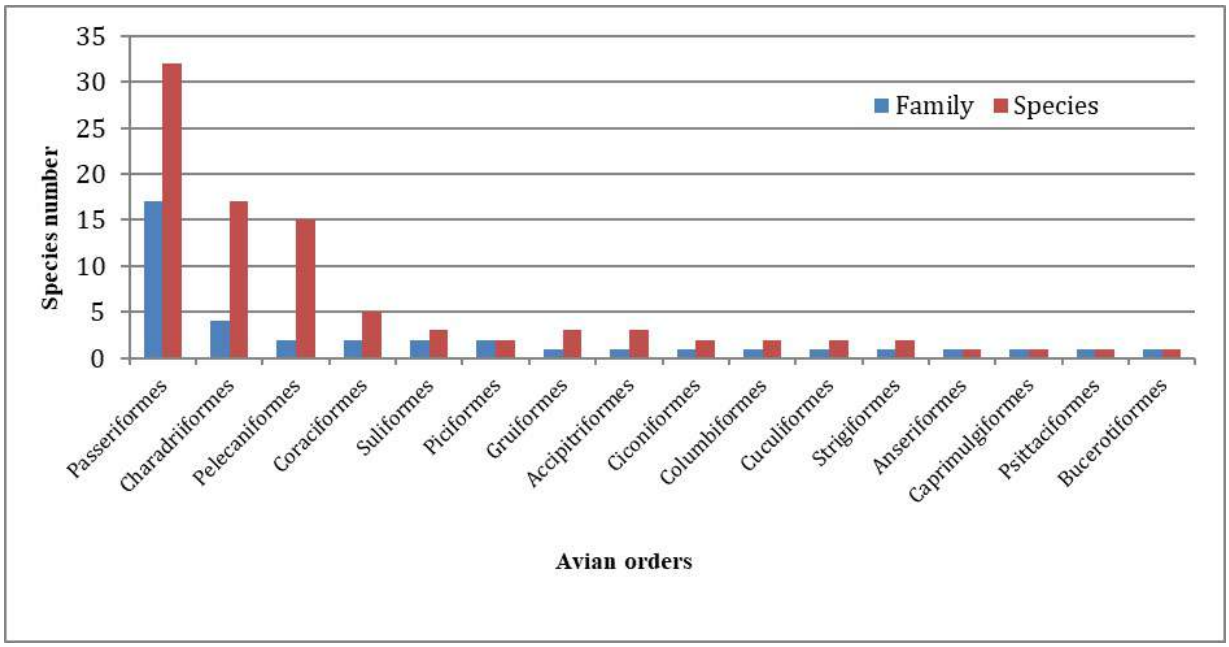


Fig 3. Resident status of avifauna of Ashtamudi wetland during Sept. 2018-Aug. 2019

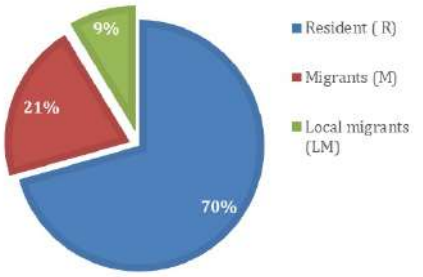


Fig 4. Abundance status of avifauna in Ashtamudi wetland during Sept. 2018-Aug. 2019

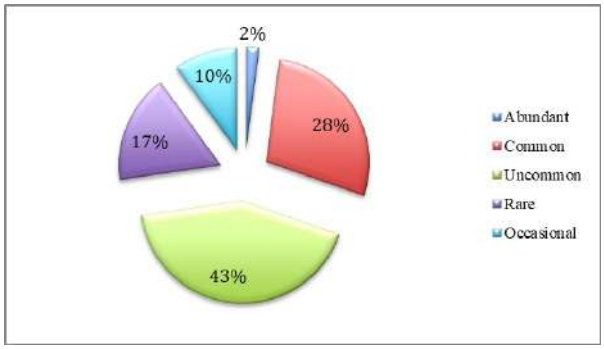
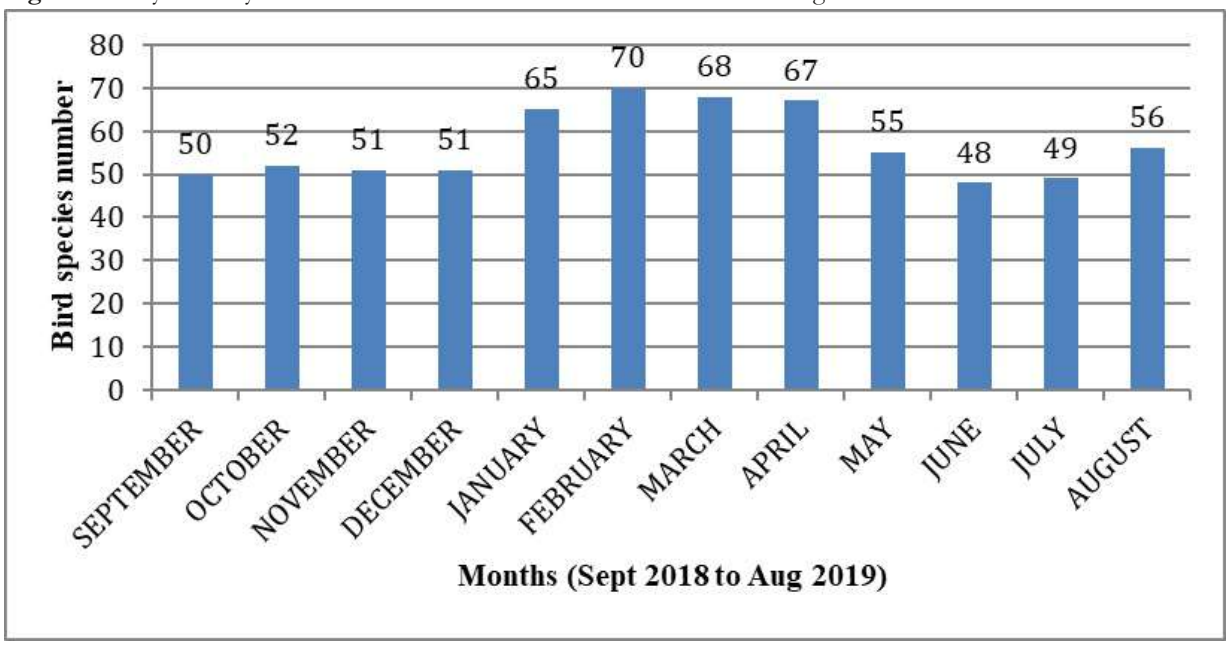


Fig 5. Monthly diversity of avifauna recorded in the Ashtamudi wetland during 2018-2019



(2010) reported Anatidae and Ardiedae as the dominant families from the avifauna of the Bhoj wetland of Madhya Pradesh. Regarding the migratory status of birds, 19 species (21%) were found to be winter migrants and eight species (9%) were local migrants and 65 species (70%) were resident birds (Fig.3). Jayson and Sivaperuman (2005) reported 68 migrant birds from Kole wetland, Thrissur. Patel and Raval (2019) reported from the Prashanavada wetland, Gujarat, India

A total of 33,906 individual birds were observed in Ashtamudi wetland during the study period. The most dominant birds were Little Egret, *Egretta garzetta* (19%); Brahminy Kite, *Haliastur indus* (17%); House Crow, *Corvus splendens* (16%); Little Cormorant, *Microcarbo niger* (8%) and Indian Pond-Heron, *Ardeola grayii* (3%). An abundance of the predatory bird Brahminy Kite was recorded along the Neendakara and Sakthikulangara fishing harbours of Kollam District and it may be due to the availability of trash fish as a food source. As per the abundance status (Fig.4), out of 92 species of birds from the Ashtamudi wetland, two of them-Little Egrets and Brahminy Kites (2%) belonged to the abundant category, 26 species (28%) belonged to the common category, 39 species (43%) were under the uncommon category, whereas 16 (17%) and nine (10%) bird species came under the rare category and occasional category, respectively.

The highest number of species from the Ashtamudi wetland were recorded during February (70) followed by March (68), April (67), and January (65), the lowest was reported in the month July (49) and June (48) (Fig. 5). Study in Kole wetlands of Thrissur district of Kerala, India reported high species richness during the migratory period

(December to March) and low during the monsoon (Jayson, 2002). Balapure *et al.* (2012) noted the maximum diversity of birds in the winter and lowest during the monsoon in the Narmada River basin, Madhya Pradesh, India. The arrival of migratory birds during the winter season results in increased avian diversity from January to March. Due to the onset of the South-West Monsoon in Kerala, the occurrence of birds was significantly low from June to September, due to the adverse field conditions for birds (Kannan and Pandiyan, 2012).

River basins with thick riparian vegetation help birds roost and nest, and also support diverse avian fauna (Bijukumar, 2006; Vincy *et al.*, 2016). The habitat heterogeneity of the Ashtamudi wetland makes it a highly productive ecosystem. The west-coast and east-coast of India come under the Central Asian Flyway and it includes the Ashtamudi wetland area, which acts as a major wintering and stop-over site for migratory birds (Hari *et al.*, 2018). The Kole wetland of Thrissur and Kadalundi-Vallikunnu Community Reserve is the other important zones in CAF (Sivperuman and Jayson, 2000; Aarif *et al.*, 2015). The winter visitors showed species-specific patterns of arrival and departure. They appear in the wetlands from the beginning of September and stay up to May. The winter population of migratory birds attains its peak during February (70) and March (68), which could be the reason for the high species diversity of birds from January (65) to April (67) at Ashtamudi wetland.

Based on the IUCN Red List of Threatened Species (2021) out of 92 species, five species (5%) were categorized as Near Threatened (NT) and 87 species (95%) fall under the Least Concern (LC) category. The Near Threatened species were Oriental Darter (*Anhinga melanogaster*), Painted Stork (*Mycteria leucocephalia*), Black-tailed Godwit (*Limosalimosa*), Black-headed Ibis (*Threskiornis melanocephalus*), and Eurasian Curlew (*Numenius arquata*). Hari *et al.* (2018) reported four birds that belong to the Near threatened category (Oriental Darter, Painted Stork, Black-tailed Godwit, Black-headed Ibis) and one bird belongs to the vulnerable category (Woolly-necked Stork) from Kandchira, which is the southernmost branch of the Ashtamudi wetland. Three Near Threatened, one Critically Endangered, and two Vulnerable species were recorded from the Prashnavada wetland, Gujarat, India (Patel and Ravel, 2019). Bijukumar, (2006) observed four Near Threatened species from the Bharathapuzha river basin, Kerala, India. Three Near threatened species were recorded in the Barna wetland of the Narmada River basin, Madhya Pradesh, India (Balapure *et al.*, 2012). The presence of five Near Threatened bird species highlights the importance of Ashtamudi wetland as a bird habitat that must be conserved.

Anthropogenic pressures adversely influence the structure of the bird community (Sharma and Saini, 2012) in Gharna Wetland, Jammu (J&K) India. Habitat destruction reduces the heterogeneity of wetland areas (Svingen and Anderson, 1998). George and Jayakumar (2011) reported a reduction in the number of birds visiting the Ashtamudi wetland area due to anthropogenic activities. Many human interventions that affect the health of Ashtamudi wetland ecosystem were identified during this study. Land reclamation, encroachment of the wetland for aquaculture activities, rampant solid waste disposal, unregulated fishing, and oil spills from fishing boats were the major concern among them. Improper disposal of abandoned nets and fishing materials and plastic wastes in the wetland leads to casualties of birds. The most important threats faced by the avifauna in the Ashtamudi wetland are the following.

Conservation concerns

Reclamation of wetland area:

One of the major threats posed to the Ashtamudi wetland is its encroachment and reclamation of non-eco-friendly activities. Unscientific land use causes salinity intrusion, ecosystem imbalance, and finally, biodiversity loss. Wetland areas were intensely reclaimed for constructing roads, buildings, and agricultural activities, as observed in Kandachira, and Munrothuruthu- the eastern part of Ashtamudi wetland. Hari *et al.* (2018) have reported on the alterations that happened in the land use pattern in Kandachira area of Ashtamudi wetland. This type

of anthropogenic activity might reduce the roosting and foraging areas available for the wetland birds (Erwin and Beck, 2007) and may ultimately result in the loss of avian diversity.

Solid waste Disposal:

The water holding capacity of the wetlands plays a significant role in maintaining the hydrological cycle (Abraham, 2015). Therefore, the uncontrolled solid waste disposal in the wetland ecosystems is hindering the natural flow of water and leading to its availability in the wetland area. Solid waste disposal also affects the physico-chemical properties of the wetland water and has a negative impact on many waterfowl (Azeez *et al*., 2008; Munisamy, 2018), as they heavily depend on these wetland bodies for their needful resources. The garbage dumps in the wetlands can shift the feeding habits of many-dependent organisms, especially waterfowl and these garbage sites have both physical and toxicological implications on these species. As these sites are vulnerable to infectious microbes, through the migratory birds, there is a chance of spreading diseases over the country. Another study also reported that municipal solid waste disposal from various means causes a serious threat to Ashtamudi Lake (Razeena *et al*., 2012; Sitaram, 2014).

Many of these disposed of plastic debris become a life-threatening agent for many waterbirds. It is really heartbreaking to observe Oriental Darter (Near Threatened) spotted in Kandachira area of Ashtamudi wetland stuck with a plastic float of the fishing net around its beak. In this study, it was observed that abandoned fishing nets were used as the nesting material by many birds, especially crows, but it could become a life-threatening issue for their nestling and young ones, which reveals a very grave situation.

Hunting:

Hunting was not reported as a major threat to the overall avifauna of Ashtamudi Lake. Still, hunting pressure was found to be intense in some areas of the Ashtamudi wetland. A small Island in the northern part of the Lake named 'Kakkathuruthu' where many incidents of hunting of Egrets and Storks using air guns (Personal information from local people) were reported in this study. Picking nestlings from heronries was a major threat faced by heronries of Little Egrets in the Neendakara harbor area.

Intrusion of feral dogs: Feral dog intrusion in the wetland area was another concern for Ducks and Egrets. Intense threats from feral dogs have been reported from Kandachira, Uliyakovil and Kumbalam sampling sites, where the dumping of slaughterhouse wastes in wetland areas was noted These feral dogs may also be responsible for driving down the population of migratory birds, often visiting the wetland areas. Thus, feral dog presence causes avifauna to be more vigilant, which reduces its feeding, roosting, and breeding success (Aarif *et al*., 2014).

Conservation action plan:

- The following action plan is proposed for the conservation of birds in the Ashtamudi wetland,
- Awareness programmes on wetland conservation and more specifically on avifauna have to be given to the local people.
- Strict implementation of Coastal Regulation Zone (CRZ) rules and the Kerala conservation of paddy land and Wetland Act-2008 to prohibit reclamation of wetlands and paddy fields may do wonders in the conservation strategies.
- Government and local bodies must take necessary actions to implement a proper scientific solid waste management action plan to avoid the solid waste-related pollution threats in the Ashtamudi wetland.
- Uninhabited small islands in the Lake can be declared as protected areas for birds, and thus encroachments and hunting can be prevented. Officials of forest and fisheries departments can do periodical patrolling in these areas.
- Patches of Mangrove forests present in the Lake need to be protected with the help of government and non-government agencies, with the collaboration of student Nature clubs or eco-clubs. Regular afforestation programmes and follow-ups need to be ensured.
- Most of the birds reported from Ashtamudi wetland come under Schedule I & IV of Wildlife (Protection) Act 1972, which are assigned as

highly protected species against hunting and poaching (Chapter III of Wildlife (Protection) Act 1972). Therefore, strict implementation of conservation acts is highly warranted for the conservation of the Ashtamudi wetland habitat and associated avifauna.

- To increase participatory effects in the conservation and management of this wetland ecosystem, there should be representation from local bodies and NGOs in the conservation bodies and authorities formed by the Government at the district level.
- Implementation of the district coastal management plan of Ashtamudi Estuary, Kollam prepared with the Centre for Earth Science Studies, Thiruvananthapuram, and ASR Limited, New Zealand (Black and Baba, 2001).
- The prioritized and integrated involvement of Government agencies, wildlife fund managers, and conservation donors is necessary for initiating and implementing conservation projects like bird sanctuaries, reserves, and protected areas for birds as well as overall biodiversity conservation and also in running responsible ecotourism projects in this Ramsar site.
- A detailed long-term study on the avifaunal diversity of Ashtamudi wetland needs to be conducted to determine the spatial and temporal pattern of bird movements with special reference to migratory birds. Our data can be used as a baseline for future monitoring and surveys.

Conclusion

The present study recorded the occurrence of 92 bird species belonging to 74 genera, 40 families, and 16 orders. Of these, 19 species (20%) were winter migrants and eight species (9%) were local migrants and 65 species (71%) were resident birds. Based on the IUCN Red List of Threatened Species, five species (5%) were categorized as Near Threatened (NT). The most abundant birds were Little Egret, *Egrettta garzetta* (19%); Brahminy Kite, *Haliasturindus* (17%); House Crow, *Corvus splendens* (16%); Little Cormorant, *Microcarboniger* (8%) and Indian Pond-heron, *Ardeolagrayii* (3%). The highest number of species was recorded during February (70) followed by March (68), lowest was in July (49) and June (48). The arrival of migratory birds was the reason for the peak in species diversity during the month of February and March due to the deposition and drying of trash fish near the Neendakara and Sakthikulangara harbour sites led to the abundance of Brahminy Kite in this region. The study clearly indicated the destructive human interference in the proper functioning of this wetland ecosystem. Therefore, much more priority must be given in to the protection of this wetland ecosystem by the strict implementation of Coastal Regulation Zone (CRZ) rules and Wild Life Protection Acts. Long-term monitoring of the avifauna of Ashtamudi wetland is needed for developing strategies for the conservation and proper management of this wetland. Additional information such as the spatio-temporal pattern of bird movements, and breeding (if any) of important wintering visitors from this wetland is still needed. Awareness programmes for the local people, on the consequences of solid waste disposal and altered land use pattern in these wetland areas, need to be implemented by local government bodies in cooperation with non-governmental organisations. A concerted effort is required to restore the original ecological features of Ashtamudi wetland to make it an abode for waterfowl.

Acknowledgement

The authors are grateful to The Principal, Sree Narayana College, Kollam for providing the facility for carrying out the study. The authors are also acknowledging the research facility provided by the Department of Science & Technology-Fund for Improvement of Science & Technology Infrastructure in Universities and Higher. Educational Institutions (DST-FIST Program, 2018-2021) and Department of Biotechnology (DBT)-Star College Scheme. The first author acknowledges the Council of Scientific & Industrial Research (CSIR) for Junior Research Fellowship for pursuing his Ph.D. programme and the University of Kerala for his Ph.D. Registration.

Conflict of interest

The authors declare that they have no conflict of interest

Table 1. Checklist of the avifauna of Ashtamudi wetland from September 2018 to August 2019. The birds were categorized into Abundance status

Sl. No.	Common name	Scientific names	Abundance	IUCN Category	Resident Status
	Order: Accipitriformes				
1	Black Kite	*Milvus migrans*	C	LC	R
2	Brahminy Kite	*Haliastur indus*	A	LC	R
3	Shikra	*Accipiter badius*	RA	LC	R
	Order: Anseriformes				
4	Lesser Whistling-Duck	*Dendrocygna javanica*	UC	LC	R
	Order: Bucerotiformes				
5	Common Hoopoe (Eurasian Hoopoe)	*Upupa epops*	OC	LC	R
	Order: Caprimulgiformes				
6	Asian Palm-Swift	*Cypsiurus balasiensis*	UC	LC	R
	Order: Charadriiformes				
7	Common Greenshank	*Tringa nebularia*	RA	LC	M
8	Common Redshank	*Tringa totanus*	UC	LC	M
9	Common Sandpiper	*Actitis hypoleucos*	UC	LC	M
10	Wood Sandpiper	*Tringa glareola*	UC	LC	M
11	Marsh Sandpiper	*Tringa stagnatilis*	RA	LC	M
12	Green Sandpiper	*Tringa ochropus*	RA	LC	M
13	Eurasian Curlew	*Numenius arquata*	RA	NT	M
14	Whimbrel	*Numenius phaeopus*	OC	LC	M
15	Black-tailed Godwit	*Limosa limosa*	UC	NT	M
16	Caspian Tern	*Hydroprogne caspia*	UC	LC	M
17	Whiskered Tern	*Chlidonias hybrid*	UC	LC	M
18	Lesser Crested Tern	*Thalasseus bengalensis*	C	LC	M
19	Black-headed Gull	*Chroicocephalus*	C	LC	M
20	Brown-headed Gull	*Chroicocephalus brunnicephalus*	RA	LC	M
21	Red-wattled Lapwing	*Vanellus indicus*	UC	LC	R
22	Pacific Golden-Plover	*Pluvialis fulva*	UC	LC	M
23	Black-winged Stilt	*Himantopus himantopus*	UC	LC	LM
	Order: Ciconiiformes				
24	Asian Openbill	*Anastomus oscitans*	UC	LC	LM
25	Painted Stork	*Mycteria leucocephala*	OC	NT	LM
	Order: Columbiformes				
26	Yellow-footed Green-Pigeon	*Treron phoenicopterus*	OC	LC	R
27	Rock Pigeon (Feral Pigeon)	*Columba livia*	C	LC	R
	Order: Coraciiformes				
28	Blue-tailed Bee-eater	*Merops philippinus*	C	LC	LM
29	Common Kingfisher	*Alcedo atthis*	UC	LC	R
30	Stork-billed Kingfisher	*Pelargopsis capensis*	UC	LC	R

Sl. No.	Common name	Scientific names	Abundance	IUCN Category	Resident
31	White-throated Kingfisher	*Halcyon smyrnensis*	C	LC	R
32	Indian Roller	*Coracias benghalensis*	RA	LC	R
33	Green Bee-eater	*Merops orientalis*	RA	LC	R
	Order: Cuculiformes				
34	Greater Coucal	*Centropus sinensis*	UC	LC	R
35	Asian Koel	*Eudynamys scolopaceus*	C	LC	R
	Order: Gruiformes				
36	White-breasted Waterhen	*Amaurornis phoenicurus*	C	LC	R
37	Rudy-breasted Crake	*Zapornia fusca*	OC	LC	R
38	Slaty-breasted Rail	*Lewinia striata*	OC	LC	R
	Order: Passeriformes				
39	Ashy Woodswallow	*Artamus fuscus*	RA	LC	R
40	Barn Swallow	*Hirundo rustica*	C	LC	M
41	Blyth's Reed Warbler	*Acrocephalus dumetorum*	UC	LC	M
42	Clamorous Reed Warbler	*Acrocephalus stentoreus*	RA	LC	M
43	Jungle babbler	*Argya striata*	C	LC	R
44	Yellow-billed Babbler	*Argya affinis*	UC	LC	R
45	House Crow	*Corvus splendens*	C	LC	R
46	Large-billed Crow	*Corvus macrorhynchos*	C	LC	R
47	Rufous Treepie	*Dendrocitta vagabunda*	C	LC	R
48	Black Drongo	*Dicrurus macrocercus*	C	LC	R
49	Pale-billed Flowerpecker	*Dicaeum erythrorhynchos*	UC	LC	R
50	Tricolored Munia	*Lonchura Malacca*	UC	LC	R
51	White-rumped Munia	*Lonchura striata*	C	LC	R
52	Scaly-breasted Munia	*Lonchura punctulata*	UC	LC	R
53	Common Myna	*Acridotheres tristis*	C	LC	R
54	Jungle Myna	*Acridotheres fuscus*	C	LC	R
55	Black-hooded Oriole	*Oriolus xanthornus*	UC	LC	R
56	Indian Golden Oriole	*Oriolus kundoo*	UC	LC	LM
57	Ashy Prinia	*Prinia socialis*	UC	LC	R
58	Plain Prinia	*Prinia inornata*	UC	LC	R
59	Common Tailorbird	*Orthotomus sutorius*	UC	LC	R
60	Oriental Magpie-Robin	*Copsychus saularis*	UC	LC	R
61	Indian paradise-Flycatcher	*Terpsiphone paradise*	RA	LC	LM
62	House Sparrow	*Passer domesticus*	UC	LC	R
63	Purple-rumped Sunbird	*Leptocoma zeylonica*	C	LC	R
64	Loten's Sunbird	*Cinnyris lotenius*	UC	LC	R
65	Purple Sunbird	*Cinnyris asiaticus*	UC	LC	R
66	Streaked Weaver	*Ploceus manyar*	UC	LC	R
67	Red-whiskered Bulbul	*Pycnonotus jocosus*	UC	LC	R
68	Red-vented Bulbul	*Pycnonotus cafer*	RA	LC	R
69	Zitting Cisticola	*Cisticola juncidis*	OC	LC	R

Sl. No.	Common name	Scientific names	Abundance	IUCN Category	Resident
	Order: Pelecaniformes				
70	Glossy Ibis	*Plegadis falcinellus*	UC	LC	LM
71	Black-headed Ibis	*Threskiornis melanocephalus*	C	NT	R
72	Little Egret	*Egretta garzetta*	A	LC	R
73	Grey Heron	*Ardea cinerea*	C	LC	R
74	Cinnamon Bittern	*Ixobrychus cinnamomeus*	OC	LC	R
75	Black Bittern	*Ixobrychus flavicollis*	RA	LC	R
76	Yellow Bittern	*Ixobrychus sinensis*	RA	LC	R
77	Cattle Egret	*Bubulcus ibis*	C	LC	R
78	Intermediate Egret	*Ardea intermedia*	UC	LC	R
79	Great Egret	*Ardea alba*	C	LC	R
80	Western Reef-Egret	*Egretta gularis*	RA	LC	LM
81	Indian Pond-Heron	*Ardeola grayii*	C	LC	R
82	Purple Heron	*Ardea purpurea*	UC	LC	R
83	Black-crowned Night-Heron	*Nycticorax nycticorax*	RA	LC	R
84	Striated Heron	*Butorides striata*	UC	LC	R
	Order: Piciformes				
85	White-cheeked Barbet	*Psilopogon viridis*	UC	LC	R
86	Black-rumped Flameback (Lesser Golden backed Woodpecker)	*Dinopium benghalense*	UC	LC	R
	Order: Psittaciformes				
87	Rose-ringed Parakeet	*Psittacula krameri*	C	LC	R
	Order: Strigiformes				
88	Spotted Owlet	*Athene brama*	OC	LC	R
89	Jungle Owlet	*Glaucidium radiatum*	UC	LC	R
	Order: Suliformes				
90	Indian Cormorant	*Phalacrocorax fuscicollis*	C	LC	R
91	Little Cormorant	*Microcarbo niger*	C	LC	R
92	Oriental Darter	*Anhinga melangaster*	UC	NT	R

(A: Aundant > 5000, C: Common– 100 ≤ 5000, UC: Uncommon – 10 ≤ 99, RA: Rare – 2 ≤ 9, OC: Occasional – sighted only once). IUCN Category (LC: Least Concern, NT: Near Threatened) Residency Status (R: Residents, M: Migrants, LM: Local Migrants)

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Chapter 4

DIVERSITY OF GENUS *RUSSULA*- AN ECTOMYCORRHIZAL MACROFUNGI

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Abstract The monophyletic genus *Russula* Pers. is the most dominant and diverse amongst the four genera with over 750 species known world over. During the study ten *Russula* species were collected from Kerala state, systematically studied and identified. Full descriptions, field photographs and illustrations of this species are provided. Most of the members show ectomycorrhizal association with Dipterocarpaceae members. Of the studied specimens two were new to Kerala and three were new to science. The genus *Russula* includes some of the most beautiful examples of gilled fungi. Many species are brilliantly coloured, and their shapes are exquisitely symmetrical. The genus is distributed worldwide, and its members are found in most, if not all, ectotrophic ecosystems of the world. Although members of the genus *Russula* are frequently a very conspicuous part of the Indian mycoflora, they are the least studied group in Kerala. In the current investigation, those areas studied most intensively lie to the southern part of Kerala. The phenology, ecology and distribution pattern of all species considered are discussed.

Keywords: *Ectomycorrhiza, Biodiversity, Mushrooms, Russula.*

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Introduction

Fungi represent one of the largest groups of living organisms on the planet (Mueller & Schmit 2007; Blackwell 2011) but the information available for most species is limited and incomplete, which means that current estimates of the number of species of fungi are significantly different depending on the contributing author. Some authors suggest that species diversity in fungi can be estimated using the diversity of vascular plant species from the same region. Efforts to better document their diversity, in order to preserve it, need to be prioritized (Mueller *et al.* 2007; Blackwell 2011). Family Russulaceae is one of the largest ectomycorrhizal families and includes agaricoid, msecotioid, pleurotoid and gasteroid forms (Buyck *et al.* 2008; Morozova *et al.* 2013). The genus is represented in India by about 158 taxa (Sharma *et al.* 2018). Seventeen species of *Russula*were known from Kerala so far. Tropical fungi are poorly studied and analysis of diversity, abundance, and taxonomy are far from complete, especially in taxonomically challenging groups such as Russulaceae, which are poorly understood and species recognition and delimitations demand a high degree of familiarity within the group.

Methodology

Gross morphological descriptions are based exclusively on fresh materials collected from Kerala State, India. Colour coding follows that of Kornerup and Wanscher (1978). Microscopic characters were studied on dried materials using hand cut sections of basidiomata revived in a 3% solution of KOH, stained with 1% Congo red and examined under a Leica DME 1000 compound microscope. The mean quotient (Q) of spore length divided by spore width was calculated from measurements of 20 spores. Line drawings were made with assistance of an attached drawing tube. All materials examined are deposited at the Mycological Herbarium of Tropical Botanic Garden and Research Institute, Trivandrum [TBGT (M)].

Result

1. *RUSSULA PURPUREONIGRA* Petch, Ann. Roy. Bot. Gard., Peradeniya 6: (3): (1917) (Plate 1. Fig. 1)

Pileus 8–9.5 cm diam., convex with a slightly depressed centre, then plano-convex, becoming infundibuliform; surface dull white with 'brownish grey' (5B2) to 'greyish brown' (5D3) tints, viscid to sticky when wet, otherwise dry, smooth and glabrous, cuticle not peeling off; margin in rolled when young, becoming uplifted when mature, still remaining incurved, non- striate. Lamellae adnate to subdecurrent, white when young, 'yellowish white' (2A2) when mature, crowded with lamellulae of different lengths, Stipe 3–4 × 1.5–2.5 cm, central, cylindric, equal, solid then stuffed; surface white, glabrous, becoming black on bruising. Context turning black on cutting. Smell not characteristic. Taste mild on lamellae. Spore print white.

Chemical reactions: SV light green on stipe surface; $FeSO_4$ on stipe surface-bluish green.

Basidiospores 6.5–7.5 × 6–7.5 µm, (Q= 1–1.33), subglobose to broadly ellipsoid, thin-walled, hyaline, with amyloid, conical warts up to 0.5 µm high, connected by more or less thick lines to form an almost complete reticulum, suprahilar plage inamyloid. Basidia 28.5–36.5 × 8–9 µm, clavate to cylindro-clavate, thin-walled, hyaline, 4-spored; sterigmata up to 6 µm long. Lamella-edge heteromorphous with crowded macrocystidia. Macrocystidia 22.5–37.5 × 10.5–19.5 µm, obclavate to cylindric, with or without a papilla at the apex, thin-walled, with granular contents. Pleurocystidia 63–103.5 × 7.5–12 µm, cylindric, capitate, sometimes with apical constrictions, thin-walled, filled with refractive contents. Stipitipellis a cutis, composed of parallel hyphae 3–4.5 µm wide, hyphae 1.5–6.5 µm broad, thin-walled, hyaline, without encrustation. Caulocystidia absent. Clamp connections absent in all parts.

Whole fruit body readily blackening on bruising or on drying.

Habitat & phenology: Scattered on the ground, in riparian vegetation dominated by *Vateria indica* L. and *Myristica malabarica* Lam. April-May, August-September.

2. *RUSSULA ADUSTA* (Pers.) Fr., Epicr. syst. mycol. (Upsaliae): 350 (1838) [1836-1838] (Plate1. Fig. 2)

Lactarelis nigricans (Pers.) Earle, Bull. New York Bot. Gard. 5: 410 (1909)

Omphalia adusta (Pers.) Gray, Nat. Arr. Brit. Pl. (London) 1: 614 (1821)

Russula adusta f. *gigantea* Britzelm., Botan. Zbl. 62: 310 (1895)

Russula adusta f. *rubens* Romagn., Bull. trimest. Soc. mycol. Fr. 61: 71 (1943)

Russula adusta var. *coerulescens* Fr. ex P. Karst., Bidr. Känn. Finl. Nat. Folk 32: 200 (1879)

Russula adusta var. *sabulosa* Bon, Cryptog. Mycol. 7(4): 306 (1986)

Russula nigricans var. *adusta* (Pers.) Barbier, So. Sci. Nat. Sâon. 33(2): 91 (1907)

Pileus 6.5–11.5 cm in diam., convex to plano-convex, depressed at the centre, slightly infundibuliform when fully expanded; surface dull white, becoming 'brownish grey' to 'greyish brown' (5D2/5E3), then 'dark brown' (6F4) and finally black on handling, bruising or ageing, non-striate, slimy when wet, soon dry, smooth, glabrous, sometimes areolately cracked in dry weather; white becoming black on bruising or handling, up to 5 mm wide, crowded, with lamellulae of different lengths, often bifurcated near to the stipe; Context firm, white, slowly discolouring grey brown and finally black on exposure. Stipe 3–6 × 1–2 cm, central, cylindric, equal or slightly eccentric, solid, brittle; surface chalky white to pale cream, turning greyish brown or dark brown and finally black on handling, smooth to finely pruinos. Odour not distinct. Taste acrid. Spore print white.

Chemical reactions: on the pileal context SV reddish purple; $FeSO_4$ pinkish (before greying).

Basidiospores 7–9 × 6–8 µm, (Q=1–1.31 µm), globose to broadly ellipsoid, strongly amyloid with an ornamentation of verrucae and connectives, finely or loosely connected, more or less distinct and irregular, ornamentations not exceeding 0.2 µm in height; plage inamyloid. Basidia 40–48 × 8.5–10.5 µm, clavate, 4-spored, sterigmata up to 5.8 µm long, with refractive contents; Lamella-edge with

crowded macrocystidia, 63–103.5 × 7–12 µm, clavate with a mucronate apex, thin-walled, hyaline, filled with oleaginous refractive contents, discolouring black. Pleurocystidia similar scattered. Pileipellis an ixocutis with cylindrical, obtuse or rarely clavate hairs at the top with abundant brown vacuolar pigment, dermatocystidia very few. Stipitipellis with erect to suberect caulocystidia, 39–62.5 × 6–7.5 µm, scattered throughout the surface, cylindrical to narrowly clavate, thin-walled, with or without a mucronate apex, with refractive contents. Oleiferous hyphae and clamp connections absent in all parts.

Habitat & phenology: Solitary to scattered on forest soil, in association with *Myristica malabarica* Lam. and *Hopea parviflora* Bedd.; April-November.

3. *RUSSULA FOETENS* Pers., Observ. mycol. (Lipsiae) 1: 102 (1796) (Plate 1. Fig. 3)

Agaricus foetens (Pers.) Pers., Observ. mycol. (Lipsiae) 2: 102 (1800) [1799]

Agaricus foetens var. *lactifluus* Corda, Icon. fung. (Prague) 4: 49, tab. 10:139 (1840)

Agaricus incrassatus Sowerby, Col. fig. Engl. Fung. Mushr., Suppl. (London)(no. 30 [no. 3 of suppl.]): tab. 415 (1814)

Russula foetens var. *minor* Singer, Bull. trimest. Soc. mycol. Fr. 54: 135 (1938)

Pileus 410 cm diam., fleshy, convex, becoming applanate and shallowly depressed, finally uplifted; surface 'greyish yellow' (4B5) at the centre, 'pale yellow' (3A3) to 'dull yellow' (3B3) towards the margin, tuberculate striate to radially wrinkled, the cuticle separable by a third of the radius, slimy-viscid when wet, otherwise dry; margin becoming uplifted with age, crenate, variously incised. Lamellae narrowly adnate, up to 7 mm wide, white then cream, crowded, fragile, without lamellulae, bifurcate towards the margin, interveined, brittle; edge concolourous with the sides, entire. Stipe 48 × 1.5–1.7 cm, central cylindric, solid, equal, narrowly tapering towards base, curved; surface white, staining yellow brown with age, smooth, brittle, dry. Context whitish, thin, unchanging but slowly discolouring brownish when cut. Odour strong, unpleasant, fishy when old. Taste acrid. Spore print pale cream.

Chemical reactions: SV no reaction; $FeSO_4$ salmon-pink in context.

Basidiospores 7.5–9 (9.3) × (6) 7.2–7.5 (9) µm, (Q= 1–1.28 µm), broadly ellipsoid, often subglobose, with an ornamentation of large, amyloid, isolated warts, projecting up to 1.3 µm in height, suprahilar plage weakly amyloid. Basidia 30–38 × 8.4–13.2, clavate, 4 - spored, rarely 2-spored. Lamella-edge heteromorphous; cheilocystidia macrocystidioid, abundant, 50–68 × 8–8.4 µm, cylindro-clavate, sometimes with a mucronate apex, thin-walled, with granular contents, not staining in SV. Pleurocystidia similar, not very abundant, 54–68 × 10–10.4 µm. Pileipellis a gelatinised epicutis, composed of parallel to interwoven thin-walled, hyaline, septate, branched hyphae; end cells 16–21.2 × 4–7.2 µm wide, obtuse or tapered, interspersed with pileocystidia, 22–64 × 6–8 µm, similar to cheilocystidia, with homogeneous contents, not staining in SV. Stipitipellis with scattered caulocystidia, similar to cheilocystidia. Oleiferous hyphae present. Clamp connections absent.

Habitat & phenology: Scattered in groups on riverine soil, associated with *Vateria indica* L., *Thespesia populnea* (L.) Sol. ex Correa.; May.

4. *RUSSULA ACICULOCYSTIS* Kauffman ex Bills & O.K. Mill., Mycologia 76(6):990(1984) (Plate 1. Fig. 4)

Pileus 3.5–8 cm in diam., hemispherical with an incurved margin when young, expanding convex to broadly convex, then plane with a central depression; surface 'greyish red' (10B6/ 10D5/11C6), 'brownish red' (10D6/11D6), 'dull red' (11A3/11B3/11B4), 'reddish lilac' (14B3) or sometimes 'pink' (11A4), with a 'greyish rose' (11B6), 'greyish ruby' (12D5), 'dark ruby' (12F6), 'violet brown' (11D5/11E4/ 11E5/11E6/11E7/11E8/11F6) or 'greyish magenta' (13E5/ 14D4) centre, sticky when wet, otherwise dry, minutely velvety, sometimes areolately cracked forming fine squamules.. Lamellae adnate, 'yellowish white' (3B2/4A2), up to 1 cm wide, ventricose, close to subcrowded. Stipe 2–5 × 0.5–2 cm, central, cylindric, equal, or slightly attenuated below, rarely with a subglobose base, longitudinally rugulose, stuffed to hollow; surface

Plate 1.
1.*Russula purpureonigra* 2.*Russula adusta* 3.*Russula foetens* 4.*Russula aciculocystis* 5. *Russula* sp. 2

white, sometimes 'pastel red' (11A4) to 'pale red' (9A4/10A4/11A3) or with a 'reddish pink' to 'greyish red' (10B5) tinge, more often entirely pink. Context white, 4–5 mm wide, firm when young, brittle in age, not discolouring when cut or bruised. Odour not characteristic. Taste mild acrid. Spore print 'pale yellow' (3A2).

Chemical reactions: SV salmon on pileal surface; $FeSO_4$ negative.

Basidiospores 6.5–7.5 × 6–7.5 µm, (Q=1–1.11 µm), subglobose to broadly ovate, ornamentation with amyloid, conical to irregular ridges or wings up to 1.5 µm high, widely spaced, connected by thick lines or ridges forming a more or less complete reticulum, plage inamyloid. Basidia 33–42 × 11.5–13.5 µm, 4-spored, clavate, thin-walled. Lamella-edge sterile with tufts of cheilocystidia, 21–67.5 × 7.5–13.5 µm, subulate, or aciculate with a basal cylindric cell, obclavate, flexuous with a rounded apex or sometimes vesiculose, thin-walled or slightly thick-walled, hyaline. Pleurocystidia abundant, pseudocystidioid, arising from the trama, 81–120 × 6.5–18 µm, projecting 80 µm beyond the basidioles, ventricose-rostrate, cylindrical or flexuous, thin-walled, hyaline, sometimes with a cylindrical basal cell. Pileipellis distinctly two layered; suprapellis a trichoderm of erect to suberect hyphae, in chains of cylindrical cells; terminal elements, 16.5–60 × 3–75 µm, lanceolate, acicular or subulate, with 5–6 basal cells; basal cells cylindric to barrel shaped, arranged in uniseriate chains with an apical aciculate hair, thin-walled, not reacting in SV, containing a purple intracellular pigment; subpellis gelatinized, composed of thin-walled, unbranched, 2.5–3 µm wide hyphae, with a purple intracellular pigment. Macrocystidioid elements lacking in the pileipellis. Stipitipellis composed of non-gelatinized interwoven to subparallel hyphae, similar to the pileipellis, giving rise to tufts of caulocystidia, similar to cheilocystidia in size and shape, without basal cells. Stipe trama heteromerous with nests of sphaerocytes and hyphae. Clamp connections absent in all tissues.

Habitat & phenology: Solitary to scattered on soil under *Myristica malabarica* Lam., *Vateria indica* L. and *Calophyllum apetalum* Willd. April-December.

5. *RUSSULA* SP. 2 (Plate 1. Fig. 5)

Pileus 4–6.5 cm in diam., plano-convex with a central depression; surface 'bright red' (9A7/10A6/10A7) or 'greyish red' (10B5/10B6), 'pale red' (10A3) or sometimes 'yellowish white' (3A2/4A2) towards the centre, sticky when wet, becoming dry, cuticle peeling moderately, radially striate up to half way towards the centre; margin straight, entire to incised, finely scalloped. Lamellae adnate, becoming adnexed in some, 'yellowish white' (3A2), up to 9 mm wide, ventricose, close to subcrowded, without lamellulae, often bifurcating towards the margin; edge concolorous to the sides, entire. Stipe 3–4.5 × 0.7–1.2 cm, central, cylindric, equal, stuffed, brittle; surface white towards the base, sprayed 'reddish white' (9A2) on a white background towards the apex, smooth. Context white, 3–5 mm wide, unchanging, readily decaying. Odour aromatic. Taste mild in all parts. Spore print 'yellowish white' (4A2).

Chemical reactions: SV purple on stipe and lamellae; $FeSO_4$ salmon.

Basidiospores 7.8–9 × 7.5–8.5 (9) µm, (Q= 1–1.2 µm), globose to subglobose, ornamentation reticulate, consisting of large amyloid, conical to irregular ridges or wings up to 3 µm high, widely spaced, connected by thick lines or ridges forming a more or less complete reticulum, plage inamyloid. Basidia 38–44 × 10–12 µm, 4-spored, clavate, thin-walled. Lamella-edge heteromorphous; cheilocystidia 75–102 × 9–14.5 µm, clavate, ventricose to lanceolate, thin-walled or slightly thick-walled, rarely multiseptate, hyaline, turning pale purple in SV. Pleurocystidia similar, 85–99 × 10.8–15.6 µm. Pileal trama heteromerous consisting of sphaerocytes and connective hyphae; sphaerocytes 30–48 µm wide, thin-walled, hyaline; hyphae thin-walled, hyaline, up to 6 µm wide. Subhymenium pseudoparenchymatous, 2–4 layered. Hymenophoral trama heteromerous, sphaerocytes 22.8–63 × 19.2–48 µm wide; connective hyphae thin-walled, hyaline, septate, branched, up to 6.6 µm wide. Pileipellis distinctly two layered; suprapellis a trichoderm of erect to suberect hyphae, in chains of cylindrical cells; terminal elements, 12–36 × 12.8–4 µm, lanceolate, acicular or subulate, with 2–4 cylindric to

barrel shaped, thin-walled, hyaline basal cells, not reacting in SV; trichodermial elements short and closely septate at the centre of the pileus with more elongate, subulate terminal elements towards the margin. Stipitipellis composed of non-gelatinized, interwoven to subparallel hyphae, similar to the pileipellis, interrupted by tufts of caulocystidia, not staining in SV, 46–56 × 6–8 µm, fusoid cylindric to ventricose lageniform with a long slender neck, similar to the trichodermial elements of the pileipellis in size and shape, without or with a single, globose, basal cell, thin-walled, hyaline. Stipe trama heteromerous with nests of sphaerocytes, 27–42 × 24–36 µm wide and connecting hyphae up to 6.6 µm wide. Oleiferous hyphae present. Clamp connections absent in all tissues.

Habitat & phenology: Scattered on soil under *Hopea parviflora* Bedd., and *Vateria indica* L. January, April-September, November-December.

6. *RUSSULA* SP. 3 (Plate 2. Fig. 6)

Pileus 1–2 cm in diam., thin, convex to plano-convex, centrally depressed, finally becoming infundibuliform; surface uniformly 'orange grey' (5B3) in the bud stage, milky white with a 'smoke brown' (4E2) to 'olive brown' (4E3) centre when expanded, tuberculate-striate, sticky when wet, smooth and glabrous; margin straight to uplifted, entire in bud, slightly uplifted at maturity. Lamellae adnexed, white, up to 2 mm wide, close to subdistant, lamellulae absent, rarely with one or two lamellulae, forked near stipe; edge concolorous to the sides, entire. Stipe 1–2 × 0.2–0.4 cm, central, cylindrical, equal, sometimes slightly tapering towards the base, curved, stuffed, brittle; surface white, smooth, dry. Context dull white, thin, soft. Odour not characteristic. Taste mild. Spore print dull white.

Chemical reactions: SV pale pink on pileus, gills, and stipe surface; $FeSO_4$ negative.

Basidiospores 7.5–9 × (6.5) 7.5–9 µm, (Q=1.1–1.31), subglobose to mostly broadly ellipsoid, amyloid, composed of isolated obtuse verrucae, up to 1.3 µm high, not connected by connectives, suprahilar plage inamyloid. Basidia 26–32 × 7.6–11.2 µm, clavate, 4-spored, rarely 2-spored, sterigmata up to 4 µm long, thin walled, with plasmatic contents. Lamella-edge sterile; cheilocystidia 12–24 × 4.8–6.8 µm, thin-walled, hyaline, rarely septate. Cheilomacrocystidia scattered, 46–56 × 432–36 µm, fusoid to lanceolate, often with mucronate or appendiculate apices, sometimes with 12 constructions, thin-walled, filled with refractive granular contents. Pleurocystidia similar, 44–56 × 6–7.2 µm, originating from the subhymenium. Pileal trama heteromerous, sphaerocytes 31–48 × 30–48 µm, thin-walled, hyaline; connecting hyphae up to 5.5 µm wide, thin-walled, hyaline, septate, branched. Pileipellis an ixotrichoderm; terminal elements narrow, hair-like, 18–40 × 2–4 µm, thin-walled, hyaline, arising from chains of globose to elongated inflated basal cells, intermixed with pileocystidia; pileocystidia 18–32 × 4.8–6.4 µm, clavate, cylindric or fusoid, often with appendiculate apices, thin-walled, with refractive granular contents. Stipitipellis an interrupted epicutis with interwoven hyphae, up to 3.6 µm, thin-walled, hyaline, septate, branched, with crowded caulocystidia, similar to macrocheilocystidia, 30–42 × 3–5.4 µm. Stipe trama heteromerous, sphaerocytes thin-walled, hyaline, 9–29 × 9–27 µm; connecting hyphae, septate, branched, thin-walled, hyaline. Oleiferous hyphae not observed. Clamp connections absent in all tissues.

Habitat & phenology: Scattered on ground, associated with *Hopea parviflora* Bedd. and *Vateria indica* L.; October.

7. *RUSSULA* SP. 5 (Plate 2. Fig. 7)

Pileus 3.5 cm diam., convex with a small depression at the centre; surface 'brownish red' (10D7) at the centre, paler towards the margin, slimy when wet, otherwise dry, appearing smooth but densely pruinose at the centre when viewed under the stereo, disrupted towards the margin; margin incurved. Lamellae adnexed, white, up to 5 mm wide, crowded, without lamellulae, not interveined; edge concolorous to the sides, smooth. Stipe 1.7 × 0.6 cm, central, cylindric, solid, equal, slightly curved; surface white, finely striate, dry. Annulus absent. Odour nil or not characteristic. Taste mild to slightly acrid. Context white, thin, unchanging.

Chemical reactions: SV salmon on pileal surface; $FeSO_4$ negative.

Basidiospores 7.5–9 (9.5) × 7–8.5 (9.5) µm (Q=1–1.2 µm), globose to subglobose, with an amyloid exosporial ornamentation, up to 1.8 µm high, consisting of elongated warts and ridges, connected together forming a nearly complete reticulum; suprahilar plage amyloid. Basidia 32–36 × 14–16 µm, clavate, thin-walled, hyaline, 4-spored. Lamella-edge sterile; cheilocystidia versiform, 20–30 × 5–8 µm, clavate, ventricose or lanceolate, often with obtuse mucronate apices, thin-walled, hyaline. Macrocystidia scattered both on the edges and sides of the lamellae; macrocheilocystidia 26–41 × 6–11 µm, inflated-clavate, with granular contents more at the apex, blackening in SV. Pleurocystidia similar, 48–56 × 10–12 µm. Pileal trama heteromerous with hyphae and sphaerocytes; sphaerocytes 15–39 × 12–27 µm, thin-walled, hyaline; connecting hyphae up to 6 µm wide, thin-walled, hyaline. Subhymenium pseudoparenchymatous. Hymenophoral trama heteromerous composed of thin-walled, hyaline hyphae, up to 4.8 µm wide, intermixed with sphaerocytes, 18–32 × 15–30 µm. Pileipellis a trichoderm, composed of tufts of erect, septate elements, forming catenulate chains of barrel shaped, globose or cylindrical elements, often with attenuate hyphal extremities, continuous at the centre, disrupted towards the margin, end cells measuring 10–19.5 × 3–4.5 µm. Primordial hyphae scattered in groups in the pileipellis, projecting out, composed of septate, globose or oval to cylindrical elements with thickened encrusted walls, blackening in Cresyl blue. Pileal cystidia absent. Stipitipellis a cutis composed of thin-walled, parallely arranged hyphae, interrupted by erect to semi erect tufts of caulocystidia, 13.5–32.5 × 4–7.5 µm, cylindric, fusoid to lanceolate with capitate to appendiculate apices, thin-walled, hyaline. Oleiferous hyphae present. Clamp connections absent.

Habitat & phenology: Scattered on the forest floor dominated by *Vateria indica* L. June.

8. *RUSSULA CONGOANA* Pat., Bull. Soc. mycol. Fr. 30(3): 336 (1914)

Russula congoana var. *djongoensis* Buyck, Bull. Jard. Bot. natn. Belg. 58(3-4): 474 (1988) (Plate 2. Fig. 8)

Pileus 2–5 (8) cm diam., at first convex then expanded with a shallow central depression; surface 'pastel red' to 'greyish red' (9A4/9A5/9B5/9B7/9C5/9C6/10A4/10A5/10B4/10B5), or 'carmine red' (11A8) soon discolouring to whitish from the centre outwards or entirely so, sulcate-striate up to three-fourth of pileus radius, shiny and lubricous when wet, sometimes sticky or glutinous and therefore bearing soil fragments, slightly pruinose in some collections; margin straight, entire to variously incised, rarely with pellicle at extreme margin. Lamellae adnexed, white, cream or pale yellow (4A2/4A3) when mature, exceeding the gills, up to 10 mm wide, close to crowded, without lamellulae, very rarely lamellulate, interveined, rarely bifurcate towards the margin, flexible; edge entire, concolourous to the sides. Stipe 1.5–5 × 0.5–2 cm, central, cylindric, equal or tapering towards the base, sometimes tapering towards the apex in some collections, slightly bulbous or with an attenuated base, curved, stuffed, brittle; slightly broader at the base, solid becoming stuffed and hollow; surface creamy white with a pinkish tinge at the base, smooth. Annulus absent. Context white, thin, soft, unchanging. Odour not characteristic. Taste nil.

Chemical reactions: SV salmon on stipe and lamellae; $FeSO_4$ negative.

Basidiospores 6–8.5 × (5.5) 6–7 (7.5) µm (Q=1–1.25 µm), subglobose to ellipsoid, mostly broadly ellipsoid, rarely globose, densely ornamented with coarse, amyloid, verrucae up to 1.5 µm high, interconnected by an incomplete to almost complete reticulate system, suprahilar plage indistinct. Basidia clavate, 28.5–31.5 × 10.5–12 µm, clavate, 4-spored. Lamella edge heteromorphous, cystidia scattered on both edges and sides of the lamellae, cheilocystidia 45–65 × 8–12 µm, macrocystidioid, clavate to fusiform, frequently mucronate, thin-walled, with refractive contents, violet brown in SV. Pleurocystidia similar, 46.5 –75 × 10.5–13.5 µm. Pileal trama heteromerous; sphaerocytes 27–42 × 23–33 µm, thin-walled, hyaline; connective hyphae thin-walled, hyaline, septate, branched, up to 7 µm wide. Subhymenium pseudoparenchymatous. Hymenophoral trama

composed mainly of thin-walled, hyaline sphaerocytes, 18–36 × 18–35 µm and filamentous hyphae. Pileipellis an ixotrichoderm composed of chains of globose to elongate inflated elements, terminal cells at the centre of the pileus short and obtuse, 6–18 × 3–6 µm, thin-walled, hyaline, septate, narrowly cylindrical to ovate, tips often narrow; terminal elements towards the margin of the pileus long, subcylindrical, filiform, 16.5–37 × 3–5 µm; subpellis up to 250 µm broad, gelatinized, hyphae interwoven, up to 3.5 µm wide; distinct irregular to zebroid encrustations often present in the hyphae of the subpellis. Pileocystidia few, scattered, cylindro-clavate to clavate or mucronate, 38–75 × 3–5 µm, arising deep from the subpellis, without or rarely with septa, filled with refractive contents, violet brown in SV. Stipitipellis a repent epicutis with thin-walled, hyaline, septate, branched hyphae and scattered caulocystidia similar to macrocheilocystidia, cylindric to cylindro-clavate 41.5–55.5 × 6–12 µm, aseptate or rarely with septa. Stipe trama heteromerous, sphaerocytes thin-walled, hyaline, 13.5–30 × 13.5–28 µm; connective hyphe thin-walled, hyaline, branched, up to 3 µm wide. Oleiferous hyphae present. Clamp connections absent in all parts of the tissues.

Habitat & phenology: Solitary on soil, in evergreen vegetation dominated by *Hopea parviflora* Bedd., *Pongamia pinnata* (L.) Pierre and *Vateria indica* L.; April-August, October.

9. *RUSSULA NOBILIS* Velen., České Houby 1: 138 (1920) (Plate 2. Fig. 9)

Russula emetica var. *mairei* (Singer) Killerm., Denkschr. Bayer. Botan. Ges. in Regensb. 20: 27 (1939)

Russula fageticola Melzer ex S. Lundell, in Lundell & Nannfeldt, Fungi Exsiccati Suecici 47-48(Sched.): 37 (1956)

Russula fageticola (Romagn.) Bon, Docums Mycol. 17(no. 65): 55 (1986)

Russula fageticola var. *strenua* Carteret & Moënne-Locc., in Moënne-Loccoz & Reumaux, Les Russules Émétiques, Prolégomènes à Une Monographie des Emeticinae d'Europe et d'Amérique du Nord (Bassens): 237 (2003)

Russula fagetorum Bon, Docums Mycol. 17(no. 67): 12 (1987)

Russula mairei Singer, Bull. trimest. Soc. mycol. Fr. 45: 103 (1929)

Russula mairei var. *fageticola* Romagn., Bull. mens. Soc. linn. Lyon 31(1): 174 (1962)

Russula mairei var. *sublongipes* Reumaux, in Reumaux, Bidaud & Moënne-Loccoz, Russules Rares ou Méconnues (Marlioz): 71 (1996)

Russula nobilis var. *semilucida* R. Socha, in Socha, Hálek, Baier & Hálek, Holubinky (Russula) (Praha): 510 (2011)

Pileus 5–6 cm diam., fleshy, hemispherical when young, later convex with a broad depression at the centre; surface 'greyish red' (9B6) sometimes with 'yellowish white' (3A2) or white tints at places, sticky when wet, translucent-striate to slightly tuberculate at the margin, pellicle peeling up to half way to the centre; margin crenate, incised. Lamellae adnexed, almost free at maturity, up to 5 mm wide, 'pale yellow' (3A2), crowded, without lamellulae, many forked; edge entire. Stipe 3.5–4 × 1–1.5 cm, central, slightly broader at the base, stuffed; surface white, smooth with a faint red colour at apex and middle. Annulus absent. Smell agreeable. Taste mild, then acrid. Context white, pink under the cuticle, up to 3 mm wide, brittle. Spore print 'yellowish white' (2A3/3A2).

Chemical reactions: FeSO4 very pale salmon on stipe and lamellae; 2% phenol deep reddish brown.

Basidiospores 6–7.5 × 5.5–6 µm, (Q= 1.021.07 µm), subglobose, ornamentation moderately thick, projecting up to 0.5 µm, consisting of warts and connectives forming a more or less complete reticulum, suprahilar area weakly amyloid. Basidia clavate, 36–45 × 11.5–12 µm, 4-spored. Lamella-edge heteromorphous; cheilocystidia and pleurocystidia few, macrocystidioid, 52.5–60 × 9–13.5 µm, broadly clavate with an apical projection, with refractive contents only at the apical portion, subhymenial or tramal in origin. Pileal trama heteromerous with nests of sphaerocytes and connecting hyphae. Subhymenium pseudoparenchymatous. Hymenophoral trama heteromerous with abundant sphaerocytes and connecting hyphae. Pileipellis a broad gelatinized zone with a trichodermial epicutis, comprising

modified elements, multiseptate, with filiform hairs at the tip, intermixed with macrocystidioid dermatocystidia similar to that of the hymenium. Stipitipellis composed of interwoven nongelatinised, septate hyphae with their tips often macrocystidioid, 58.5–73.5 × 6.5–9 µm, filled with refractive contents and tufts of thin-walled, hyaline, aciculose caulocystidia, 25.5–45 × 6–7.5 µm.

Habitat & phenology: Solitary on soil under *Vateria indica* L. and *Hopea parviflora* Bedd. April.

Specimens examined: Kerala State, Thiruvananthapuram Dist., Kallar: 18 Apr. 1996, TBGT (M) 2897; 28 April 1998, TBGT (M) 4295.

10. *RUSSULA LUTEOTACTA* Rea, Brit. basidiomyc. (Cambridge): 469 (1922) (Plate 2. Fig. 10)

Russula luteotacta f. *alba* Fillion & Frund, in Frund & Reumaux, Bull. Mycol. Bot. Dauphiné-Savoie 46(no. 180): 16 (2006)

Russula luteotacta f. *griseoalb*a Bidaud & Frund, in Frund & Reumaux, Bull. Mycol. Bot. Dauphiné-Savoie 46(no. 180): 16 (2006)

Russula luteotacta var. *cyathiformis* Reumaux & Frund, in Frund & Reumaux, Bull. Mycol. Bot. Dauphiné-Savoie 46(no. 180): 16 (2006)

Russula var. *duriuscul*a Reumaux & Frund, in Frund & Reumaux, Bull. Mycol. Bot. Dauphiné-Savoie 46(no. 180): 16 (2006)

Russula var. *intactior* Jul. Schäff., Annls mycol. 36(1): 37 (1938)

var. *semitalis* J. Blum ex Bon, Cryptog. Mycol. 7(4): 303 (1986)

Russula luteotacta var. *semitalis* J. Blum, Bull. trimest. Soc. mycol. Fr. 72(2): 143 (1956)

Russula luteotacta var. *serrulata* J. Blum, Bull. trimest. Soc. mycol. Fr. 72(2): 142 (1956)

Russula luteotacta var. *terrifera* Reumaux & Frund, in Frund & Reumaux, Bull. Mycol. Bot. Dauphiné-Savoie 46(no. 180): 16 (2006)

Pileus 4.5–6.5 cm diam., initially convex, becoming plano-convex, finally applanate with a shallow depression at the centre, sometimes becoming uplifted with age; surface 'greyish red' (10C5), discolouring by the rain, becoming 'pale red' (10A3) or with pale red patches at places, sometimes becoming completely white with a reddish sheen, very finely striate at the margin, slimy and viscous when wet, cuticle attached, difficult to separate; margin straight, entire. Lamellae adnate when young becoming subdecurrent to decurrent when fully expanded, white when young, 'pale yellow' (3A3) when old, up to 2 mm wide, close to crowded, with lamellulae of 2–3 lengths, sometimes bifurcating towards the margin; edge concolorous to the sides, entire. Stipe 4.5–5.3 × 0.8–1.2 cm, central, cylindric, slightly tapering towards the base, solid becoming spongy; surface white, becoming pale yellow when bruised, smooth, dry. Context white, up to 3 mm wide, soft, brittle, unchanging. Odour not characteristic. Taste bitter. Spore print white.

Chemical reactions: SV carmine red on lamellae; FeSO4 pale greyish pink on flesh.

Basidiospores 7.5–9 (9.6) × 6.9–7.5 µm, (Q=1–1.33 µm), mostly broadly ovate ellipsoid, sometimes subglobose, composed of amyloid, isolated verrucae, with obtuse tips, up to 0.6 µm high, devoid of any connections; suprahilar plage slightly amyloid. Basidia 28–38 × 4–10 µm, clavate, 4-spored. Lamella-edge heteromorphous; cheilocystidia and pleurocystidia similar, very numerous, macrocystidioid. Cheilocystidia 42–62 × 6–8.8 µm, narrowly clavate to clavate or ventricose, often with an apical protrusion or appendage, thin-walled, filled with granular refractive contents, staining black in SV. Pleurocystidia 52–75 × 6–7.5 µm, similar but slightly larger than the cheilocystidia. Pileal trama heteromerous with sphaerocytes and hyphae; sphaerocytes 20–42 × 18–38 µm, thin-walled, hyaline; connecting hyphae thin-walled, hyaline, septate, branched, up to 4 µm wide. Subhymenium pseudo-parenchymatous. Hymenophoral trama heteromerous, composed of sphaerocytes, 18–27 × 15–24 µm; connecting hyphae thin-walled, hyaline, up to 3 µm. Pileipellis distinctly two layered; suprapellis a trichoderm, of short cylindric to ventricose elements, 10–15 × 2.5–4 µm, intermixed with abundant clavate to narrowly clavate macrocystidioid pileocystidia, 18–60 × 4.5–6 µm, blackening in SV; subpellis a zone of gelatinized interwoven hyphae, thin-walled, hyaline, up to 4 µm wide, intermixed with numerous pseudocystidia, similar to the pileal cystidia,

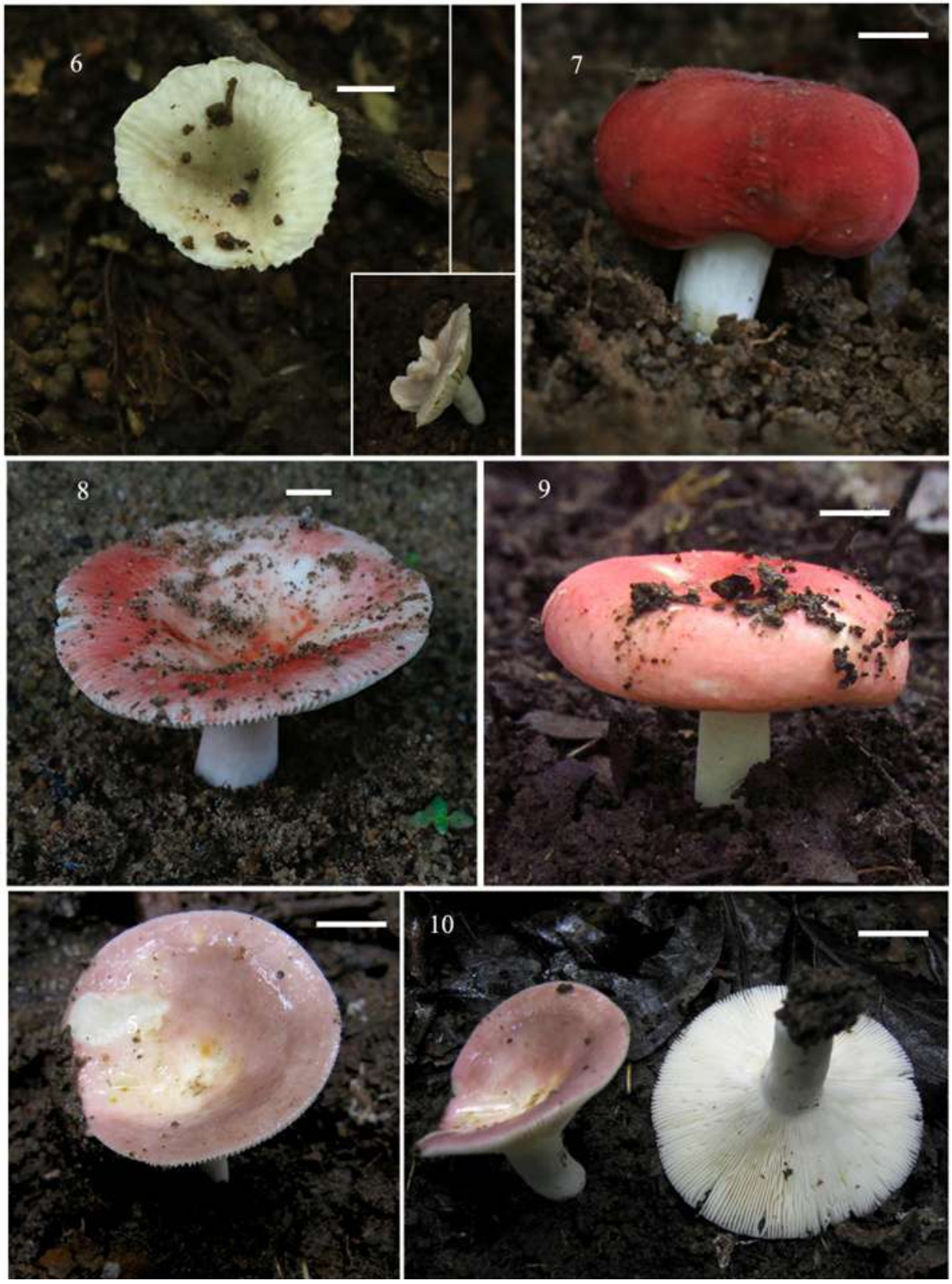

Plate 2.
Fig. 6. *Russula* sp. 3 Fig. 7. *Russula* sp. 5 Fig. 8. *Russula congoana* Fig. 9. *Russula nobilis* Fig. 10. *Russula luteotacta*

remaining embedded in the subcutis, which are just the undifferentiated terminations of the vascular hyphae. Stipitipellis a repent epicutis of parallely arranged hyphae, intermixed with abundant macrocystidioid caulocystidia, with refractive granular contents, blackening in SV, 22–60 × 3–5.5

µm. Oleiferous hyphae present. Clamp connections absent in all parts of the tissues.

Habitat & phenology: Scattered on sandy soil in association with *Myristica malabarica* Lam.; April-May, September.

Specimens examined: State, Thiruvananthapuram Dist., JNTBGRI campus: 28 Apr. 1998, TBGT(M) 4296; 3 May 1998, TBGT(M) 4298; 03 Sep. 2012, TBGT(M) 14225.

Discussion and conclusion

A total of 143 individual collections were made from various localities of Kerala. Among these collections *R. aciculocystis* (Subgenus *Amoenula*) hit top position with 67 collections followed by *R. congoana* (Subgenus *Russula)* 22 collections and *R. purpureonigra* (Subgenus *Compactae*) with 17 collections.

Most of the *Russula* species collected from Kerala were associated with Dipterocarpaceae members, while few others formed ectotrophic associations with other family members of higher plants belonging to Fabaceae, Guttiferae, Malvaceae, Moraceae and Myristicaceae.

The genus *Russula* has a good diversity in Kerala representing all the six subgenus. Among these two species are new record to Kerala and three species are new to science.

Of the 10 species of *Russula* treated in this study, some are very common and widely distributed, while others are rare or have a limited distribution area. Factors like occurrence of their mycorrhizal symbionts (s), climatic conditions and soil conditions seem to be very important in delimiting the distribution pattern of the individual species.

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Chapter 5

DISTRIBUTION OF TOTAL HETEROTROPHIC BACTERIA IN BIVALVES OF ASHTAMUDI ESTUARY, A RAMSAR SITE IN KERALA, SOUTH WEST COAST OF INDIA

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Abstract A systematic investigation of total heterotrophic bacteria (THB) was conducted in Ashtamudi Estuary (8°53'0 19.083"N and 76°32' 6.157"E), a Ramsar site known as the gateway to Kerala's backwaters. The estuary that played a crucial role in the hydrological, biological and ecological resources of the region nowadays has become a dump site for sewage which is slowly choking the wetland to death. Accumulation of THB in edible bivalve species, *Marcia recens* (Yellow clam), *Perna viridis* (Asian green mussel), *Villorita cyprinoides* (black clam), and *Crassostrea madrasensis* (Indian backwater oyster) from three sites viz., Neendakara, Dalavapuram and Kureepuzha is seasonally analysed using serial dilution pour plate method. Basic physicochemical parameters like pH, DO and salinity was also analysed and compared with the THB. The results displays that the THB in bivalves are more during monsoon season, though without spatial variation. However, there was a slight temporal variation in the THB in water and sediment. Pearson's correlation shown a significant positive correlation of THB in water and sediment with their counterparts in the organisms under study indicating the influence of water and sediment in the bioaccumulation of bacteria in the lake system. Pollution prevention procedures and sanitation packages need to be applied to increase consumer health.

Key Words: *Ashtamudi Lake, Accumulation, THB, Marcia recens, Perna viridis, Villorita cyprinoides, Crassostrea madrasensis*

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Introduction

Ashtamudi lake, the second largest Ramsar notified wetland in Kerala, has rich avi-faunal and aqua-faunal diversity, ecosystem services and aesthetic assets (RIS Information Sheet on Ramsar Wetlands, 2002). It is the second biggest fish-landing centre and around 30,000 fishermen depend on for their livelihood (Chackacherry and Jayakumar, 2011). Oysters collected from it form a vital source of meat for consumption and the lime-shell for cement and calcium carbide industries (Appukuttan *et al.*, 1987).

Even though the aquatic system has inferred to major values and functions, growing anthropogenic activities on the coast cause serious pollution, environmental degradation and shrinkage. As the water body is slowly becoming fallow, it threatens the livelihood of fishermen. Clam beds are disappearing at alarming rates due to hydrological regime change, invasive species and illegal fishing practices.

Entry of bacterial pathogens through sewage and river runoff also causes danger. The pathogens reaching the estuary may enter the food chain like bivalves via detritus feeders. Widespread filter feeding behaviour, diverse geographical scattering and limited movement make bivalves useful bio-indicators of the marine environment (Zuykov *et al.*, 2013). The present study focuses on the accumulation of total heterotrophic bacteria in *Marcia recens* (Yellow clam), *Perna viridis* (Asian green mussel), *Villorita cyprinoides* (black clam), and *Crassostrea madrasensis* (Indian backwater oyster) from three sites viz., Neendakara, Dalavapuram and Kureepuzha.

Materials and methods

Study area and sampling locations

Six sampling stations are selected for the collection of surface water, sediment and oyster samples covering the entire estuary (Fig.1).

Figure 1. Showing the sampling sites selected for the present study

Sampling and analysis for bacteriological parameters

Seasonal samples were collected from April 2019 to February 2020 (Table 1). Water samples were collected in sterilized BOD bottles and sediment and live clam samples in clean polythene bags using Ekman's grab and from local fishermen respectively. The samples transported to the laboratory in an icebox aseptically were analysed for Total Heterotrophic Bacteria (THB) on the same day (Anonymous, 2017).

Analysis of THB

THB analysis was performed using the pour plate method. Bivalves samples cleaned by scrubbing followed by washing under running water were shucked with a sterile knife to extract the flesh (Chinnadurai *et al.*, 2016). Water, sediment and homogenized flesh samples were serially diluted with sterile peptone water and 1ml of 10^{-4}, 10^{-6} and 10^{-7} dilutions respectively were plated in duplicate on petri plates over which sterilized and cooled plate count agar (Hi-Media, Mumbai, India) was poured and uniformly mixed. After solidification, the plates were inverted and incubated at 370°C for 24-48h. Plates having 30 - 300 colonies were counted and THB expressed as cfu (colony forming units) per ml or g (Anonymous, 2017).

Statistical analysis

TPC values were converted to $\log_{10}$ values prior to analysis. Statistical analysis of Bio-Accumulation Factor (BAF) of THB was carried out by dividing its count in oyster by that in water. Pearson's correlation was applied to determine the relationship of bio-accumulation of THB in water and sediments in various Oyster species studied.

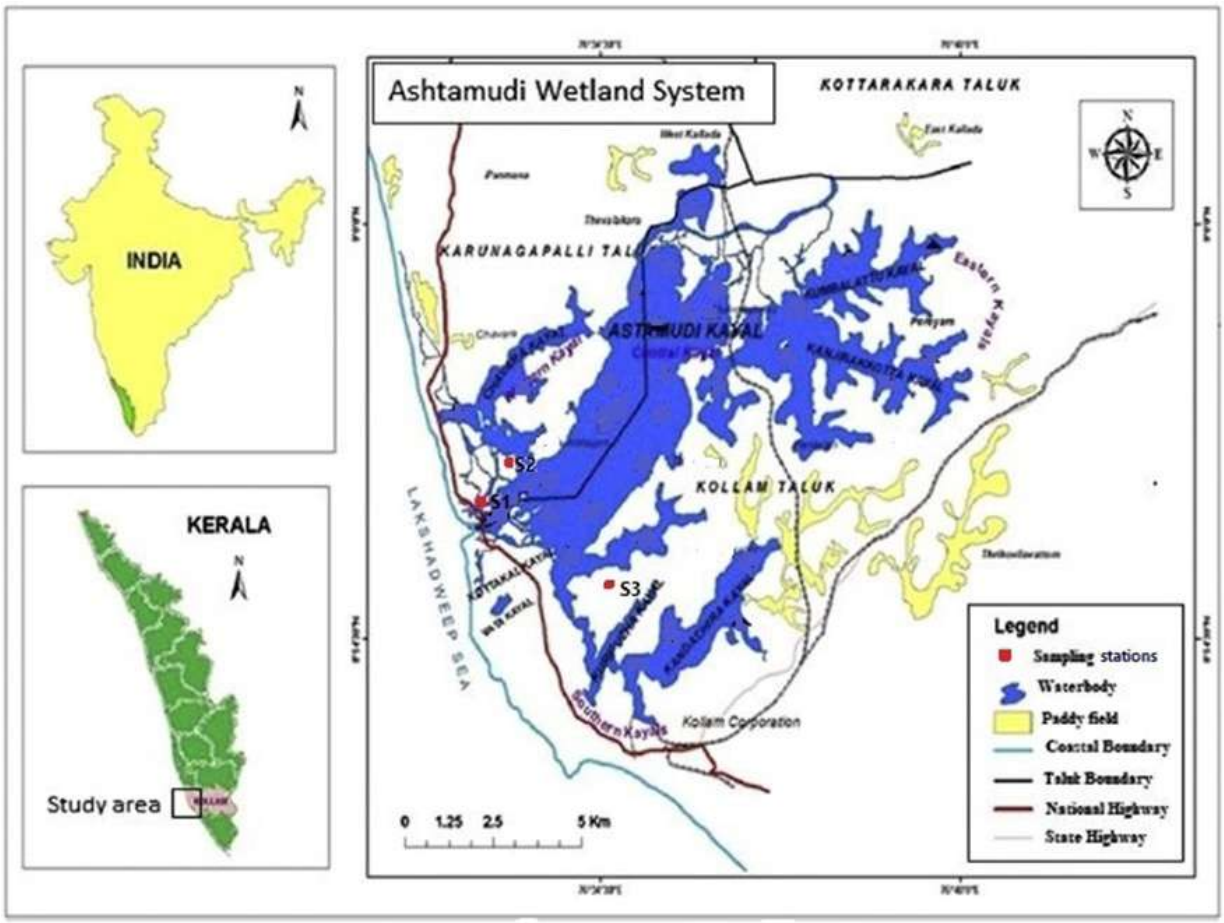

Fig. 1. Showing the sampling sites selected for the present study

Table 1. Seasonal mean log values of TPC in water and sediments in Ashtamudi Lake

Season	NKA	DPM	KPZ	NKA	DPM	KPZ		
	TPC log10water			TPC log10sediment			F value	P value
PRM	5.293±0.001	5.101±0.006	5.239±0.003	6.108±0.0001	6.088±0.0001	6.169±0.0005	0.275	0.563
MON	5.368±0.001	5.426±0.003	5.533±0.033	6.325±0.0011	6.357±0.0005	6.394±0.019	0.586	0.631
POM	4.833±0.001	4.717±0.033	5.122±0.004	5.815±0.0009	5.788±0.0150	6.015±0.015	0.148	0.247

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha SD- Standard Deviation

Table 2. Seasonal mean log values of TPC in oysters in Ashtamudi Lake

	Mean ± SD			Mean ± SD			Mean ± SD		
	NKA			DPM			KPZ		
	PRM	MON	POM	PRM	MON	POM	PRM	MON	POM
M. recens	8.191± 0.0007	8.230± 0.0004	8.154± 0.0012	8.120± 0.0086	8.320± 0.0008	8.067± 0.0041	8.140± 0.0004	8.370± 0.0041	8.154± 0.0005
C. madrasensis	7.122± 0.043	7.316± 0.050	7.076± 0.041	7.104± 0.312	7.402± 0.055	7.051± 0.023	7.166± 0.019	7.411± 0.029	7.108± 0.049
P. viridis	7.106± 0.021	7.212± 0.043	7.046± 0.030	7.102± 0.033	7.214± 0.034	7.064± 0.032	7.106± 0.050	7.231± 0.031	7.009± 0.041
V. cyprinoides	7.104± 0.024	7.157± 0.041	7.021± 0.037	7.104± 0.036	7.207± 0.035	7.007± 0.032	7.105± 0.023	7.240± 0.028	7.118± 0.039
F Value	**0.658**			**0.865**			**2.049**		
P value	**0.664**			**0.532**			**0.149**		

PRM-Premonsoon, MON-Monsoon, POM-Post monsoon, NKA-Neendakara, DPM-Dalavapuram, KPZ-Kureepuzha SD- Standard Deviation

Results

Total Plate Count

In water and sediments, the maximum THB counts were observed at Kureepuzha in monsoon and the minimum at Dalavapuram in post monsoon (Table 1). THB count in *M. recens* was the maximum at Kureepuzha in monsoon and the minimum value was at Dalavapuram in post monsoon. In *C. madrasensis*, the highest and lowest count was recorded respectively from Kureepuzha in monsoon and Dalavapuram in post monsoon. The highest and the lowest counts of THB in *P. viridis* was seen at Kureepuzha in monsoon and post monsoon respectively and in *V. cyprinoides* at Kureepuzha in monsoon and at Dalavapuram in post monsoon (Table 2).

Bio Accumulation Factor of THB

The BAF broadly varied from 1.307 in *P. viridis* in Dalavapuram to 1.710 in *M. recens* in Kureepuzha among different oyster species of Ashtamudi lake. In *M. recens*, BAF varied from 1.513 at Dalavapuram in post monsoon to 1.710 in Kureepuzha in post monsoon. Accumulation of THB in *C. madrasensis* fluctuated from 1.339 to 1.495 at Dalavapuram in post monsoon and at Kureepuzha in monsoon. *P. viridis* showed BAF varying from 1.307 at Dalavapuram in post monsoon to 1.498 at Kureepuzha in monsoon and *V. cyprinoides* from 1.309 at Dalavapuram in monsoon to 1.485 at Kureepuzha in monsoon (Table 3).

Pearson's correlation revealed significant positive correlation between THB in water and sediment with THB counts in oysters.

Discussion

Estuarine habitats are generally more productive due to the accrual of nutrients from fresh water runoff. They act as breeding ground for a number of prawn and shrimp species, oysters and fish and offer good inland fishing grounds for the fisherman due to their shallow depth. The

Table 3. Variation in Bio-accumulation Factor of THB in Oysters in Ashtamudi Lake

	M. recens			*C.madrasensis*			*P. viridis*			*V. cyprinoides*		
Season	NKA	DPM	KPZ	NKA	DPM	KPZ	NKA	DPM	KPZ	NKA	DPM	KPZ
PRM	1.547	1.533	1.687	1.344	1.363	1.464	1.343	1.344	1.458	1.342	1.333	1.453
MON	1.592	1.533	1.710	1.393	1.364	1.495	1.392	1.330	1.498	1.393	1.328	1.485
POM	1.554	1.513	1.592	1.368	1.339	1.388	1.356	1.307	1.368	1.356	1.309	1.390

Table 4. Correlation Coefficient of THB in water, sediment and oysters in Ashtamudi Lake

	Water	sediment	*M.rec*	*C.madr*	*P. viridis*	*V. cipr*
Water	1					
sediment	0.972	1.000				
M.recens	0.909	0.908	1.000			
C.madr	0.843	0.907	0.944	1.000		
P. viridis	0.803	0.892	0.824	0.908	1.000	
V. cipri	0.948	0.955	0.964	0.917	0.795	1.000

fishermen who depend on the inland fishery resources are the immediate stakeholders who suffer from the change in the quality of the system.

The current study showed that benthic organisms accumulate total heterotrophic bacteria with respect to their counts in water and sediments. THB counts showed increasing trend in water, sediment and oysters. Among the oysters under study, THB count was the highest in *C. madrasensis*. Seasonally, monsoon season showed the maximum THB indicating the entry of the contaminants through rain water. Higher TPC in sediments than the water may be due to the fact that the estuarine sediments play a significant role in the demineralization of organic content which supports the growth of microbes (Swarnakumar *et al.*, 2008) and the lesser dwelling time of microbes in water column than in sediments. The density of TPC in shellfish samples was higher than that in water and sediments because they accumulate microbes from the surrounding water and maintain their levels for longer periods (Sasikumar and Krishnamoorthy, 2010). However, TPC count in shellfish showed positive correlation with that in water and sediment (Table 4). High filtration rates in clams in monsoon may increase the uptake of microbes along with the particulate organic matter from the sediments (Murali *et al.*, 2019). This is evident from the higher THB values during monsoon in all the stations. In general, the occurrence of TPC indicates the presence of heterotrophic bacteria in the lake water due to various human, agricultural and industrial activities. The TPC number recommended for good quality bivalve molluscs is 500,000/g (European Communities, 2004) and the counts in bivalves from the current study were found to be below this threshold limit. In the present study, the distribution of TPC in water, sediment and bivalves is found to have seasonal variation.

Conclusion

Higher TPC in shellfish samples in all seasons indicated the general trend of microbial contamination in the estuary from the surroundings and higher accumulation during monsoon season indicates re-suspension of sediments during monsoon along with runoff contaminants. It is clear from the study that the relative occurrence of heterotrophic bacteria in water and sediment is reflected in the BAF and their correlation is highly positive.

Acknowledgment

The authors thank the Management of SN College, Kollam for the facilities during the research work.

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Chapter 6

ANATOMICAL AND PHYTOCHEMICAL INVESTIGATION ON *MYXOPYRUM SMILACIFOLIUM* (WALL.) BLUME

Ratheesh N, Anagha S S

Abstract In the present study, *Myxopyrum smilacifolium* (Wall.) Blume, was collected from Ezhukone, Kollam District, Kerala to determine the anatomical characters, the phytochemical analysis, antioxidant activity and antibacterial activity using selected bacterial species. The anatomical study was conducted on leaf, petiole and stem. The preliminary phytochemical analysis using ethanolic extract of leaves showed the presence of phenols, flavonoids, alkaloids, tannins, glycosides, saponins, steroids, terpenoid and quinones. Quantitative analysis of the ethanolic extract of leaves resulted in high phenolic content of 74.85µg/ml of gallic acid equivalents of extract and flavonoid content of 60.67µg/ml of Quercetin equivalents of extract and terpenoid content of about 1 %. The antioxidant activity increases with increasing concentrations of the extract. The results support the efficiency of natural phenolics of plant origin offering protection against oxidative injury. The antibacterial activity of two different strains of bacteria *Bacillus subtilis* and *Staphylococcus aureus* was assayed by disc diffusion method. The two bacterium do not shows any zone of inhibition indicating that they are resistant to the plant extract and hence did not possess any antibacterial activity against these two bacterium. The presence of phytochemicals contribute the medicinal properties and it could be used as a source of useful drug to treat various diseases.

Key words: *antioxidant activity, quantitative estimation, glycosides, flavonoids*

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Introduction

The medicinal properties of plants have been studied due to their potential pharmacological activities, low toxicity and economic viability which eventually leads to drug discovery and developments. The plant derived products however, have long been used as a source of drugs. The primary benefits of using plant derived medicine are that, they are relatively safer offer profound therapeutic benefits and more affordable treatments (Thilagavathi *et al.*, 2015). Moreover, phytopharmaceuticals are used as an alternative medicine to prevent many diseases. The medicinal effects of plant are due to the presence of phytochemicals and it is useful for healing as well as curing human diseases. Over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Parekh and Chanda, 2007). However, it is necessary to conduct research whose objective is the production of active components from the plant through various chemical methods.

The usage of plants in the treatment of various disease has been reported in Vedic scriptures. Thus plants paved a way for new therapeutic agents. Now a days, the demand for plant derived compounds are increasing because of their lesser side effects and low cost. So, drugs developed from plants can replace the demerits of modern synthetic drugs. Phytochemical screening not only helps to reveal the constituents of the plant but also helpful in searching bioactive compounds which can be also in the discovery of useful drugs. The medicinal value of the plant is due to its bioactive compounds such as terpenoids, flavonoids, phenols and alkaloids. The discovery of phytochemical compounds leads to the production of new drugs for the treatment of various diseases. This revival of interest in plant

based drugs is due to the current widespread belief that "green medicine" is safe and more dependable than the costly modern synthetic drugs, many of which have side effects (Parekh and Chanda,2006). *Myxopyrum smilacifolium* (Wall.) Blume is an important medicinal plant used in indigenous system of medicine. The leaf of this plant is used for the treatment of cough, rheumatism, cephalagia, otapathy, febrifuge, fever, cut and wounds (Siju, *et al.*, 2016). The roots are used in the treatment of cough, scabies and fever. The plant has also been studied for its antimicrobial, wound healing and anti-inflammatory activity. Studies have shown the presence of triterpenoids, ursolic acid and iridoid glycoside myxopyroside in leaves (Franzyk *et al.*, 2001. Hence the present study was conducted with the aim of analysing the morphological and anatomical characters, phytochemical constituents and antioxidant activity of *M. smilacifolium* with the following aim of investigating the morpho-anatomical characteristics, to perform the preliminary phytochemical screening to identify the major phytochemicals in the plant extract by specific phytochemical tests and to determine the *in vitro* antioxidant activity of ethanolic extract.

Materials and Methods

For the present investigation, *M. smilacifolium* belongs to the family Oleaceae was selected and analysed. The plant material required for the entire study was collected from Ezhukone, Kollam district on the month of February. The plant is a large woody twinning shrub with quadrangular stem growing in exposed areas. Leaf 7-13x3-5 cm, elliptic to ovate, membranous to subcoriaceous, base rounded, apex acuminate. Margin usually obscurely serrated towards apex. Venation triplinerved with additional 3 pairs of lateral veins, secondary veins prominent below, tertiary veins conspicuous above in dried state. Petiole 0.5- 1.5cm thickened. Inflorescence terminal, paniculate 7.5-19 cm. Bracts apiculate, 1mm. Flowers yellow, subsessile. Calyx1-1.5 cm long, ovate -acute, pubescent. Corolla tube companulate, 1-1.5mm long. Stamens less than 1 mm long, subsessile. Anthers are elliptic. Ovary globose, less than 1mm long. Stigma sessile, bilobed. Fruit is globose measures about 5x5-10x13 mm. Pericarp rugose when dry. Seeds 1-2.

Hand sections of leaf, petiole, and stem were taken with the help of sharp razor. The sections were stained in saffranin. The stained sections are placed on a clean slide and mounted on glycerine and covered with a coverglass. It is then observed under a light microscope for microscopic observation and photomicrographs were taken.

For the screening of phytochemicals soxhlet extraction method was employed (Saj and Vinny, 2011). Dried leaves were powdered to obtain a coarse powder. Dried leaf powder is placed inside a thimble, which is loaded into Soxhlet extractor. The extraction of weighted leaf powder with respective volume of the solvent was carried out with its boiling point. Selected solvents were carried for the extraction depending on their increasing polarity. The soxhlet extractor is positioned into a flask containing the extracting solvent. The Soxhlet is then fitted with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation tube and condensed into the extractor housing the thimble holding the solid. The condenser ensures that solvent vapour condenses and drips back down into the chamber housing the plant material. The chamber containing the material slowly fills with warm solvent. Some of the desired compound then dissolves in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the extracting flask. This cycle may be allowed to repeat many times, over hours or days, until the solvent gets colourless in extracting chamber. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. After extraction the solvent is removed typically by means of a rotary evaporator yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded after extraction running with all the selected solvents.

Qualitative analysis

(Ejikeme *et al.*, 2014; Sasikumar *et al.*, 2014)

1. Test for phenols (Lead acetate test): 2ml of

plant extract was taken in a test tube and add 1% lead acetate solution. Formation of white precipitate indicates the presence of phenolic compounds.

2. Test for Flavonoids (Alkaline reagent test): 2ml of plant extract was treated with 2ml of 10% ammonium hydroxide solution. Appearance of yellowish green colour which turns colourless when conc. HCl was added.

3. Test for alkaloids (Wagner's reagent test): Take 2 ml of plant extract and add 2ml Wagner's reagent. Test tubes were observed for the appearance of reddish-brown precipitate.

4. Test for tannins (Ferric chloride Test): About 0.5 g of plant extract was boiled in 20 ml of distilled water in a test tube and then filtered. 1ml of the leaf extract added with 5 % $FeCl_3$ (1 ml) was added to the filtrate. Appearance of brownish green coloration showed the presence of tannins.

5. Test for glycosides (Kellar-Killani Test): In 5ml plant extract, 2ml glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added. Brown ring appears, indicating the presence of glycosides

6. Test for saponins (Foam Test): To about 1ml of each extract was added to 2ml of distilled water in a test tube and shaken vigorously with few drops of olive oil. Foam which persisted was taken as a evidence for the presence of saponins.

7. Test for steroids (Liebermann-Burchard Test): 1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

8. Test for terpenoids (Liebermann-Burchard Test): 2ml of each extract of plant samples was mixed with 2ml of chloroform. Then allow to evaporate and add 2ml of concentrated sulfuric acid, then heat for 2 minutes. Greyish colour indicates the presence of terpenoids.

9. Test for quinones (HCl method): 2ml of each extract of plant was mixed with 3 or 4 drops of concentrated HCl. A yellow colour precipitate indicates the presence of quinones.

Quantitative analysis

Estimation of phenols (Meda *et al.*, 2005): Different extract of samples was pipette out and the volume in each tube was made up to 3.0 ml with distilled water. Folin-Ciocalteau reagent (0.5mL) and 2mL 20% Na_2CO_3 were added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 750nm in a spectrophotometer against a reagent blank. Standard gallic acid solutions (2.5-100µg/mL) were also treated as above.

Estimation of flavonoids (Lee wei and Ismail, 2012): Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1mg of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of Quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/ mg of extract.

Estimation of terpenoids (Ferguson, 1956): Powered sample of 100 mg was soaked in 10ml of ethanol for about 24 hours and it was filtered and extracted using petroleum ether using separating funnel. The collected petroleum ether extract was allowed to dry and % of Terpenoid content was estimated using the following formula;

Percentage terpenoid content = (Weight of terpenoid extract /weight of the sample)100

Antioxidant activity (DPPH Radical scavenging assay- Kevin and Mahmoud, 2013, Brand – William *et al.*, 1995): For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ ml; w/v). A 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800µg/ml). The plates were kept in the dark for 15 minutes at room temperature and

the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula;

Percentage inhibition = ((Absorbance of Control -Absorbance of test)/Absorbance of control)X100

Result and Discussion

Anatomical studies

The microscopical examination of leaf revealed the presence of outermost single layered, thick walled, Epidermis covered with cuticle. It includes upper and lower epidermis. Followed by the epidermis is the mesophyll which is differentiated into 1-2 layers of upper palisade parenchyma and lower spongy parenchyma. Asicular crystals may found in the spongy parenchyma. Vascular bundles are slightly concave, conjoint, collateral and closed. Xylem lies towards the upper side and phloem lies towards the lower side. Vascular bundles are surrounded by a compact layer of thin wall parenchyma cells called bundle sheath. Bundle sheath extensions are also found in leaf. While stomatal type of *M. smilacifolium* was anomocyticie, the size of the subsidiary cells and epidermal cells are equal (Plate 1). While in T.S of petiole shows the outermost layer is the single layered epidermis covered with thick cuticle. The epidermis is followed by several layered collenchymatous cortex. Vascular bundles are similar to that of leaf. They are slightly concave, conjoint, and collateral occupied at the center. Few to many brachysclereids are associated with vascular tissue

The stem exhibits normal secondary growth. The T.S of the stem is deeply grooved and winged above in outline. Stem consist of single layered Epidermis covered with cuticle. Followed by the epidermis is the compactly arranged 2-3 layers of collenchymatous hypodermis. Below the hypodermis is the parenchymatous cortex. The cortex is separated from the vascular tissue by means of single layered Endodermis. Usually, the cells contain starch grains and the endodermis may term as starch sheath. Between the endodermis and the vascular bundle is the pericycle. Vascular bundles are conjoint, collateral and open. The interfasicular cambium present in the vascular bundle produces secondary phloem towards outside and secondary xylem towards inside. The central region is occupied by a large pith

Preliminary Phytochemical Analysis

Plant derived compounds are of great demand because of their versatile applications (Baris *et al*., 2006). Phytochemical screening is one of the necessary steps to isolate the chemical compounds. In the present investigation study nine phytochemical screening test have been carried out included phenols, flavanoids, alkaloids, tannins, glycosides, saponins, steroids, terpenoids and quinones. The preliminary phytochemical examination of ethanolic extract of M. smilacifolium indicated the presence of all these nine constituents (Plate 2). The result obtained in the study was presented in Table 1. A white precipitate is formed on adding 1% lead acetate solution indicates the presence of phenols. A yellowish green colour indicates the presence of Flavonoids. When the extract is treated with Wagner's reagent a reddish brown precipitate is formed because of the presence of Alkaloids. The formation of brownish green colour when 5% $FeCl_3$ was added to the filtrate of the plant extract which is boiled with distilled water showed the presence of Tannins. On adding 2ml glacial acetic acid, one drop of 5% ferric chloride and concentrate sulphuric acid to the plant extract a brown ring appears, indicating the presence of glycosides. The presence of Saponins is confirmed by the formation of persisted foam when distilled water is added to the plant extract and shaken vigoursly with few drops of olive oil. When the extract was dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid was added by the sides of test tube, the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence because of the presence of steroids. When the plant extract was mixed with 2 ml of chloroform, then allowed to evaporate and added 2 ml of con. sulphuric acid then heated for 2 minutes. The formation of greyish colour showed the presence of Terpenoids. A yellow precipitate is

formed when the plant extract is treated with con. HCl indicated the presence of quinones.

In the phytochemical compound extracted in polar solvents are pharmaceutically important because of their high antioxidant, and free radical scavenging activity (Nawaz, *et al*., 2020). Preliminary phytochemical analysis revealed the presence of phenols, Flavonoids, Alkaloids, Tannins, Glycosides, Saponins, Steroids, Terpenoids, and Quinones in ethanolic extract of *M. smilacifolium*. The pharmacological effect exherted by polyphenols on human body is strongly related with their high antioxidant capacity (Hainal, *et al*., 2011). Phytochemical investigation of *M. smilacifolium* carried out so far has reported the presence of Iridoid glucoside-myxopyroside (Franzyk, *et al*., 2001).

Ethy alcohol extract of the leaves revealed the presence of terpenoids, flavonoids, tannins, and polyphenols (Jolly, *et al*., 2014) which is comparable to our result. Preliminary phytochemical screening was conducted on root extracts of *M. smilacifolium* revealed the presence of alkaloids, phenols, flavonoids, tannins, and glycosides (Praveen and Ashalatha, 2014) which is in agreement with our result.

Quantitative Analysis

In quantitative analysis we were estimated the total Phenolics Flavanoid and Terpenoid content in ethanolic extract of *M. smilacifolium*.

Table 1. Preliminary phytochemical analysis of ethanolic extract of *M. smilacifolium*

Sl. No	Secondary metabolites	Test	Observation	Result
1	Phenol	Lead acetate test	Formation of white precipitate	+++
2	Tannin	Ferric chloride test	Formation of brownish precipitate	+++
3	Flavonoid	Alkaline reagent test	Formation of yellow green colour	+++
4	Saponin	Foam test	Persistant foam	++
5	Terpenoid	Liebermann – Burchard test	Formation of greenish colour	+++
6	Alkaloid	Wagner's reagent	Appearance of reddish brown precipitate	+
7	Glycoside	Kellar - Killani test	Formation of brown ring	+
8	Quinones	HCl method	Appearance of yellow precipitate	+ +
9	Steroid	Liebermann – Burchard test	Upper reddish layer and greenish yellow acid layer	+

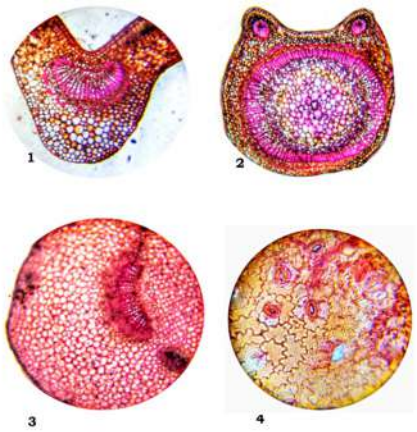

Plate 1. Microscopical examination of *M. smilacifolium* leaf
1. Vascular bundle arrangement 2. Petiole 3. stem 4. Stomatal type

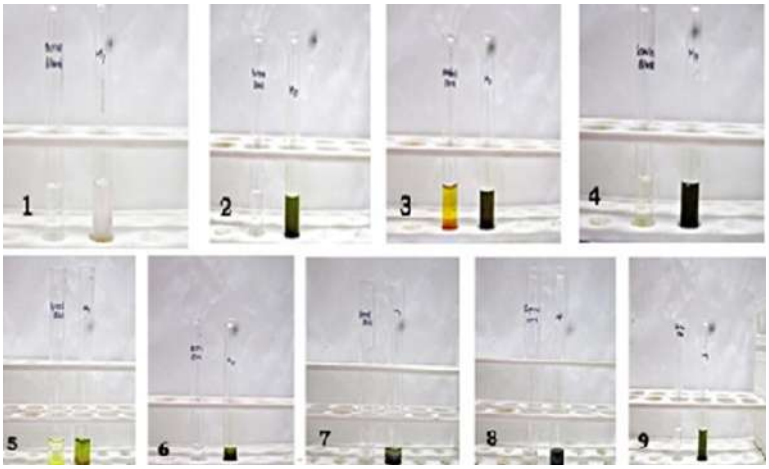

Plate 2. Preliminary phytochemical analysis of *M. smilacifolium* leaf
1. Phenol 2. Flavonoid 3. Alkaloid 4. Tannin 5. Glycoside 6. Saponin 7. Steroid 8. Terpenoid 9. Quinones

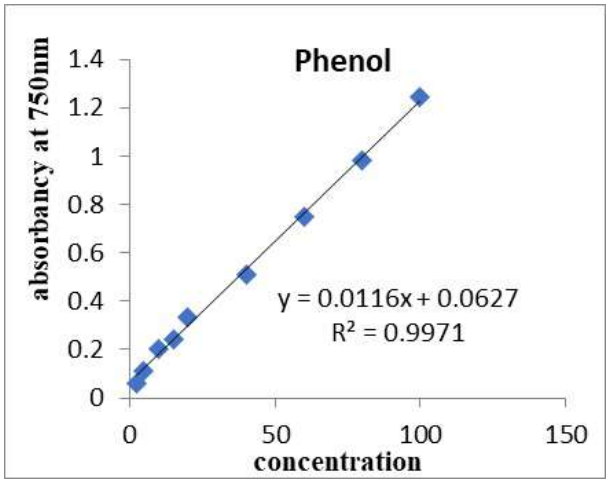

Fig.1. Standard graph for total phenolic content

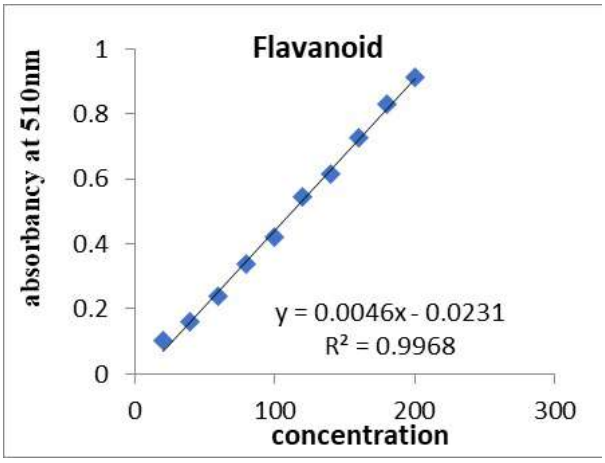

Fig. 2. Standard graph of total flavanoid content

Estimation of total phenolics

The total phenolics content of ethanolic extract was determined using Folinciocalteu reagent (Fig. 1). The phenolic content observed in *M. smilacifolium* leaf extract was 74.85 µg/mg of Gallic acid equivalents of extract.

Estimation of Flavonoids

The total flavonoid content was determined using aluminum chloride ($AlCl_3$) according to known method with quercetin as a standard (Fig. 2). The total flavanoid content in leaf extract was found to be 60.67µg/mg of quercetin equivalents of extract.

Estimation of terpenoids

From the formula the percentage of terpenoid content in the ethanolic extract of leaf of *M. smilacifolium* was estimated to be 1. In the quantitative analysis of ethanolic extract of *M. smilacifolium* resulted in high phenolic content of 74.85µg/ml of gallic acid equivalents of extract and total flavonoid content of 60.67µg/ml of quercetin equivalents of extract. Quantitative analysis of Myxopyrum species revealed that the leaf extract poses a great content of total phenol and flavonoid (Sheelarani *et al*., 2019), which is comparable with the present investigation.

Antioxidant activity

Total antioxidant activity of the plant extract and standard compound was determined by DPPH Assay. The extract showed effective antioxidant activity. The% of inhibition of various concentrations (1.56, 3.12,6.25,12.5,25,50,100,200,

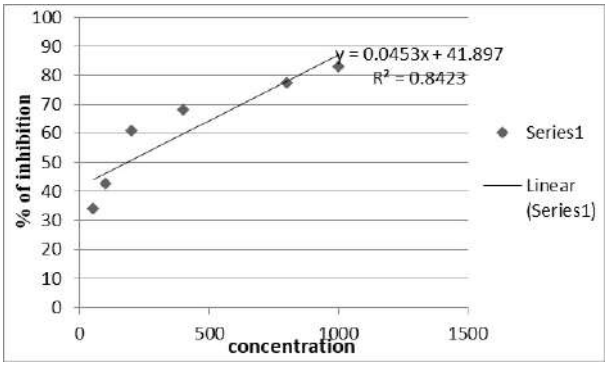

Fig. 3. Total antioxidant activity of the extract

400,800,1000) of the extract were found to be 4.19%, 14.08%, 21.26%, 25.54%,30.89%, 34.25%, 42.68%, 60.92%, 68.23%, 77.51%, 83.24% respectively as shown in Fig. 3.

During the present investigation, the antioxidant activity of the leaf extract was estimated, the result showed that it is a potential source of antioxidant. A previous study conducted by (Siju *et al*., 2015) indicated the similar results. The antioxidant activity of plant extracts can be correlated to its phenolic, non-phenolic and the unidentified compounds (Shahidi *et al*., 1992). These compounds have beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations. As reported by Litty and Anusha (2019) the phenolic and flavonoids compounds present in the extract is responsible for its antioxidant activity. Ingestion of natural antioxidants has been associated with reduced risk of cancer, diabetes and other diseases associated with ageing.

Present study revealed that the phytochemical analysis of the ethanolic extract of leaves shows the presence of phytochemicals such as phenols, flavonoids, alkaloids, tannins, glycosides, saponins, steroids, terpenoids and quinones. The extract shows high antioxidant activity and the activity increases with increase in concentration of the extract and the increased antioxidant activity may be due to the presence of various phytochemicals.

Acknowledgement

The authors are thankful to the Principal, S N College Kollam and Head Department of Botany S N College Kollam for providing facility to fulfil the research work.

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Chapter 7

DOCUMENTATION OF ETHNOBOTANICAL DIVERSITY OF MEDICINAL PLANTS USED BY THE PANIYA TRIBES OF WAYANAD DISTRICT, KERALA

Gayathri G, Aswathy K

Abstract The legacy of plant and human relationships is as old as human history. Plants are sources of food, shelter, medicine and nutrition to us. Indigenous people live in harmony with their surroundings and they depend on plants for most of their livelihood. Documentation of traditional knowledge (TK) related to medicinal plants is a cue to new areas of research which helps in the conservation of biodiversity. Documentation of this TK helps to prevent the loss of valuable folk knowledge. The present study attempts to identify the knowledgeable persons and the diversity of plants that are utilised for medicinal purposes, by the Paniya tribes of Padinjarathara village, Wayanad District, Kerala. Only a very few respondents were perceptive about the medicinal plants used by their progenitors. Most of the plants used by them for medicinal purposes belong to angiosperms and pteridophytes. It was noticed that the Paniya community has moved to a civic life and depends on nearby towns and cities rather than forests or forest products, unlike their ancestors. Due to this, their knowledge on forest resources is depleting and it becomes unavailable to the scientific world.

Keywords: *Ethnobotany, Paniya tribes, Wayanad, Medicinal plants*

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Introduction

Humans and plants have a complex relationship extending far back into our joint evolutionary history. This legacy can be seen today as plants provide nutrition, fibre, pharmaceuticals and energy for people and animals across the globe. Plant domestication and agriculture allowed human society to develop and our settlements to become more complex. As such our modern cities and cultures rely in part on the stable and reliable production and distribution of food. The present work examines how the changes affec the globe may impact upon plant human relationship. Plants are central to our well-being, not only as food, but also as key components of our cultures, religions and medicines. We do not just get nourishment from plants, they are central to our societies (Schaal, 2019).

Traditional medicine and ethnobotanical information play an important role in scientific research, particularly when the literature and field work data have been properly evaluated (Awadh *et al.*, 2004). Tribes are considered as the traditional society consisting of families with limited numbers. The nature of the tribes, also called Adivasis, has changed over the centuries. The tribal population in India is more than 10,000. Their cultural, social and political entry differs from the other people. There are 645 tribal groups in India. The tribal communities differ from state to state, especially from Andhra Pradesh to Kerala. Some of the states have the same community of tribes also. The total population of scheduled tribes is 10.43 crore as per the 2011 census. But now the population has almost declined. The states with major tribal populations are Maharashtra, Karnataka, Gujarat, Andhra Pradesh and the states with the smallest population

of tribes are Assam, Tripura, Bihar and Manipur (Ministry of Tribal Affairs, 2011).

In Kerala 35 tribal communities are present. Adiyan, Arandan, Kanikaran, Kattunayakan, Eravallan, Hill Pulaya, Irulan, Kadar, KochuVelan, Konda Kapus, Koraga, Kondareddis, Kudiya, Kota, Malasar, Malaivedan, Malakkuravan, Malaipandaram, Malaiarayan, Mahamalasar, Kurumbas, Kurumanskurichchan, Malayan, Malayarayar, Mannan, Marati, Mudugar, Palleyan, Palliyan, Palliyar, Paniyan, Ulladan, Uraly. Among them, Paniyas, Kurumas, Adiyars, Kurichyas, Ooralis, Kattunaikkans and Uraalikurumas are the tribal communities present in Wayanad. They constitute almost 20% of the total population of the district. Paniya tribes in Wayanad is a major tribal community (Ministry of Tribal Affairs, 2011).

The World Health Organization (WHO) has emphasized the importance of traditional indigenous medicines since a large majority of rural people in developing countries still use these medicines as the first defense in health care (Arshad *et al*., 2010). Ethnobotanical studies to document indigenous knowledge will aid in the conservation of bioresources along with its sustainable utilization. In the present study, documentation of the diversity in ethnobotanicals of Paniya tribes at Padinjarathara village of Wayanad district was attempted.

Study area

Wayanad is in the north- east of Kerala state with the administrative headquarters at the municipality of Kalpetta. The district is situated at a height between 700 metres and 2100 metres above the mean sea level. The places are nested among the mountains of Western Ghats on the Eastern portion of the North Kerala and on the sides of Tamil Nadu and Karnataka states (www.wayanad.gov.in). Wayanad is situated at 11.6854° N and 76.1320° E longitudes and latitudes. The district has a green cover of 74.18% (Forest Survey of India, 2017), there are three main rivers, altitude is 1000 msl with mean rainfall being >300cm, quite often affected by the odds during monsoon, with water scarcity during summer (Sushanth and Anooj, 2020). The major study area for the present work was Padinjarathara section, Kuttyamvayal forest area, Mangalam colony near Banasurasagar.

Materials and Methods

Fieldwork in the Mangalam colony was conducted in September 2021 for this study. Ethnobotanical data were collected according to Jain, 2001. The data was collected through questionnaires, interviews and discussions among the Paniya group and a local tribal medical practitioner. A Questionnaire was prepared to bring out the ethno-botanical uses of Paniya tribes. The data was collected from the elder people of the Paniya population. The details of the Mangalam colony and surrounding area were collected from the Padinjarathara section office. Plant collection was done based on the information given by the respondents. The reliability of the information was assessed after repeated verification of the plants.

Results and Discussion

The present study includes the identification of plants of medico- ethnobotanical importance used by the Paniya tribal community. Documentation of local healthcare traditions of tribes and specific use of each of the medicinal plants and the plants used in religious rituals, cultural activities, entertainments, festivals and household implements were done during this study.

Mangalam colony in Kuttyamvayal forest area near Banasurasagar, Padinjarathara panchayath is a small tribal colony which is surrounded by the dam water, because of which, the colony looks like an Island. This colony is located on the forest border. The location details, borders and coordinates of the study area are given in Table 1. The map showing the study area (Picture Courtesy: Saleem Khan, Handbook of Climate Change and Biodiversity) and the GPS coordinates of the area of study are shown in Fig 1.

Table1. Location details of Mangalam Colony

Total area	42-44 acres
Altitude	819 m
Latitude	11°39'40" N
Longitude	75°56'35" E
South and East	Banasurasagar Dam
North and West	Forest and Forest section office

On visit, it was observed that the colony consisted of 3 major tribal communities; Paniyar, Kattunaikar, Kurichyar. The three communities consisted of 68 families (2 Kurichyar families, 7 Kattunaikar families and 59 Paniya families). The community included 5 Kurichyars, 21 Kattunaikars and 193 Paniya people making it a total population of 219 residents. The Paniya tribe was found to be dominant in this area.

The Paniyatribes dwell on the hill slopes surrounded by evergreen forest. The area was rich in floral diversity with natural and cultivated vegetation, with tall trees, epiphytic plants, herbs and shrubs. In addition to the angiosperm diversity, the study area was rich in lower groups of plants as well. The soil was found to be rich in humus and seems to be ideal for the luxuriant growth of the floral population. This area has not been subjected to any recent explorations.

There were a total of six respondents from the colony. The people who responded gave details about the plants they used for their daily requirements. The details were collected and the plants were enlisted with its ethnobotanical uses.

The Paniya tribes live in concrete houses as different from their ancestors (Fig. 2a). They have their own way of presenting themselves with some ornaments. They use ornaments of typical pattern with beads of different colours and for the making of some chains they use coins also (Kaashumala) (Fig. 2b, 2c, 2d).

From the survey details Paniya people use many

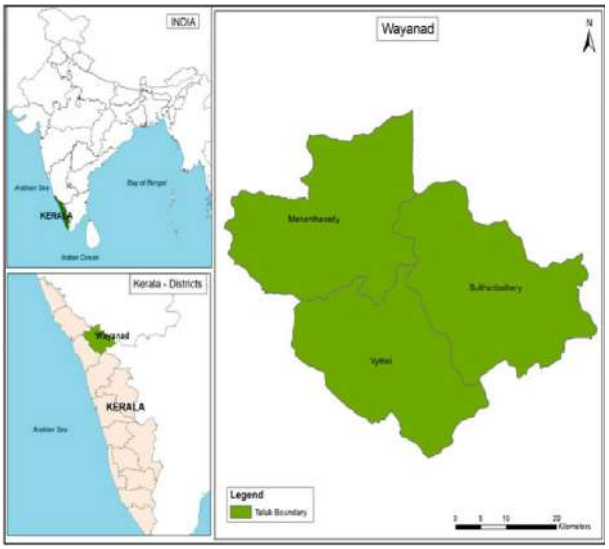

Fig. 1. Study area

Fig. 2a Paniya Family

Fig. 2b Bracelet

Fig. 2c Neck Chain

Fig. 2d. Kaashumala

plants for their basic and daily needs. Data obtained from field surveys is represented in Table 2. The Paniya tribes use different plant products as their ornaments, viz. earrings made from Abrus precatorius seeds (Fig. 2e). They use 22 plants commonly for their needs. They use these plants for food and medicine purposes (Fig. 3a- 3p). Some of them are leafy vegetables and most of them are tubers and fruits which are directly consumed. They make porridge in the rainy season for health care using roots and leaves of medicinal plants. They cultivate some vegetable crops in their house

Table 2: Ethnomedicinal plant species, plant parts used and ailments cured by the Paniyar tribes in Mangalam Colony of Wayanad dist.

Sl. No.	Common name	Botanical name	Family	Part used	Uses
1	Thumba	*Leucas aspera* (Wild.) Link	Lamiaceae	Whole plant	Head ache, cough
2	Kandavanakuthi	*Bidens pilosa* L.	Asteraceae	Whole plant	Wound healing
3	Muyalcheviyan	*Emelia sonchifolia* (L.) DC. ex DC.	Asteraceae	Whole plant	Wound healing
4	Neerakatti	*Chenopodium album* L.	Chenopodiaceae	Whole plant	Swellings and pain
5	Kolithal	*Colocasia esculenta* (L.) Schott.	Araceae	Whole plant	Leafy vegetable
6	Mukkutti	*Biophytum reinwardtii* (Zucc.) Klotzsch	Oxalidaceae	Whole plant	Swellings and pain
7	Modungach-appu	*Solanum nigrum* L.	Solanaceae	Leaf	Asthma
8	Thottavadi	*Mimosa pudica* L.	Fabaceae	Leaf	Knee pain
9	Thulasi	*Ocimum sanctum* L.	Lamiaceae	Leaf	Head ache
10	Anathakara	*Senna alata* (L.) Roxb.	Fabaceae	Leaf	Skin diseases
11	Veepu	*Azadirachta indica* A. Juss	Meliaceae	Leaf	Pain and Measles
12	Vathakodi	*Naraveliya zeylanica* (L.) DC.	Ranunculaceae	Leaf	Arthritis
13	Vathakandal	*Blumea lanceolaria* (Roxb.) Druce	Asteraceae	Leaf	Arthritis, pain remover
14	Thakarachappu	*Crotalaria retusa* L.	Fabaceae	Leaf	Vegetable
15	Paadakizhangu	*Dioscorea belophylla* (prain) Voigt ex Haines	Dioscoreaceae	Tuber	Consumed as food
16	Chathavari	*Asparagus gonoclados* Bakel	Liliaceae	Tuber	Consumed as food
17	Kaattukizhangu	*Dioscorea oppositifolia* L.	Dioscoreaceae	Tuber	Boiled and consumed
18	Alppalpam	*Thottea siliquosa* (Lam.) Ding Hou	Aristolochiaceae	Leaf, Fruit	Stomach ache
19	Njaval	*Syzygium cumini* (L.) Skeels	Myrtaceae	Fruit	Edible
20	Karinechi	*Vitex negundo* L.	Verbenaceae	Shoot, Root	Wound healing, cough, pain reliever

Fig 3m:
***Naravelia zeylanica* (L.) DC.**

Fig 3n:
***Crotalaria retusa* L.**

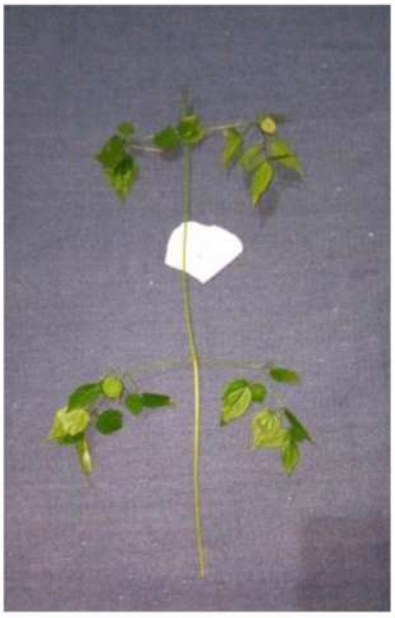

Fig 3o:
***Clematis gouriana* Roxb.**

Fig 3p:
***Bambusa vulgaris* Schard.**

premises. But,it was noticed that most of them depend on grocery shops and markets to buy vegetables and fruits. In addition, Paniya people were not engaged in forest honey collection, like their ancestors. They depend on forest only for tuber and firewood and not for any other needs. Most of the plants they currently use belong to the Angiosperm group. It was surprising to notice that Paniya tribe children of very young age were chewing tobacco.

Paniya people use the roots, leaves and stems of medicinal plants for their disease management.

Most of the medicinal plants are used for pain relief, fever, arthritis, swelling and wound healing. These plants belong to the families Asteraceae and Fabaceae. Good rainfall with other geographical and soil conditions lead to peculiar habitat and the distribution of angiosperms and other floristic elements in that area. The area consists of low temperatures (ranging from 20 to 24 during daytime and 15 to 19 during night) and high moist conditions. The flora which needs highly moist conditions and shady habitats are grown abundantly. They also domesticate cattle and dogs for their needs. Their life seems stable but not in a similar way their ancestors lived.

As per the discussions with the respondents, it was understood that the Government provides subsidies for food and clothes for people of Paniya community. The cereals, pulses and other food products are freely available for them through ration shops and other government shops. They depend on private hospitals to get treatments, rather than following their traditional medicine practices. They depend on shops more than forests. They are unemployed, there is no other way for income generation for them. They spent the day fishing from the dam and doing their daily household chores. Change in the way of living from forest life to civic life, made them rely more on public resources and supermarkets. This situation raises the question of whether this tribal community is slowly moving away from their own traditions and natural way of living. This draws urgent attention to this concern and efforts should be made to document the valuable traditional knowledge of ethnobotanically important plants and plant products, before they get completely wiped off from the knowledge of the Paniya community.

Conclusion

Ethnobotanical research is very much progressing nowadays. It leads to the emergence of new research areas and also focuses on the losing of traditional knowledge in society and in tribal communities. The lack of traditional knowledge leads to the depletion of their age- old customs, rituals and other activities. During this study, it has been noticed that they have moved to a civic life and depend on nearby towns and cities rather than forests, like their ancestors. Due to this, their knowledge on forest resources is depleting and it becomes unavailable to the scientific world. Ethnobotanical surveys are very crucial and important to protect this traditional knowledge, their ethnobotanical know-how, and information about forest and forest products.

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Vilash V, Ratheesh N, Latha S (eds.). Biodiversity Challenges and Threats; Current Scenario 2023 *ISBN 978-81-958369-2-5*

Chapter 8

SALVIA HISPANICA L. – A PROMISE TOWARDS FOOD SECURITY

Vijitha Vijayan, Latha Sadanandan

Abstract *Salvia hispanica* L. (Chia) is a biannually cultivated plant under the mint family Lamiaceae, prominently grown for its seeds. The seed contains 25% to 40% oil with 60% of it comprising ω-3 alpha-linolenic acid and 20% of ω-6 linoleic acid. Both essential fatty acids are required by the human body for good health and they cannot be artificially synthesized. Various active ingredients including essential fatty acids and phenolic compounds have been identified in chia seeds, they contribute to the health benefits. Chia seeds are often referred to as a superfood or functional food. The nutritional properties of chia seeds, such as the high content of polyunsaturated fatty acids, vegetable protein, dietary fibre, vitamins, minerals and bioactive substances resulted in numerous studies on these seeds for their therapeutic properties. Superfoods in health conscious diet contain high vitamins and minerals. It helps to keep our body always healthy and also promotes a balanced diet, weight loss, improve energy level and decreased the effect of aging. It has anti-inflammatory, anticancerous, antibacterial and antiglycemic properties.

Keywords: *Salvia hispanica L., ω-3 alpha-linolenic acid, fatty acids*

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Introduction

Salvia hispanica L. is a small annual herbaceous plant also known as chia, originally from Southern Mexico and Northern Guatemala belongs to the mint family Lamiaceae. The genus *Salvia* consists of approximately 900 species widely distributed thousands of years around several regions of the world including Southern Africa, Central America, North and South America and South-East Asia. It is mainly grown for seeds and produces white and purple flowers. The plant itself is sensitive to daylight and can grow up to 1 m tall. Leaves are reverse petiolate and serrated, and are 4 to 8 cm long and 3 to 5 cm wide. Chia seeds are generally very small, oval-shaped. The colour of the seed varies from black, grey, or black spotted to white. (Knez Hrncic *et al.*, 2018)

Systematic position of *S. hispanica* L.

Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Eudicots
Clade : Asterids
Order : Lamiales
Family : Lamiaceae
Genus : *Salvia*
Species : *S. hispanica* L.

The genetic variability of chia (*S. hispanica* L.) has been decreasing. Thus, it is important to valorize the present diversity of Mesoamerican native chia species for better conservation and use planning. The morphological variation of 22 cultivated and wild *S. hispanica* accessions were evaluated in 1999. Two plants of each chia accession were transplanted in randomized blocks design with four replications, under greenhouse conditions at Chapingo Mexico. Twenty-three morphological characters of leaf, stem, inflorescence, flower and seed were recorded. Principal components and cluster analyses indicated six groups of chia accessions which were associated considering the similarity to width and length of corolla, width of calyx, seed scatter habit, stem thickness, time of flowering and branching. The cultivated chia groups from Jalisco, Puebla and

Central America developed bigger, wider and more exposed corollas, longer, wider and compact inflorescences, indehiscent seeds and heavier seeds than wild chia group. On the other hand, the size of corolla, weight of seed and shatter habit of the cultivated group from Guerrero were similar to the wild chia group (Metcalfe and Chalk, 1972).

Chia seed is mainly used for its oil. Currently, chia seed is used as a healthy oil supplement for humans and animals. Seed from *S. hispanica* L. is a traditional food in Central and Southern America. Currently, it is widely consumed for various health benefits, especially in maintaining healthy serum lipid levels. This effect is contributed by the presence of phenolic acid and omega 3/6 oil in the chia seed (Da silva *et al.*, 2019). Although the presence of active ingredients in chia seed warrants its health benefits, the safety and efficacy of this medicinal food or natural product need to be validated by scientific research.

History

S. hispanica was used beside corn, bean, and amaranth by ancient Mesoamerican cultures Aztecs and Mayans in the preparation of folk medicines and food. In pre-Columbian societies, it was the second main crop after beans. In the Aztecs communities, chia was used for food, cosmetics, and religious rituals (Munoz *et al.*, 2013).

The benefits of chia seeds in medicinal, pharmaceutical, and food industry.

As worldwide public health awareness and the demand for functional food with health benefits has increased. chia seeds have gained popularity in recent years due to its numerous nutritional characteristics including high concentrations of extractable fatty acids, large quantities of Polyunsaturated fatty acids (ω3 and ω6), extraordinary mucilaginous fiber content, vitamins, minerals and antioxidants (Peiretti and Meineri, 2008)

Chia seeds analyzed for total phenolic compounds, antioxidant activity, and quantification of phenolic acids and isoflavones by ultra-high performance liquid chromatography (UHPLC), in order to obtain a phenolic phytochemical profile. The total phenolic concentration was higher than previous reports and the antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay showed 68.83% inhibition, which was higher than the values reported previously for chia and different plant foods. Additionally, a simple reproducible and rapid UHPLC method was proposed for the analysis of phenolic acids and isoflavones in chia. The method was demonstrated to perform well with regard to linearity, limits of detection and quantification, precision, accuracy, and sensitivity. The detection limits ranged from 0.05 to 0.4 ng/mL and the recovery percentage from 23.62 to 162.48%. With this method the major compounds rosmarinic acid 0.92, protocatechuic ethyl ester 0.74, caffeic acid 0.02, gallic acid 0.01, and daidzin 0.006 mg/g identified and quantified in seed. In brief, this study demonstrates that chia could be considered a seed with high antioxidant capacity and novel isoflavone source that can be incorporated in human diet. (Suri *et al.*, 2016).

Various active ingredients including essential fatty acids and phenolic compounds have been identified in chia seed. Chia seed is composed of protein (15–25%), fats (30– 33%), carbohydrates (26–41%), high dietary fiber (18–30%), ash (4-5%), minerals, vitamins, and dry matter (90–93%). It also contains a high amount of antioxidants (Ixtaina *et al.*,2012). Heavy metal analysis showed that chia seed contains them at safe levels, not exceeding the maximum metal levels for food safety, and the seed is also free from mycotoxins (Bresson *et al.*, 2009). Another key feature of chia seed is that it does not contain gluten (Bueno *et al.*, 2010).

Recent studies on chia seeds have focused on phytochemicals and their extractions from the seeds. Functional foods have gained tremendous attention worldwide over the past few years due to the wave of healthy lifestyle changes. One of the reasons for the interest in a healthier lifestyle is the increasing number of people suffering from cardiovascular diseases (CVDs), high blood pressure, obesity, diabetes, and other related diseases. These conditions are commonly due to an inactive lifestyle and poor diet where the food consumed daily contains high amounts of saturated fatty acids (SFAs). There are numerous studies that reported

on the correlation between high saturated fatty acids, particularly palmitic acid, and low Polyunsaturated fatty acid intakes with cardiovascular diseases (Ayerza *et al.*, 2002).

Traditionally, the so-called functional foods have been consumed based on their availabilities as daily staple foods. At present, many studies have been done to increase their functionality as high nutrient food supplements. The benefits of functional foods primarily come from the presence of active ingredients and the bioactivities of compounds originally present in the plant being still present in the food products after they have been processed to make them suitable for human consumption. Recently, chia has regained its popularity by becoming one of the main oil sources that contain high levels of cardiovascular diseases. Chia, which used to be the major food crop of the indigenous peoples of Mexico and Guatemala, is now widely cultivated and commercialized for its (omega) ω-3alpha-linolenic acid (ALA) content and antioxidant properties. Today, its cultivation is not only limited to the Americas but is also extended to other areas such as Australia and Southeast Asia (Ganzaroli, 2017).

Market Potential and Commercial Application of Chia Seed

At present, chia seeds are used as a healthy oil supplement for humans and animals. Human consumption of chia in diet is mainly from the extracted oil through its incorporation into cooking oil, confections, or supplements. In 2000, the US Dietary Guidelines recommend that chia seed can be used as a primary food not exceeding 48 g/day (Munoz *et al.*, 2013). Chia is commonly consumed as salad from chia sprout, in beverages, cereals, and salad dressing from the seed, or it is eaten raw. The European Commission approved the use of chia seed in bread products with a limit of not more than 5%. Other than bread, the food industry of various countries around the world including US, Canada, Chile, Australia, New Zealand, and Mexico has widely used chia seeds or its oil for different applications such as breakfast cereals, bars, cookie snacks, fruit juices, cake, and yoghurt. Although chia seed has been commercialized for a long time in Argentina, however, due to the comparatively small-scale production there, problems in its availability and sustainability as an edible oil source in the global market exist (Munoz *et al.*, 2013). The current planting and production of chia seed oil are yet to fully meet the world market demand

Summary

Chia oil is today one of the most valuable oils on the market. Chia seeds contain healthy ω-3 fatty acids, polyunsaturated fatty acids, dietary fiber, proteins, vitamins, and some minerals. Besides this, the seeds are an excellent source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, myricetin, quercetin, and others. Subsequently chia seeds are rich of omega-3 fatty acids, protein, fibre, vitamins, minerals, and phytochemicals, they can be used as nutritional additives in food, a nutritional supplement, and a base for beverages. Fresh chia leaves used as a salad bedding, in stir fries, and in green smoothies. Raw or dried chia leaves steeped in hot water and sweetened with honey makes a fantastic therapeutic tea, traditionally used to relieve pain, fever, and sore throats.

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Chapter 9

WATER QUALITY ASSESSMENT OF VARATTUCHIRA TEMPLE POND PERINADU, KOLLAM, KERALA

Parvathy Chandran and Ratheesh N

Abstract A study was carried out at Varattuchira temple pond, Perinad, Kollam, Kerala, to ass the water quality. The pond having 80 feet length 36 feet width and 9 feet depth. The depth of the pond varies from place to places. Water quality was studied over a period of 6 months from September 2020 to February 2021. The water samples were analyzed for eight parameters: temperature, phenolphthalein alkalinity, total alkalinity, free carbonates, pH, dissolved oxygen, hardness and BOD. The surface water temperature is closely related to the variation of atmospheric temperature and fluctuates between 26-32°C at all the sites. The temperature at all the sites showed a peak value in April. The pond water remained slightly acidic during the study period. The recorded average pH values were lower than the permissible limit. Phenolphthalein alkalinity was completely absent in the pond. While the total alkalinity was ranging from 0.5-0.9 mg/l. The highest value were recorded in the monsoon season and the lowest value were recorded in the pre-monsoon season. The observed values were within the permissible limit of 600 mg/ l. Free CO2 was present throughout the period of study at all the sites. It varied from 0.5-0.9 at site 1 from 0.6-1 at site two and from 0.5 to 0.7 from site three and from 0.5 to 0.9 from site four. The maximum hardness was observed in January and the minimum was observed in September. The maximum hardness was observed in October and the minimum was observed in December. The average value of hardness obtained from this site 0.4. The dissolved oxygen was present in the river throughout the study period.DO varied from 0.2-0.7 mg/ l with an average of 0.45 mg/ l. At all the sites dissolved oxygen was within the recommended limits of WHO and ICMR. The maximum BOD was recorded in October. BOD values at all sites were within the maximum permissible limit. As per the water quality rating scale, all the sites were belongs to 26-50 scale range. Hence having good water quality to domestic use.

Key words: *water quality, dissolved oxygen, hardness, BOD*

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Introduction

A pond is a small area of still, fresh water. It is different from a river or a stream because it does not have moving water and it differs from a lake because it has a small area and is no more than around 4.8m deep. A pond is a fascinating habitat to study, a good one teeming with a great variety of both animal and plant life. The community or all the species of animals and plants present in one pond may be quite different from that in another, even if the ponds are close together. This is because most pond animals cannot travel from one pond to another. Also the water temperature, oxygen content, water cleanliness and the material of the pond bottom have an influence on the kind of life present. In any pond it is essential that there is a balance of different kinds of organisms so that there is enough food for them all to live and reproduce.

Pond is a body of shallow water with aquatic plants and animals. Biota in the ecosystem depends on physical and chemical parameters such as water level, nutrient levels, etc. Regular monitoring of physico – chemical and biological parameters would aid in assessing the status of the water body. Favorable water quality maintains the primary production with appropriate levels of secondary and tertiary production, which helps in maintaining

the balance. Parameters such as temperature, pH, light penetration, total dissolved solid etc. affect the water quality and these factors are influenced by the anthropogenic as well as natural dynamics such as geological, hydrological, etc. (Dhanam *et al.*, 2016).

The planktons are the microscopic organisms which wander at the mercy of winds, currents and tides. They are the base of the aquatic ecosystem. Due to short life cycle, they respond quickly to environmental changes hence, they are used as pollution indicators. Distribution of plankton is closely related to water quality parameters (Baruah and Kakati 2012). Planktons are divided into two categories- phytoplankton and zooplankton. Phytoplanktons are the chlorophyll bearing microorganisms consisting of algae with representatives from all major taxonomic phyla. They have the unique ability to convert inorganic material to organic hence all other aquatic organisms are depending for food on phytoplankton

The main aim of the current investigation is to draw attention to the large proportion of diversity that exists in the fresh water environment it's importance, and the need for its conservation. In this aspect the present study was undertaken in a fresh water pond on their immunological aspects and water quality with the following aims and objectives. The present study aimed to estimate the physio- chemical parameters of Varattuchira Temple pond water and to evaluate the water quality index of Varattuchira Temple pond.

Materials and Methods

Study area

The present study was carried out in the Varattuchira Temple Pond. The Varattuchira Bhagavathy Temple is a Hindu Temple to the Goddess Durga Bhagavathy or Aadi Shakthi, located in the Village of Perinadu, Kollam District, Kerala, India. The Varattuchira Temple Pond situated nearby the temple, owned by the Perinad Grama Panchayath. In earlier times, the pond exist as a small water body surrounded by marshy area's covered with grass. During days of rainy season a stream originated and making the surroundings areas fertile and cultivable. As this place was full of grass and pure water, the cow -herders from the neighboring area would congregate there with their cattle.

Varattuchira Temple Pond with a length of 80 feet and a width of 36 feet and 9 feet depth. The depth of the pond is different in different regions. It was the major water source of many families living around the pond. Right now the water body was contaminated by the waster deposition (fig 1). The pond was constructed in the shape of a rectangle and it is separated into two parts, the middle portion is for the Varattuchira Temple for conducting the traditional rituals. The rest of the portions of the pond is now utilized for cultivation of fishes.

The study has been carried out for a period of six months from September 2020 to February, 2021.

Fig 1. Varattuchira temple pond

Water samples were collected from the pond at regular intervals. The water samples were collected and brought to the laboratory in sterile plastic bottles. These samples were kept in room temperature until the analysis was completed. Algal samples were preserved in 4%

formalin. Both qualitative and quantitative analysis of algae were made from fresh as well as preserved samples. The physical and chemical parameters like body parameters such as surface water temperature, pH, phenolphthalein alkalinity, total alkalinity, free CO2, dissolved oxygen ,BOD and total hardness were analysed following standard methods (ALPHA,2005).

Methods

Surface water temperature

The temperature of surface water was recorded every time of visit with the help of mercuric

thermometer.

2.Hydrogen ion concentration (pH)

Water pH was measured by a Systronic digital pH meter

Phenolphthalein Alkalinity

Phenolphthalein Alkalinity was estimated by titrating 100 ml of sample with a strong acid HCL(0.1) in presence of phenolphthalein indicator. There was no color change occurred indicated the absence of phenolphthalein Alkalinity.

Total Alkalinity

To the above sample 2-3 drops of methyl orange was added and continued the titration.At the end point the yellow colour was changed to pink.T hus alkalinity was measured.

Free CO2

Free CO2 was estimated by titrating the sample using a strong alkali(such as carbonate free NaOH to PH 8.3.At this PH all the free CO2 were converted into bicarbonates.For this took

100 ml of sample in conical flask and added a few drops of phenolphthalein indicators and coloured turned pink , indicated absence of free CO2.

Dissolved oxygen

Dissolved oxygen content was estimated following Wrinkler's method. For this, water samples were collected in a glass stopper 300 ml capacity BOD bottle. To this 3 ml each of Manganous Sulphate and potassium iodide solutions were added using separate pipettes. A precipitate was formed in the sample. Then 4-6 ml of concentrated H2SO4 was added through the sides of the bottle for the settlement of the precipitate. 100 ml of the reaction samples was poured out into a conical flask and it was titrated against the pre-standardized sodium thio- sulphate (0.025) using starch as indicators. As titration progressed, the dark blue colour gradually vanished. At the end point it became colourless.Once the end point was reached calculations were done to get the results.

Biological Oxygen Demand (BOD)

It is the measure of degradable organic material present in the water samples, and can be defined as the amount of the oxygen required by the microorganisms to decompose biologically active and unstable organic matter under aerobic method, it is specified in mg/ l. This test is conducted after 25 hrs. BOD was measured using Wrinkler's method as described in APHA(1998). The principles of the method involves the difference of O2 concentration between the sample and after incubating it for five days at 20 degree Celsius.

Total Hardness

Total hardness was estimated by EDTA method. For estimation 50 ml samples was taken in conical flask and 1 ml of buffer solution was added. Then 100-200 mg Eriochrome black T

was used as an indicator, the solution turned wine red and titrated against 0.01M EDTA solutions. At the end point colour changed from wine red to blue.

Water quality analysis

Water quality index and water quality status were calculated using the expression

WQI= Σ Sli

Sli =Wi*Qi

Where ,

Sli is the sub index of ith parameter

Qi is the rating based on concentration of ith parameter n is the number of parameters

Where,

Wi = wi /∑ wi (here i=n)

Wi is the relative weight

wi is the weight of each parameter n is the number of parameters.

Qi = (Ci /Si)*100

Where,

Qi is the quality rating

Ci is the concentration of each chemical parameter in each sample in milligrams per litre

Si is the world health organization standard for each chemical parameters in milligram per liter according to the guidelines

In addition to the above, ANOVA and correlation analysis were carried out using Microsoft Excel.

Result and Discussion

Physico-chemical parameters body of Varattuchira Temple Pond at four sites were analysed for a period of 6 months from September 2020 to February 2021. The results are as following;

Surface temperature

The surface water temperature did not show much difference among the study sites .The surface water temperature varied from 26-32°c with an average value of 28.7° C. The maximum temperature was recorded in September and minimum in November 2020.

Water pH

Monthly variations in pH values at the 3 sites were shown in figure 3. It varied from 5.99-

6.89 at site one and from 6.71-7.6 at site two and from 6.65-7.4 at site three and from 6.56-

7.2 at site four. The minimum values were obtained in December 2020 at all the sites. The average pH values were 6.44, 7.15, 7.02, and 6.88 respectively from the four sites studied (Fig 2).

Fig. 2. Average water pH from four study sites

Free CO2

Free CO2 varies at different sites were shown in the fig 3.It varied from 0.5-0.9 at site one and from 0.6-1 at site two and from 0.5 to 0.7 from site three and from 0.5 to 0.9 from site four.The average free carbonates values were 0.7, 0.8, 0.6 and 0.7 at sites one ,two ,three and four respectively (Fig 3).

Phenolphthalein Alkalinity (PA)

PA was completely absent in the pond water

Total Alkalinity

Total alkalinity fluctuated from 0.5-0.9 mg/l at site one, from 0.5-1 mg/l at site two, and from 0.3-0.7 mg/l at site three and from 0.5-0.9 mg/l at site four. The average values noted were 0.7, 0.75, 0.5, and 0.7 at site one, two, three and four respectively. The highest value were recorded in November and lowest value were recorded in December 2020 at all the sites (Fig 4).

Hardness

Hardness of river water varied from 0.1-0.2 from site one .The maximum hardness was observed in January and the minimum was observed in September.The average value obtained from this site was 0.15. At site second hardness varied from 0.2-0.6.The maximum hardness was observed in October and the minimum was observed in December. The average value of hardness obtained from this site 0.4. At site three hardness varied from 0.2-0.4.The maximum value of hardness was observed in November and minimum was observed in January. The average value of hardness obtained from this site was 0.3. At site four hardness varied from 0.1-0.3.The maximum hardness was observed in February and minimum was observed in October. The average value of hardness obtained from this site was 0.2 (Fig 5)

Biological Oxygen Demand (BOD)

Monthly variations in BOD was observed. BOD values varied from 2.5-3.1 mg/l at site one. The highest BOD was observed in October and lowest was in December. The mean BOD at this site was 2.8mg/ l. At site two BOD values varied from 2.5-3.6 mg/l. maximum value were recorded in November and lowest was in February. The mean value at this site was 3.05 mg/ l. At site three BOD varied from 2.5-3.1 mg/ l. The highest value was noted in January and lowest was in September. The mean BOD from this site was 2.8 mg/ l. (Fig 6)

Dissolved oxygen (DO)

Fluctuations in DO content in the pond water were as follows. Dissolved O2 level showed only a slight difference during the sampling period among the sites. DO concentration varied from 0.2-0.6

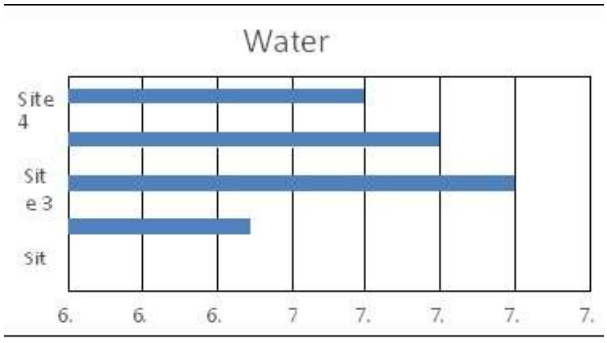

Fig. 2. Average water pH from four study sites

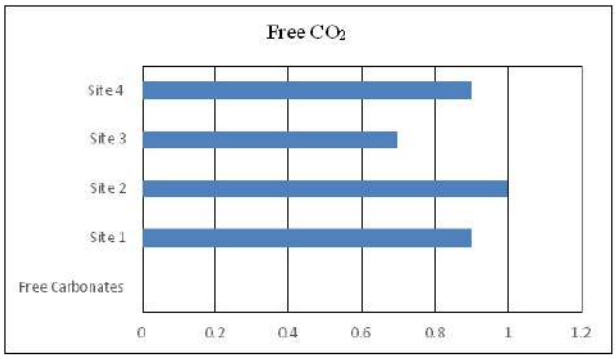

Fig. 3. Average Free CO_2 resulted from the study

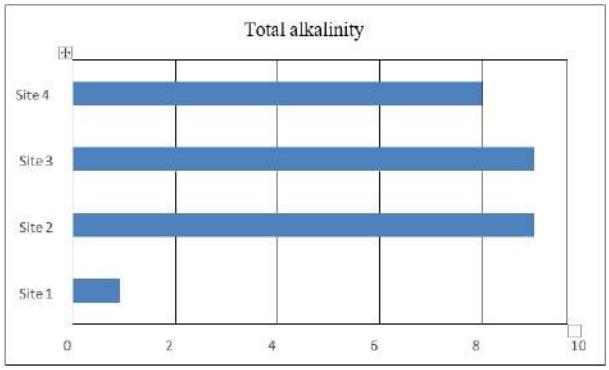

Fig. 4. Average total alkalinity obtained from four sites.

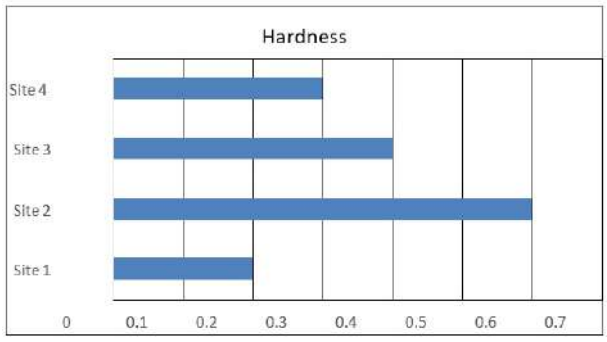

Fig.5. Average hardness resulted from four sites

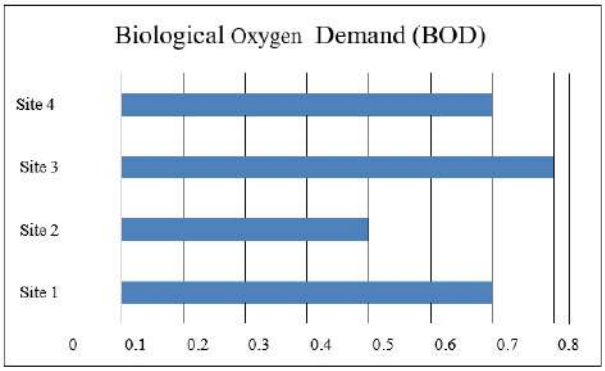

Fig. 6. Average BOD resulted from four sites

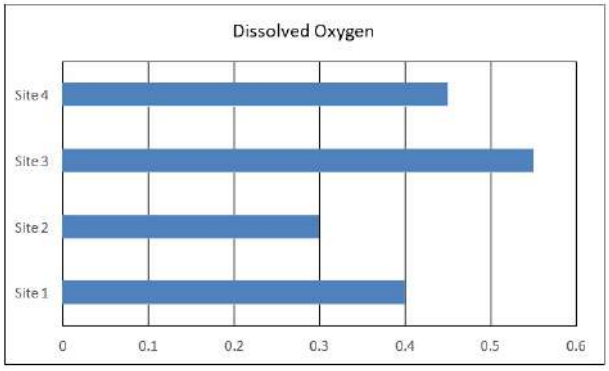

Fig. 7. Dissolved oxygen content

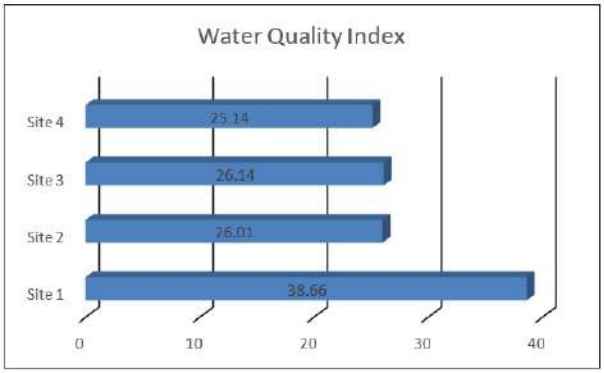

Fig. 8. Water quality index of the four study sites in the Pond

Table. 1. Water quality index and status of water quality

WQI	Water quality status
0-25	Excellent water quality
26-50	Good water quality
51-75	Poor water quality
75-100	Very poor water quality
>100	Unfit for drinking

mg/ l at one site one. The highest DO was recorded in October. Average value at this site was 0.4 .DO at the site second varied from 0.2-0.4 mg/ l. The highest concentration was recorded in December and the lowest concentration was observed in November. The mean DO value at this site was 0.55 mg/l. DO at site three varied from 0.4-0.7 mg/l. The highest concentration was at September and lowest concentration was at January. Mean DO concentration at this site was 0.55 (Fig 7).

Water Quality Index Analysis

The quality rating scale for each parameter was calculated by dividing it's concentration in each water samples by its respective standards and multiplied the results by 100. The standard value are given the Table 1.

Water quality index is measure of overall quality of water which has been statistically arrived at from a number of parameters and is in use more to describe the quality of fresh water systems (Mohanta and Patra, 2000). In the present study WQI of four sites were determined separately. For the calculation of WQI selection of parameters has great importance. Since selection of too many

parameters might widen the WQI and importance of various parameters depends on the intended use of water, 5 physico chemical parameters viz. pH, TA, BOD, Hardnes, and DO were used to calculate WQI. The calculated WQI value for four study sites were shown in the figure 8. Fig. 8. Water quality index of the four study sites in the Pond

As per the water quality rating scale, all the sites were belonging to 26-50 scale range. Hence having good water quality. For the present study four sites of Varattuchira temple pond was selected. The main sources of pollution include agricultural wastes, domestic wastes, municipal wastes, slaughtery wastes and detergents used for washing of clothes and bathing etc. Specific study was carried out on water quality at the four sites in relation to Algal community showed a marked seasonal fluctuations which is connected with the seasonal changes in the climatic conditions, Biological and other activities in the waters. Temperature is one of the important factors which regulates the biochemical activities in the aquatic ecosystems. The surface water temperature and the atmospheric temperature followed a linear pattern throughout the annual cycle as reported by earlier workers (Anitha, *et al*; 2005). The surface water temperature of all the four sites varied from 26 to 32°C with an average of 28.68°C. The maximum surface water temperature was recorded in pre- monsoon season and the minimum

values were observed in the months of rainy period. A similar observation has been made by earlier workers (Anitha, *et al*; 2005). During pre-monsoon water temperature was higher because of low water level, clear atmosphere and greater solar radiation. The minimum water temperature in the rainy season may be due to frequent clouds, high relative humidity, and high Water levels.

Conclusions

The water quality index of the Varattuchira Temple Pond ranged 26-50 of the standard scale. Hence, the water in the pond is of good quality for domestic purposes. This study can offer the requisite information for the authority to protect and conserve the small water bodies in the Kollam District. The water quality index was a very efficient and useful tool to summarize available data to the decision makers in order to fully understand the type of water quality and to have a chance for better use in the future as well.

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Chapter 10

PROTECTION OF TRADITIONAL KNOWLEDGE, BIORESOURCES AND FOLKLORE UNDER THE TRIPS AGREEMENT: RECENT DEVELOPMENTS IN LIGHT OF NAGOYA PROTOCOL

Ijinu T P, Vilash V, Vimal Kumar C S, Navas M, Rajasekharan S, George V, Pushpangadan P

Abstract Biodiversity, encompassing the variety and variability of all life on earth, is the product of over 3.5 billion years of evolutionary history. Growing concern over the monopolisation of benefits led countries that provided genetic resources to restrict access to genetic resources and associated traditional knowledge. The ratification of the Convention on Biological Diversity (CBD) in 1993 by the members of the UN brought forward the agenda of Access and Benefit Sharing (ABS) from the use of genetic resources. CBD (1993), WTO-TRIPS (1995), ITPGRFA (2001), the Bonn Guidelines (2002), and the Nagoya Protocol (2014) provide a broad framework for ABS procedures. The main features of ABS mechanisms include Prior Informed Consent (Article 15.5), Mutually Agreed Terms (Article 15.4), Material Transfer Agreements (Article 16.3) and Benefit-Sharing Agreements (Article 15.7) through monetary and non-monetary means. The entry into force of the Nagoya Protocol is the most recent iteration of international legal instruments that affirm sustainable development principles. With its substantive focus inclusive of TK and indigenous communities, the Protocol exemplifies a trend towards utilising legal measures to clarify rights and drive sustainable development through the use of market mechanisms such as contracts and IPRs and mutually supportive institutions at the national, regional, and community level. Conversely, the present challenge is how the Parties implement the provisions of the Protocol so that legal, regulatory, and administrative measures contribute to and fulfil the CBD objectives while facilitating the emergence and scale-up of BioTrade. This article provides a glance through historical aspects, recent developments in the ABS regime, and associated traditional knowledge.

Keywords: *Biodiversity, Traditional Knowledge, Intellectual Property Rights, Access and Benefit Sharing, Nagoya Protocol, Sustainable Development*

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Introduction

The history of human civilization and the development of economic systems are all inherently and inveterately interwoven with our biological resources (Ravi and Pushpangadan, 1998; Pushpangadan and Ijinu, 2017). The economic activity of humankind continues to derive its sustenance directly or indirectly from the biological resources. The unknown potentials of genetic diversity found in the biological organisms,

particularly plants represent a never-ending biological frontier of inestimable value. Genetic diversity will enable breeders to tailor crops to meet the increased productivity, adapt changing climatic conditions, disease resistance and to meet the other essential needs and future aspirations of humankind. Thus, biodiversity is the biological capital of our planet and it forms the foundation upon which human civilization is built (Ijinu *et al.*, 2023).

Throughout history, biodiversity has been the commons of local communities, with both resources and knowledge being freely exchanged. The concept of sovereign rights or property rights in genetic resources was almost alien to the traditional communities. Less than a year after CBD came into force, the World Trade Organization (WTO) in 1994 was established with a different agenda. The convention is founded on the principle that local communities are dependent on biodiversity and should continue to benefit from it. The WTO administers a global trading system, much of which is founded on the private monopoly rights of transnational corporations over biodiversity. Thus, we observe a paradigm shift in the world view on biodiversity and its utilization in the 21st century.

Biodiversity and Traditional Knowledge

Biodiversity is the variety and variability of living organisms on the planet and it forms the bedrock for sustainable economic development. The total annual global markets for products derived from genetic resources are estimated to be worth between US$500 billion and US$800 billion (Kate and Laird, 2021). Most of the countries located in these regions are, interestingly the TWCs are blessed with almost all known types of topographic and climatic conditions ranging from tropical to temperate and alpine zones (Pushpangadan and Nair, 2005). India is rated as one of 17 megadiverse countries of the world, with about 7-8% of the total species of plants, animals, fungi, and microorganisms. The floristic spectrum of India comprises approximately 45,500 species, of which over 5285 species are endemic (NBA 2018). The rich biodiversity of India is matched with equally rich cultural diversity and a unique wealth of traditional knowledge system, developed, preserved, and practiced by millions of ethnic and indigenous people living in the rural and forest areas (Anonymous, 1998; Pushpangadan, 1998). Local and Indigenous people used about 9,500 plant species for Indigenous medicinal preparations and more than 3,900 plant species as food, fibre, fodder, insecticides, pesticides, gum, resins, dyes, perfumes, and timber (Anonymous, 1998; Pushpangadan and Pradeep, 2008; Pushpangadan *et al.*, 2017, 2018; NBA, 2018). Many of the plants are still unknown around the world, and the majority has never been studied for their therapeutic use. There are an estimated 500,000 known plant species worldwide (Corlett, 2016), with a total number of angiosperm species at around 450,000, of which 10-20% are still unknown (Joppa *et al.* 2011; Pimm and Joppa 2015). Out of this, only about 1% of plants have been extensively studied in the laboratory (Newman and Cragg, 2007; Newman *et al.*, 2008).

Traditional knowledge (TK) is a community-based system of knowledge that has been developed, preserved, and maintained over many generations by the local and indigenous communities through their continuous interactions, observations, and experimentations with their surrounding environment. It is unique to a given culture or society and is developed as a result of the co-evolution and coexistence of both the indigenous cultures and their traditional practices of resource use and ecosystem management. TK is a general term, which refers to the collective knowledge, beliefs, and practices of indigenous/ local people on sustainable use and management of their ambient resources. Through years of observations and analysis, trial, error or experimentations, the traditional communities have been able to identify useful as well as harmful elements of their ambient flora and fauna. Such knowledge (acquired through ages) has always remained as part of their life, culture, traditions, beliefs, folklores, arts, music, dance, etc. TK covers a broad spectrum of the local and indigenous people's traditional life and culture, art, music, architecture, agriculture, medicine, engineering, and

a host of other spheres of human activity. TK thus can be of direct or indirect benefit to society as it is often developed, in part as an intellectual response to the necessities of their life. Protection and maintenance of TK of local and indigenous communities is vital for their wellbeing and sustainable development and for their intellectual and cultural vitality (Anonymous, 2004; Pushpangadan and Nair, 2005).

A study of TK system of traditional societies about the plant world is the subject matter of the science 'ethnobotany.' Modern drug hunters consider ethnobotany as a cost-effective means of locating new and useful compounds of great pharmaceutical value. It is well accepted that the possibility of finding a potential bioactive compound through random screening of plant samples is 1 in 10,000 and that of hitting a marketable drug is 1 in 4. In contrast, the success rate of finding a bioactive molecule through selective screening based on ethnobotanical leads is 1 in 100 and that of the discovery of a drug is 1 in 2. Many plants derived drugs employed in modern medicine were first 'discovered' through ethnobotanical investigation. The traditional societies in India as well as in other Third World Countries have always considered the natural resources and the associated TK system developed by them as commonly owned properties to be cared and shared by all and never to be commodified for the purpose of selling or marketing. It was with the coming of the westerners that the process of commodification and trading of bio-resource and associated knowledge started.

TK is experiencing increased attention worldwide in light of global healthcare demand and the significant role of traditional medicine in meeting the public health needs of developing countries. Traditional medicines already comprise a multi-billion-dollar, international industry, and the biomedical sector is increasingly investigating the potential of genetic resources and traditional knowledge. Documenting and protecting these medicines are becoming a greater priority. Many problems associated with the protection of traditional medical knowledge lack clear solutions. In attempting to protect traditional medicine, traditional knowledge holders are confronted by a confusing and diverse group of national and international policies, regulatory systems designed primarily to accommodate pharmaceutical medicines, safety and efficacy concerns, and challenges to ownership (Abbot, 2014).

The WTO-TRIPS and Protection of TK

The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) sets down the minimum standards for many forms of intellectual property (IP) regulation. It contains requirements that nations' laws must meet for intellectual property and it specifies the enforcement procedures, remedies, and dispute resolution procedures (Jiang, 2008). Article 27 of TRIPS deals with the patentable subject matter and Section 1 states that patents have to be available for any inventions, whether products or processes, in all fields of technology, provided that said inventions are new, inventive and capable of industrial application. Moreover, Section 3 defines those plants and animals other than micro-organism, and essential biological processes for the production of plants or animals other than non-biological and microbiological processes may be excluded from patentability, but a *sui generis* system should be envisaged in order to protect plant varieties. In this context, a *sui generis* system consists of a set of nationally recognised laws and ways of extending plant variety protection other than through patents (Stephen and Justin, 2003; Armour and Harrison, 2007). The use of TK for the purification or characterization of active drugs and/or the development or the modification of a molecule, i.e., the inventions TK derived, lays in article 27 of TRIPS. Consequently, WTO signing countries will have to allow the patent protection of such a kind of inventions (Ruiz, 2002).

Nagoya Protocol on ABS and its Objectives

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) to the Convention on Biological Diversity is a supplementary agreement to the Convention on Biological Diversity. It provides a transparent legal

framework for the effective implementation of one of the three objectives of the CBD: the fair and equitable sharing of benefits arising out of the utilization of genetic resources. The Access and Benefit-sharing Clearing-House (ABS Clearing-House) is a platform for exchanging information on access and benefit-sharing established by Article 14 of the Protocol, as part of the Clearing-House of the Convention established under Article 18, paragraph 3 of the Convention. The ABS Clearing-House is a key tool for facilitating the implementation of the Nagoya Protocol, by enhancing legal certainty and transparency on procedures for access and benefit-sharing, and for monitoring the utilization of genetic resources along the value chain, including through the internationally recognized certificate of compliance. By hosting relevant information regarding ABS, the ABS Clearing-House will offer opportunities for connecting users and providers of genetic resources and associated traditional knowledge.

The objective of the Nagoya Protocol is the fair and equitable sharing of benefits arising from the utilization of genetic resources, with a view to contributing to the conservation of biodiversity and the sustainable use of its components. Benefit-sharing is envisaged through appropriate access to genetic resources, the transfer of relevant technologies, and funding. Benefit-sharing obligations also arise from the use of traditional knowledge associated with such genetic resources and genetic resources held by indigenous and local communities. In this regard, the Nagoya Protocol is particularly innovative: it is the first time that such obligations are triggered by the use of traditional knowledge for research and development purposes in an international legally binding instrument. The Protocol is also innovative in detailing measures to ensure compliance with ABS-related obligations – an aspect that was neglected under the CBD (Tsioumani, 2015).

Background of Nagoya Protocol

On November 1988 the United Nations Environment Program (UNEP) created a special Ad Hoc work-group of biological diversity experts to address the issue of continuous damage to ecosystems and species caused by human actions. By February 1991, the Ad Hoc Working Group had become known as the Intergovernmental Negotiating Committee. Its work culminated on 22nd May 1992 with the Nairobi Conference for the Adoption of the Agreed Text of the Convention on Biological Diversity. The Convention was opened for signature on 5th June 1992 at the United Nations Conference on Environment and Development (the Rio 'Earth Summit'). It remained open for signature until 4th June 1993, by which time it had received 168 signatures. To date, it has been ratified by 196 Parties. The main declared aims of the CBD were:

1. Conservation of biological diversity;
2. Sustainable use of components of biological diversity; and
3. Fair sharing of the benefits arising out of the utilization of genetic resources.

The CBD itself established only a general obligation on access to genetic resources and the sharing of the benefits arising from their utilization. The respective provisions, namely Articles 15 (Access to Genetic Resources) and 8(j) (Traditional Knowledge) left significant ambiguity as to the implementation of that general obligation. Seventeen years after the CBD entered into force, few effective and efficient ABS measures or regimes are in place. Only a limited number of Parties, mostly provider countries, have adopted comprehensive ABS legislation, mostly focusing on access. Because of this ambiguity at the international level, the conditions for access in some provider countries have become very restrictive. Based on the recognition of the importance of genetic resources for achieving food security worldwide, sustainable development of agriculture in the context of poverty alleviation, and climate change and the acknowledgment of the interdependence of all countries with regard to genetic resources for food and agriculture, a first specialised ABS international agreement, called the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), was developed in harmony with CBD and concluded in 2001 under the auspices of the FAO (Food and Agriculture

Table 1. CBD Provisions for ABS and protection of TK (Pushpangadan *et al*., 2017).

Article 8 (j)	Protection of Traditional Knowledge	Promote the wider application of the knowledge, innovations, and practices of indigenous and local communities with their approval and involvement and encourage the equitable sharing of the benefit arising out of sustainable use of the knowledge, innovations, and practices of indigenous and local communities.
Article 15.1	Authority to determine access	Party has the authority to determine access to genetic resources, subject to national legislation.
Article 15.2	Access for environment-friendly uses	Each Party shall endeavor to facilitate access to genetic resources for environmentally sound uses by other contracting Parties and not imposes restrictions that run counter to the objectives of the Convention.
Article 15.4	Mutually Agreed Terms	Access to genetic resources shall be on mutually agreed terms.
Article 15.4	Mutually Agreed Terms	Access to genetic resources shall be on mutually agreed terms.
Article 15.5	Prior Informed Consent	Access shall be subject to Prior Informed Consent of the Contracting Party providing such resources, unless otherwise determined by that Party.
Article 15.6	Scientific research and development	Party receiving genetic resources from another Party shall endeavor to develop and carry out scientific research based in genetic resources provided by other Contracting Parties with full participation of, and where possible in, such Contracting Parties.
Article 15.7	Equitable Benefit Sharing	Parties to take legislative, administrative or policy measures, as appropriate, with the aim of sharing in a fair and equitable way the results of research and developments and the benefits arising from the commercial or other utilization of genetic resources with the Contracting Party providing such resources, and such sharing shall be upon mutually agreed terms.
Article 16.3	Transfer of Technology	Access to and transfer of technology using genetic resources to countries providing the genetic resources.
Article 19.1	Participation of genetic resource providing countries in biotechnological research	The Contracting Party receiving a genetic resource shall take legislative, administrative or policy measures as appropriate, to promise effective participation by providers of genetic resources, especially developing countries in biotechnological research activities.

Organisation); it entered into force in 2004.

The Bonn Guidelines, adopted by the 6th Session of the Conference of the Parties (COP 6) in 2002, were intended to guide both users and providers of genetic resources in the implementation of the access and benefit-sharing provisions of the CBD, for instance by offering guidance regarding the procedures that can be established in a provider country on granting access to genetic resources. They also provide an indicative list of mutually agreed terms (MAT), and possible monetary and non-monetary benefits. Although comprehensive regarding the need for compliance with the provisions of the CBD, these voluntary guidelines were not considered very effective with regard to concrete measures by providers or users.

Many countries from the south felt that while the Bonn Guidelines elaborated on access, they had left the benefit-sharing aspect relatively unspecific. The voluntary nature of the Guidelines has been judged as insufficient for implementing the ABS provisions of the CBD. The World Summit on Sustainable Development (WSSD) (Johannesburg, September 2002) called for the negotiation of an international regime, within the framework of the CBD, to promote and safeguard the fair and equitable sharing of benefits arising from the utilization of genetic resources. In 2004, the CBD's seventh meeting of the Conference of the Parties responded by mandating an 'Open ended Ad Hoc Working Group on ABS' (OEWG ABS) to elaborate and negotiate, in consultation with the Working Group on Article 8(j), an international regime on access to genetic resources and benefit-sharing. The objective of the new regime was to effectively implement Articles 15 and 8(j) of the

CBD (Decision VII/19).

After six years of CBD negotiations, a legally binding instrument dedicated to ABS was agreed in Nagoya, Japan on 29 October 2010. On that date, the 'Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention of Biological Diversity' (hereafter referred to as the Protocol) was formally adopted by the 10th Conference of the Parties (COP 10) to the CBD. Agreement on the ABS Protocol was a sine qua non for reaching an overall agreement at Nagoya and the 20 targets under the CBD Strategic Plan 2011-2020. The European Union and its Member States had been very active in the negotiations and are regarded as having been instrumental in bringing about consensus among the 192 Parties to the CBD. The Nagoya protocol entered into force on 12 October 2014. To date it has been ratified by 93 parties, which includes 92 UN member states and the European Union.

Alongside the Nagoya protocol, the WIPO has its Intergovernmental Committee (IGC) on Intellectual Property and Genetic Resources, Traditional Knowledge, and Folklore, which has sought also to develop legal instruments for the protection and promotion of TK. Moreover, in the WTO, some countries have sought to include checks and balances in the disclosures made by patent applicants, where they may have used genetic or biological resources and associated TK in their innovations. These negotiations in the WIPO-IGC and WTO are still ongoing. The WIPO voluntary fund has been established and utilised to support the attendance of representatives from Indigenous Peoples and Local Communities in the WIPO-IGC negotiations, and least developed countries are member states of WIPO and can attend and contribute to the negotiations shaping TK policy globally (Robinson and Raven 2019).

Salient Features of the Nagoya Protocol

1. Access to genetic resources shall be subject to the PIC of the Member countries providing such resources that is the country of origin of such resources or a member country that has acquired the genetic resources. This will be proceeded according to the domestic access and benefit-sharing legislation or regulatory requirements of the Member country.
2. TK associated with genetic resources held by indigenous and local communities shall be accessed with the PIC or approval and involvement of these indigenous and local communities. This will be in accordance with the domestic law.
3. PIC is not mandatory to regulate access to genetic resources. It is up to the Member countries, whether or not, to provide for PIC procedure via domestic legislation.
4. If a country decides to regulate access to genetic resources subject to PIC, it has to enact a domestic law. It must also provide a mechanism for PIC or approval system with the help of a Competent National Authority. The mechanism must have legal certainty, clarity and transparency and should possess fair and non-arbitrary rules and procedures for accessing genetic resources.
5. Access to genetic resources and TK shall be based on MAT, in addition to PIC or approval and involvement of the indigenous and local communities who hold such knowledge.

Conclusion and Future Prospects

Sustainable development needs to be established on mutually beneficial pillars of mutual respect and legal clarity, with the active participation of indigenous and local communities, and must be aimed at protective, pragmatic, and proactive measures designed to address poverty and inequality. The issues of community rights/TK, ABS transfers, and intellectual property rights protection related to genetic resources and traditional knowledge are the most contentious ones that keep the developed and developing countries divided in their attitude and approaches. The intricate imbalance in the core objectives and directives of CBD and TRIPS is a major concern for the Parties or members of these international laws. Among all the issues being debated between CBD Secretariat and TRIPS Council, the question of providing legal protection to genetic resources and associated traditional knowledge continues to

crop up at the inter-sessional meetings of CBD, TRIPS and WIPO. It is a matter of grave concern that certain countries with advanced technologies are still reluctant to become a Party to CBD, but continue to oppose the plea of the developing or the least developed countries for evolving an enabling and equitable legal mechanism for implementing the international trade and intellectual property laws in relation to biodiversity, genetic resources, and traditional knowledge systems.

The new thinking centered on the concept of 'Knowledge Engineering' for building up future 'Knowledge Assistance' and 'Knowledge Industries' is now gaining attention and acceptance both nationally and internationally. Knowledge based development of value-added products from genetic resources and its commercialization has become one of the fastest developing economic activities in the world. The United Nations 2030 agenda for Sustainable Development with 17 Sustainable Development Goals (SDGs) and 169 associated targets seeks to facilitate a cohesive international response to address the 2.2 billion global citizens facing 'multi-dimensional poverty', the monumental threat of destabilisation to agricultural and economic markets posed by climate change, and mainstream ecosystem integrity and conservation of natural capital. Among the 230 indicators of SDGs, two indicators directly refer to Indigenous peoples (2.3.2 and 4.5.1) and several other indicators that are relevant for Indigenous peoples, particularly 1.4.2 and 5.a.1 on land rights. Among the 169 associated targets of SDGs, 73 have substantial links to the UNDRIP. Through recognition of Indigenous and Local Communities as holders of IPRs in TK relating to biodiversity and natural capital, the development of complementary legal infrastructure to support equitable benefit-sharing, facilitating community governance and self-determination, and protecting against misappropriation, TK as a technology of biodiversity conservation and innovation can be preserved for future generations and respectfully shared based on mutually equitable terms in support of sustainable development. The entry into force of the Nagoya protocol represents a step in this direction. The new instrument, however, cannot reach these goals alone and so much will rely on functional implementation for moving forward.

Acknowledgements

The authors ITP, VG and PP express their sincere thanks to Dr. Ashok K. Chauhan, Founder President, Ritnand Balved Education Foundation (RBEF) and Amity Group of Institutions, and Dr. Atul Chauhan, Chancellor, Amity University Uttar Pradesh (AUUP) for facilitating this work.

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Chapter 11

ETHNOMEDICINAL IMPORTANCE OF *RHINACANTHUS NASUTUS* (L.) KUNTZE, A PLANT USED BY TRIBAL COMMUNITIES: EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL.

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Abstract Ethnic communities played a vital role in the conservation of biodiversity as they posses rich traditional knowledge related to medicinal plants. *Rhinacanthus nasutus* (L.) Kuntze (Acanthaceae) (Snake Jasmine), is a medicinal plant, traditionally used to treat a wide range of disorders including hepatic disorders, ringworm, abscess pain, pruritic rash, skin diseases, inflammatory disorders, etc. The root *R. nasutus* is used in Thai traditional medicine as an antidote against snake venom. The present investigation sought to explore the comparative *in vitro* antioxidant potential and *in vitro* anti-inflammatory activity of ethanolic, hydroethanolic, and aqueous root extracts of *R. nasutus*. *In vitro* antioxidant activity of ethanolic, hydroethanolic, and aqueous extracts of these plants were determined by nitric oxide radical scavenging assay, and ferric reducing antioxidant potential. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity at different concentrations with Aspirin, and Diclofenac sodium as standard drugs. The hydroethanolic extract had shown very significant activity when compared to other extracts. In nitric oxide radical scavenging assay IC50 value of alcoholic, hydroalcoholic, and aqueous root extracts was found to be 238, 117.48, 440.74 µg/mL respectively. The total ferric reducing antioxidant potential of ethanolic, hydroethanolic, and aqueous extracts were 30.536, 46.829, and 27.317µmol trolox equivalent/g of extract. In anti-inflammatory study, the hydroalcoholic extract of *R. nasutus* (RNHE) at a concentration range of 100-800µg/mL was tested with the standard. It is evident that, the hydroalcoholic extract at the concentration 400 to 800 µg/ml have significant activity for Inhibition of albumin denaturation and antiproteinase action, but concentration of 100 and 200 µg/ml did not show significant activity. Heat induced haemolysis of erythrocyte was significantly inhibited at the concentration of 600 to 800µg/ml. Hypotonicity induced was significantly inhibited at the concentration range of 500,600,700 and800µg/ml respectively. According to the current study's findings, the ethnomedicinal plant *R. nasutus* has significant antioxidant and anti-inflammatory potential, which indicates an urgent need forits conservation and sustainable utilization.

Key words: *Rhinacanthus nasutus, biodiversity, ethnic communities, antioxidant, anti-inflammatory.*

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Introduction

Traditional medicine and biodiversity are intimately connected, and for it to be as successful as it can be, our lands must be in much better condition. From olden times onwards, in order to protect themselves from the disease, people began to search for drugs in nature. As in the case of animals, the use of medicinal plants was also instinctive at the beginning (Stojanoski, 1999). There were times during which the utilization of plants was done purely on the basis of experience because they neither had necessary information regarding the cause of the illness nor regarding the plants and how they can be exploited. In course of

time, the motive for using specific medicinal plants was discovered for the treatment of particular diseases; thus, the medicinal plant's usage gradually denied the practical experience and became founded on logical and theoretical facts (Kelly., 2009). It is well known that ethnobotanical investigations are efficient tools for finding significant plants for the development of crude medicines. Plants with high antioxidant potential are responsible for their medicinal properties, used by indigenous communities for their primary healthcare needs.

Antioxidants aid organisms to handle oxidative stress, caused by free radical damage. Free radicals are chemical species, which consist of one or more unpaired electrons due to which they are extremely unstable and to attain stability they extract electrons from other molecules and cause damage. Plants are considered to be a major source of exogenous (i.e., dietary) antioxidants. It is considered that the world's two-thirds of the plant species have essential medicinal properties, and among them, almost all have remarkable antioxidant potential (Krishnaiah *et al*., 2011). The antioxidants isolated from medicinal plants were found to be far more active than those found in fruits and vegetables (Chodak *et al*., 2011). Therefore, plant extracts are investigated for their antioxidant potentials and also to isolate the antioxidants that are more active and less harmful to living organisms (Chodak *et al*., 2009). Knowing the antioxidant capacity of various edible and medicinal plants is crucial for the general population, medical professionals, dietary specialists, and nutrition researchers.

The flowering plant *Rhinacanthus nasutus* (L.) Kuntze, which is a member of the Acanthaceae family, is widely known for its therapeutic properties. *R. nasutus* is also sometimes known as Snake Jasmine because of the way its blossoms are shaped. In addition, the root of this plant has been reported to be used in traditional medicine to counter the effects of snake venom (James *et al*., 2011). Different parts of this plant have also been traditionally used for the treatment of various diseases such as diabetes, eczema, pulmonary tuberculosis, herpes, hypertension, hepatitis, and several types of skin diseases. In Thailand, *R. nasutus* has been traditionally used for the treatment of various cancers such as colon (Kupradinum *et al*., 2009), cervical, and liver cancers (Rojanapo *et al*., 1990). Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al*., 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al*., 2010). Inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs (Kumarappan *et al*., 2006). A wide range of substances found in medicinal plants can be used to find novel anti-inflammatory compounds. The purpose of this study is to assess the antioxidant potential and *in vitro* anti-inflammatory activity of *R. nasutus*, a member of the Acanthaceae family, in order to comprehend the ethnomedicinal significance of this medicinal plant.

Materials and Methods

Plant material

The fresh roots of *R. nasutus* of the Acanthaceae family were collected from Kollam, Kerala, India.

Preparation of extracts

100 g of powder of shade-dried roots of *R. nastus* was extracted successively with ethanol (1000mL), hydroethanol (500mL ethanol +500mL distilled water), and distilled water (1000mL) in an orbital shaker. The extract was then filtered and the filtrate was concentrated under reduced pressure using rotary evaporator, to get the crude extract.

Antioxidant assays

1. Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was measured using the standard procedure of Bose *et al*., 2008. Sodium nitroprusside (1mL of 10 mM) was mixed with 1 mL of different concentrations (20 to 320 µg/mL) of plant extract/serial fractions in phosphate buffer (pH-7.4). The mixture was incubated at 25°C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent (1% sulphanilamide, 2% O-phosphoric acid and 1%

napthyl ethylene diamine dihydrochloride) was added. Ascorbic acid was used as standard.

Absorbance was read at 546 nm and the percentage of inhibition was calculated using the formula: [1- (absorbance of sample/absorbance of control)] × 100.

2. Ferric reducing antioxidant potential

The ability of plant extract to reduce the ferric ion was measured using the modified version of the method described by Benzie and Strain, 1996. 200 µL of plant extract/serial fractions (mg/mL), was added to 3 mL of FRAP reagent (10 part 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM-2,4,6-tripyridyl-s-triazine (TPTZ) solution and one part 20 mM- FeCl3 6H2O solution) and the reaction mixture was incubated in a water bath at 37°C for 30 min. The absorbance was measured at 593 nm. The antioxidant capacity of the plant extracts was calculated from the calibration curve of Trolox and expressed as µmol Trolox equivalent/g of extract.

3. In vitro anti-inflammatory activity

3.1. Inhibition of albumin denaturation

The anti-inflammatory activity of *R. nasutus* was studied using inhibition of albumin denaturation technique which was studied according to Mizushima *et al.*,1968 and Sakat *et al.*, 2010 followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction. A little quantity of 1N HCl was added to the mixture to alter the pH. After 20 minutes at 37 °C and 20 minutes at 51°C, the sample extracts were cooled, and the turbidity was measured at 660 nm. The experiment was carried out in triplicate and average calculated. The following formula was used to compute the percentage inhibition of protein denaturation:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.

3.2. Antiproteinase action

The test was performed according to the modified method of Oyedepo *et al.*, 1995 and Sakat *et al.*, 2010. Reaction mixture (2 ml) containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations (100 – 800µg/ml). After 5 minutes of incubation at 37 °C, 1 ml of 0.8% (w/v) casein was added to the mixture. An extra 20 minutes were spent incubating the combination. To stop the process, 2 ml of 70% perchloric acid was added. After centrifuging a cloudy suspension, the absorbance of the supernatant was measured at 210 nm using a buffer as blank. The experiment was carried out three times. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

4.Membrane stabilization

4.1.Heat induced haemolysis (Sakat *et al.*,2010) (Sadique *et al.*, 1989)

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 800 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All centrifuge tubes containing the reaction mixture underwent a 30 minute incubation in a water bath at 56 °C. The tubes were cooled under running water after the incubation period. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatant was measured at 560 nm. For each test sample, the experiment was carried out three times. The percentage inhibition of haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

4.2.Hypotonicity-induced haemolysis (Azeem *et al.*, 2010)

Different concentrations of extract (100-800µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All of the test solutions were centrifuged at 3000 rpm after 30 minutes of incubation at 370 °C. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560nm. The percentage of hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100- (OD sample/OD control) x 100.

Results and Discussion

1.Nitric oxide radical scavenging assay

The result showed that, the Nitric oxide radical scavenging activity of ethanolic hydroethanolic and aqueous extracts of *R. nasutus* were compared with ascorbic acid. Hydroethanolic extractof *R. nasutus* roots has got profound antioxidant activity when compared with other extracts (Fig 1). The IC50 value of the ethanolic hydroethanolic and aqueous roots extracts of *R. nasutus* and ascorbic acid were found to be at 238 µg/ml, 117.48 µg/ml, 440.74 µg/ml, and 85.80 µg/ml respectively (Table 1).

2. Ferric reducing antioxidant potential

Results of Ferric Reducing Capacities of ethanolic, hydroethanolic and aqueous extracts are presented in Table 2. The hydroethanolic extract showed a very strong ferric ion reducing activity (46.829 µmol Trolox equivalent/g of extract) when compared with other extracts.

3.In vitro anti-inflammatory activity

3.1.Inhibition of protein denaturation

By applying an external stressor or substance, such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat, proteins can become denaturized, losing both their secondary and tertiary structures. When denatured, the majority of biological proteins cease to function biologically. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. At 800µg/ml, a maximum inhibition of 73.49% was noted. Aspirin, a common anti-inflammatory medication, had the highest level of inhibition (67.19%) as compared to control at a concentration of 100µg/ml (Table 3).

3.2.Antiproteinase action

RNHE exhibited significant antiproteinase activity at different concentrations, maximum inhibition of 64.64%at800µg/ml. Aspirin showed the maximum inhibition 54.41% at 100µg/ml.

4.Membrane stabilization

4.1.Heat Induced Haemolysis

At various doses, the extract proved efficient in preventing heat-induced hemolysis. The findings

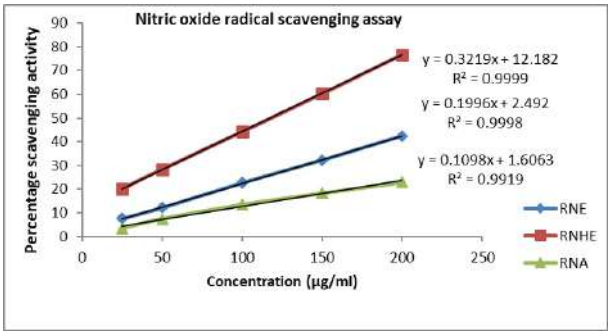

Fig 1: Graph shows Nitric oxide radical scavenging activity of Ethanolic, Hydroethanolic, and Aqueous extracts

RNE :Rhinacanthus nasutus Ethanolic Extract, RNHE :Rhinacanthus nasutus Hydroethanolic Extract, RNA :Rhinacanthus nasutus Aqueous Extract

Table 2: Ferric Reducing Antioxidant Potential of ethanolic, Hydroethanolic, and Aqueous extracts.

Concentration of test (µg/ml)	Ferric reducing antioxidant potential
Ethanolic extract (0.5ml)	30.536
Hydroethanolic extract (0.5ml)	46.829
Aqueous extract (0.5ml)	27.317

Table 1: IC50 value of ethanolic, Hydroethanolic, Aqueous extracts and standard.

	IC50
Ethanolic extract	238
Hydroethanolic extract	117.48
Aqueous extract	440.74
Standard	85.80

Table 3: Effect of RNHE on heat induced protein denaturation.

Concentration of hydroethanolic extract (µg/ml) (RNHE)	Absorbance at 660 nm	Percentage inhibition of protein denaturation
100	0.28±0.03	26.50
200	0.242±0.02	36.48
300	0.185±0.03	51.44
400	0.154±0.01	59.58
500	0.134±0.04	64.82
600	0.128±0.02	66.40
700	0.113±0.04	70.34
800	0.101±0.02	73.49
Standard (Aspirin) (100 µg/ml)	0.125±0.01	67.19
Control	0.381±0.03	

Table 4: Effect of RNHE on proteinase inhibitory action.

Concentration of hydroethanolic extract (µg/ml) (RNHE)	Absorbance at 210 nm	Percentage inhibition of proteinase action
100	0.284±0.02	21.54
200	0.269±0.05	25.69
300	0.245±0.03	32.32
400	0.216±0.04	40.33
500	0.197±0.01	45.58
600	0.169±0.02	53.31
700	0.145±0.04	59.94
800	0.128±0.02	64.64
Standard (Aspirin) (100 µg/ml)	0.165±0.01	54.41
Control	0.362±0.05	

Table 5: Effect of RNHE on heat induced haemolysis of erythrocyte

Concentration of hydroethanolic extract (µg/ml) (RNHE)	Absorbance at 560 nm	Percentage inhibition of proteinase action
100	0.226±0.04	25.20
200	0.203±0.03	32.78
300	0.184±0.01	39.07
400	0.165±0.05	45.36
500	0.159±0.02	47.35
600	0.128±0.04	57.61
700	0.102±0.01	66.22
800	0.092±0.03	69.53
Standard (Aspirin) (100 µg/ml)	0.09±0.05	70.19
Control	0.302±0.05	

Table 6: Effect of RNHE on Hypotonicity-haemolysis of erythrocyte

Concentration of hydroethanolic extract (µg/ml) (RNHE)	Absorbance at 560 nm	Percentage inhibition of proteinase action
100	0.205±0.04	37.00
200	0.184±0.02	43.38
300	0.163±0.01	49.84
400	0.154±0.04	52.60
500	0.140±0.02	57.00
600	0.103±0.03	68.30
700	0.098±0.01	69.84
800	0.085±0.05	73.84
Standard (Aspirin) (100 µg/ml)	0.127±0.05	61.00
Control	0.325±0.02	

demonstrated that RNHE effectively prevents heat-induced lysis of the erythrocyte membrane at concentrations of 600, 700, and 800 µg/ml. A large amount of protection from the detrimental effects of heat solution was provided by aspirin 100 µg/ml.

4.2.Hypotonicity-induced haemolysis

The findings demonstrated that RNHE at concentrations between 300 and 800 g/ml considerably shields the erythrocyte membrane against lysis brought on by a hypotonic solution. A large amount of defense was provided by diclofenac sodium (100 µg/ml) against the destructive effects of hypotonic solution. When compared to control, RNHE provided the greatest level of protection at 800 µg/ml, while Diclofenac sodium (100 µg/ml) inhibited RBC hemolysis by 61%.

Nitric oxide radical scavenging activity and ferric reducing antioxidant activities of ethanolic hydroethanolic and aqueous extracts of *R. nasutus* were compared with ascorbic acid and Trolox respectively. When compared to the ethanolic and aqueous extracts of *R. nasutus* roots, hydroethanolic extract exhibits a significant antioxidant potential in both procedures. Protein denaturation, a process in which proteins lose their tertiary and secondary structures due to external stress or compounds like strong acids or bases, concentrated inorganic salts, organic solvents, or heat, is a component of RNHE's in-vitro anti-inflammatory effect. When denatured, the majority of biological proteins cease to function biologically. Protein denaturation is a well-known contributor to inflammation. The potential of plant extract to suppress protein denaturation was investigated as part of the inquiry into the mechanism of the anti-inflammation action. It was effective in inhibiting heat induced

albumin denaturation. Maximum inhibition of 800 µg/ml, a maximum inhibition of 73.49%. Neutrophils are known to be a strong source of serine proteinase and are localised at lysosomes in proteinase inhibitory action. Leukocyte proteinase has been implicated in the development of tissue damage during inflammatory responses, and proteinase inhibitors have been shown to give a considerable amount of protection (Das *et al.*, 1995). RNHE exhibited significant anti-proteinase activity at different concentrations, maximum inhibition of 64.64% at 800µg/ml. Because the erythrocyte membrane is similar to the lysosomal membrane (Shenoy *et al.*, 2010) and its stabilisation means that the extract may well stabilise lysosomal membranes, it has been employed as a technique to examine the *in vitro* anti-inflammatory efficacy. The RNHE was effective in inhibiting the heat induced haemolysis at different concentrations. The result showed that, RNHE effectively prevents heat-induced lysis of the erythrocyte membrane at concentrations of 600, 700, and 800 µg/ml. Hypotonicity-induced haemolysis provided the greatest level of protection at 800 µg/ml, that is 73.84%.

Conclusion

India is home of a vast array of medicinal plants that have long been employed by tribes for both their health and prosperity. However, the scientific approval of these plants is crucial for their medicinal use. *R. nasutus* is a medicinal plant that is frequently utilised in tribal communities for conditions such as inflammatory disorders, hepatic disorders, ringworm, abscess pain, itchiness, and skin illnesses. Based on the findings of the current investigation, it is concluded that *R. nasutus* root hydroethanolic extract has strong antioxidant and anti-inflammatory activities. Ascorbic acid and Trolox were the standards used against *R. nasutus* ethanolic hydroethanolic and aqueous extracts in antioxidant tests such as the Nitric oxide radical scavenging assay and the ferric reducing antioxidant potential, respectively. Hydroethanolic extract of *R. nasutus* roots has got profound antioxidant activity in both methods when compared with the ethanolic and aqueous extracts. The hydroethanolic extract exhibited significant dose-dependent inhibition of Nitric oxide radical scavenging activity. The IC50 value of the ethanolic hydroethanolic and aqueous roots extracts of *R. nasutus* and ascorbic acid were found to be at 238, 117.48, 440.74 µg/mL and 85.80 µg/ml respectively. The total Ferric reducing antioxidant potential of ethanolic, hydroethanolic, and aqueous extracts were 30.536, 46.829, and 27.317 µmol Trolox equivalent/g of extract. Due to the inclusion of polyphenolic components such alkaloids, flavonoids, tannins, steroids, and phenols, RNHE have anti-inflammatory effects. The hydroethanolic extract prevented heat-induced albumin denaturation, proteinase activity, and maintained the membrane of red blood cells by acting as free radical scavengers, inhibitors, or perhaps primary oxidants. RNHE has substantial antiproteinase activity at various concentrations of extracts, with the greatest inhibition of 64.64% at 800 µg/ml, and significantly protects against heat-induced protein denaturation at concentrations between 400 and 800 µg/ml. RNHE at concentration 600, 700 and800µg/ml protect significantly the erythrocyte membrane against lysis induced by heat. In hypotonicity-induced hemolysis, RNHE concentration at 800 µg/ml demonstrated the greatest protection (73.84%). These *in vitro* experiments imply that this plant is a significant source of natural antioxidants, which may be helpful in reducing the development of various oxidative stresses. Tribal people value their ethnobotanical knowledge, but their scientific validation is very essential for their therapeutic application for various disorders. The current study therefore came to the conclusion that *R. nasutus* is a medicinal plant widely utilised in the tribal culture, which demonstrates high antioxidant component accountable for their therapeutic uses, and anti-inflammatory potential, which indicates an urgent need for its conservation and sustainable utilization. The conservation of medicinal plants for therapeutic purposes and the protection of biodiversity are therefore vital.

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Chapter 12

ETHNOBOTANICAL STUDY OF KANI TRIBES IN ORUPARAKARKKAKAM SETTLEMENT IN PERINGAMMALA GRAMAPANCHAYATH, THIRUVANANTHAPURAM DISTRICT, KERALA, INDIA

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Abstract The indigenous people of Kani have an extensive knowledge of edible and medicinal plants. An ethnobotanical study was done in the Oruparakarikkakam settlement, Peringammala Gramapanchayat. The study area was visited frequently, and ethnobotanical data (local name, useful part, uses, method of preparation, etc.) were collected using semi-structured questionnaires in the local language and discussion with the assistance of village leaders (MoottuKani) and local villagers and tribal practitioners in the settlement area. The ethnomedicinal information was collected through interviews with the Kani traditional healers. The collected data were analysed through use value (UV), informant consensus factor (Fic), etc. Information was received from 93 respondents, ranging in age from 20 to 90 years old. In the current study, the tribes use 60 species from 28 families for food and medicine. Among these were herbs (26), shrubs (12), small trees (6), large trees (4), and climbers (12). The herbalist treated diabetics, snake bites, jaundice, body pain, piles, ulcers, swellings, weight loss, coughs and colds, diarrhoea, and other ailments as anti-inflammatory and anti-cancerous. The informant consensus factor (ICF) and use value (UV), etc., were calculated. Detailed information was gathered on the list of plants and their food and medicinal uses among the Kani tribal people. The number of wild plants used by the Kani community, as well as knowledge of plants, is of the utmost importance in the present-day global scenario of food security and diminishing food diversity. The information gathered will also be useful for the development of nutritionally rich products in the future.

Keywords: *Ethnobotany, wild edible plants, Kani Tribe*

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Introduction

India has rich vegetation with a wide variety of plants, because of the extreme variations in geographical and climatic conditions prevailing in the country. Plants have been utilised to heal a variety of diseases since antiquity. Traditional and folk systems of medicine continue to serve a substantial segment of the population, particularly in rural regions, despite the arrival of modern medicine (Handa, 1998)..

From time immemorial, man has exploited natural resources for all his basic needs, such as food, clothing, medicine, and shelter. The indigenous communities of the world that are living close to nature have, over the years, acquired unique experience and knowledge about wild flora and fauna. The traditional knowledge, which was transferred orally for centuries, the plant-people interaction, and the communication of resulting knowledge in these traditional communities are presently threatened for various reasons, such as technological developments, modernization, and the western worldview (Ganesan et al., 2004).

India is a major origin and diversity center for a diverse range of food and medicinal plants. Because of the rich floral diversity of wild plants in the Southern Western Ghats. Today, about 25% of modern medicines contain ingredients derived from

wild plants (Sher *et al*., 2000). Wild plant uses have long been important in human life. Man has always been in close contact with his surroundings. India has the earliest evidence of plants used in the traditional system of medicine, and it dates back over 5000 years. Ayurveda records over 8000 plant-based remedies. India officially recognises over 2500 plants as having medicinal importance. It has been estimated that over 6000 plants are used in indigenous folk medicines (Huxley, 1984). Since ancient times plants have been known as a source of diverse biologically active chemicals, essential for maintaining health and useful for treating and preventing disease. It is only recently that several scientific studies have been performed to find potential health-protective phytocompounds that might prevent disease and improve general health. It is because we are becoming increasingly aware that various nutrients in the diet play a crucial role in maintaining an optimum immune response.

The indigenous people's medicinal plant knowledge has also been very high. They knew the habitat of each of the species, sustainable harvesting and sustainable utilization of plants. The valuable wealth of plants based on the knowledge of the indigenous people is passed from generation to generation through word of mouth. Unfortunately, in the recent past, there is an alarming trend noticed in the tribal colonies that the younger generation does not show enthusiasm and interest in acquiring this tremendous knowledge and hence will be vanished from the indigenous people over some time. But due to the profit gained by marketing this forest wealth they are forced to lear the traditional knowledge from the elders. Tribal people throughout the world, including India, have developed their own cultures, customs, taboos, myths, medicine, food etc. They are the repository of experience and knowledge of the traditional system; hence they can be utilized for tribal development. Nowadays, greater emphasis is being given to the traditional knowledge and usage of natural products as a new food source and medicine (Ganesan *et al.* 2007).

The Kanikaran, sometimes known as Kani, tribe can be located in the study area. Historically, they have lived as nomads. Malayalam and Tamil are the native tongues of the Kanikaran tribes. They are often short people with dark skin who depend on farming for their livelihood. They used to reside in caves and rock shelters, which offered protection to these people. Due to encounters with outsiders, their behaviours and manners have changed. There is a tribal chief for each tribe. They currently reside in a number of tribal hamlets, each with 5 to 20 families. Most Kani tribal people have a very good knowledge of medicinal plants that are used as first aid remedies, to treat coughs, colds, fevers, headaches, poisonous bites, and some other simple ailments. The tribals residing in the deep forest areas are still dependent on medicinal plants for their primary healthcare and treatment of various diseases. Kanis still supplement their food by gathering wild plants from the nearby forest areas. They are extremely hard workers and can survive without the help of modern facilities. They are socioeconomically backward and most of them are very poor. They are also engaged in the seasonal collection of honey, bee wax, and some minor forest produce. They cultivate edible plants, like tapioca, banana, millets, and cash crops, such as pepper, coconut, etc. Only a few scattered studies on edible plants research has been done among the Kani tribal people of southern India.

Materials and methods

Frequent field visits were made to the study area (Oruparakarikkakam settlement, Peringammala Gramapanchayat) and ethnobotanical data (local name, useful part, uses, method of preparation, etc.) were collected using semi-structured questionnaires in the local language and discussion with the assistance of village leaders (MoottuKani) and local villagers and tribal practitioners in the settlement area.

Data analysis

The information was arranged in alphabetical order of the scientific names of the plants along with the family, local name, used plant parts, mode of application, habit, habitat and name of the diseases they are indicated for. The results were further analysed and presented on the basis of their use and disease categories.

Use Value (UV)

According to Phillips *et al.* (1994), the UV was calculated using the following formula:

$UV=\sum U/N$

Where, "U" refers to the number of uses mentioned by the informants for a given species and "N" refers to the total number of informants interviewed. If a plant secures a high UV score that indicates there are many use reports for that plant, while a low score indicates fewer use reports cited by the informants.

Informants Consensus Factor (FIC)

The level of homogeneity among information provided by different informants was calculated by the Informants' Consensus Factor, FIC (Trotter and Logan, 1986) using the following formula: FIC = Nur – Nt / (Nur – 1)

Where, Nur = number of use reports from informants for a particular plant-use category; Nt = number of taxa or species that are used for that plant use category for all informants.

Fig. 1. Interviews conducted in the study area

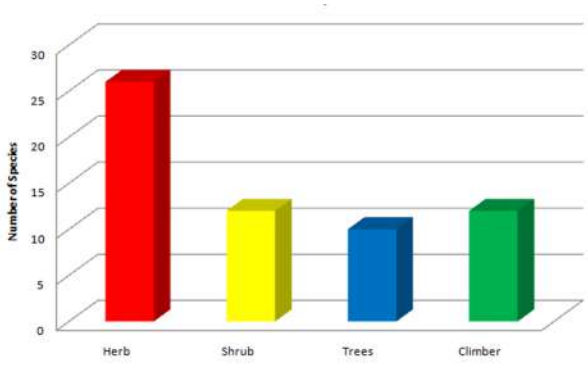

Fig. 2. Habit Study

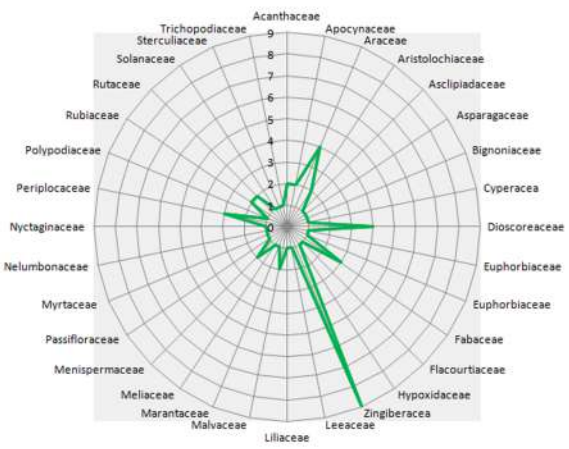

Fig. 3. Family abundance

FIC Values range between 0 and 1, where '1' indicates the highest level of informant consent.

Results

There were 54 women and 39 men among the informants. Out of 93 informants, 82 were indigenous people, and 11 were tribal healers (Fig-1). The majority of informants (38.82%) were between the ages of 50 and 60; the remaining interviewees were between the ages of 40 and 50 (29.64%), next between the ages of 20 and 40 (18.67%), and finally between the ages of 70 and 90. (12.87). Most of those interviewed were illiterate. The tribal healers described the time or season for plant gathering, the technique of medicine preparation, the schedule, and the administration dosage of medicine for the patient. In the current study, the tribes use 60 species from 28 families (Table 1) for food and medicine. Among these were herbs (26), shrubs (12), trees (10), and climbers (12)(Fig-2). The herbalist treated diabetics, snake bites, jaundice, body pain, piles, ulcers, swellings, weight loss, coughs and colds, diarrhea, and other ailments as anti-inflammatory and anti-cancerous.

The most commonly represented families were Zingiberaceae (9) Araceae (4) and Dioscoraceae (4) (Fig 3). They were using these plants to cure diseases like skin disorders, cold, fever, cough, headache, rabies, diarrhoea, fertility problems, tooth diseases, stomach ache, wounds, rheumatism, hair falling and poison (snake, scorpion and insect) bites. The information was received from 93 respondents, ranging in age from 20 to 90 years old.

In the current investigation, high use value (UV) values were observed for *Cycas circinalis* (0.925), *Trichopus zeylanicus* (0.924), *Coscinium fenestratum* (0.914), *Dioscorea pentaphylla* (0.886), and *Aristolochia acuminata* (0.886), indicating their extensive usage in the ethnobotanical practises (food and medicine) in the study area. The lowest UV were obtained for *Calophyllum inophyllum* (0.129), *Abrus precatorius* (0.183), and *Hydnocarpus pentandrus* (0.204), (Table 3). Some plants may have a high use value because of their wide spread distribution throughout the research area, making them the first choice for treatment. These plants are frequently used by indigenous and tribal people in their nation for a number of purposes. In this study the seed of *Cycas circinalis*, the plant with the highest use value content, is taken as food and for stomach pain, gastric disorders, etc. *Coscinium fenestratum* crushed wood is used to treat acute headach and *Dioscorea pentaphylla* tuber is used for thire staple food and health tonic while Aristolochia acuminata root and leaves are used for against poisonous bits.

Medicines were prepared in the form of powder, decoction, paste and juice etc. It was also observed that some plants were used in more than one form of preparation. Several plants were used in the form of powder: examples are roots of *Hemidesmus indicus*, *Adenia hondala*, *Holostemma ada-kodien* and stem of *Coscinium fenestratum*, whole plant of *Boerhavia diffusa*, some plants were used in the form of decoction: examples are leaves of *Justicia adhatoda*, and stem bark of *Aegle marmelos*, *Saraca asoca* and whole plant of *Desmodium gangeticum*. Some plants were used in the form of paste: examples are seed of *Cycas circinalis*, *Entada rheedei*. Some plants were used in the form of juice: In some cases, fruits (*Trichopus zylanicus*, *Solanum torvum*, *Ricinus communis*, *Phyllanthus emblica*) are used as medicine both in fresh and dried form. Among different plant parts used by Kani tribes in Peppara wild life sanctuary, the leaves are most frequently used for the treatments. External applications and internal consumption are involved in the treatment of wounds, rheumatism, poisonous bites, headache, skin diseases and hair falling. For diseases like cold, fever, cough, diarrhoea, fertility problems, tooth diseases and stomach-ache only internal consumption is adopted. In the present study, some of the medicinal plants are endemic to Western Ghats. For example *Trichopus zeylanicus* is abundantly found in this area and very rarely found in other places of Western Ghats it is also an endangered plant. Herbal medicines prescribed by tribal healers are either preparation based on single plant part or a combination of severalplant parts. The Kani tribals usually prepare medicines in a combination of several plant parts. They believe that combination of several plant parts cures diseases rapidly. The

Slide 1. Some ethnopharmacological leads obtained based on Kani traditional knowledge

Trichopus zeylanicus subsp. *travancoricus* Burkill ex Narayanan (Arogyapacha) – Instant energy etc.

Dioscorea pentaphylla L. Tubers used as food, cure body pain and stomach problems

Cyclea peltata (Lam.) Hook. f. & Thoms. (Padathali)- fresh root juice used to cure piles, stomach disorders, constipation

Holostemma adakodien Dried root powder –stomach problem and increase lactation

Saraca asoca (Roxb.) De Wilde (Asokam) – Internal growth (tumers)

Decalepis arayalpatra Joseph and Chandrasekharan (Amrithapala) – Internal growth (tumers)

number of wild plants used by the Kani community, as well as knowledge of plants, is of the utmost importance in the present-day global scenario of food security and diminishing food diversity. The information gathered will also be useful for the development of nutritionally rich products in the future.

Scientific research that relies on traditional knowledge must be reliable. In ethnobotanical research, consensus analysis provides the same assurance of reliability for all claims supported by evidence. The product of FIC ranges from 0 to 1. In ethnobotanical research, consensus analysis provides the same assurance of reliability for all claims supported by evidence. Whereas a low value indicates disagreement (Ragupathy *et al*., 2008). The availability of plant species in the study area typically determines the FIC of local knowledge for disease treatment. (Rajakumar and Shivaanna, 2009). The FIC values in our study ranged from 0.18 to 0.92 (Table 2). The 10 ailment categories are Snake bites, dog bites, and insect bites (71 reports, 6 species); Microbial infection: jaundice, a fungal infection (29 reports, 4 species), and Swellings, cuts, and wounds (84 reports, 12 species) *Aristolochia acuminata*, were very commonly used for the treatment of snake bite. Thus, the study indicates that the degree of knowledge shared by the users in the study area regarding the use of medicinal plants in the treatment of ailments is high.

Table 1. List of wild edible plants of the Peppara wild life sanctuary.

Botanical name	Common name	Family	Habit used	Part	Use	Use value
Abrus precatorius L.	Vellakkunni	Fabaceae	S	R	M	0.183
Abutilon indicum (L.)	Oorppam	Malvaceae	S	R	M	
Acorus calamus L.	Vayambu	Araceae	H	Rh	M	0.226
Adenia hondala (Gaertner) de Wilde	Muthakku	Passifloraceae	C	Tr	F, M	0.387
Adhatoda vasica Nees	Adalodakam	Acanthaceae	S	L	M	0.731
Aegle marmelos (L.) Corrêaex Roxb.	Koovalam	Rutaceae	T	Fr, L	M	0.484
Alocasia macrorrhizos (L.) G.Don	Madantha	Araceae	H	Rh	F, M	0.494
Alpinia calcarata (Haw.) Roscoe	Kolinji	Zingiberaceae	H	Rh	M	0.698
Amorphophallus commutatus (Schott) Engl.	Kattu chena	Araceae	H	Rh	F, M	0.280
Andrographis paniculata Nees	Kiriyathu	Acanthaceae	H	Wp	M,F	0.333
Aristolochia tagala Cham	Valiyaarayan	Aristolochiaceae	C	R	M	0.885
Asparagus racemosus Willd.	Sathavari	Asparagaceae	C	R	M	0.699
Averrhoa carambola L.	Anappulinchi	Oxalidaceae	T	Fr	F,M	0.431
Azadirachta indica A. Juss.	Arya veppu	Meliaceae	T	L	L	0.742
Boerhavia diffusa L.	Thazhuthama	Nyctaginaceae	H	Wp	M	0.527
Clausena anisata (Willd.) Hook.f.	MAlaveppu	Rutaceae	T	L,R	M	0.316
Colocasia esculenta (L.) Schott	Chembu	Araceae	H	Rh	F	
Colebr.						
Coscinium fenestratum (Gaertn.)	Maramanjal	Menispermaceae	C	W	M	0.914
Costus speciosus (J.Koenig) Sm.	Channakoova	Zingiberaceae	H	Rh	M	0.280
Curculigo orchioides Gaertn.	Nilappana	Hypoxidaceae	H	Rh	M	0.495
Curcuma amada Roxb.	Mangainchi	Zingiberaceae	H	Rh	F, M	0.505
Curcuma aromatica Salisb.	Kasthurimanjal	Zingiberaceae	H	Rh	F,M	0.634
Curcuma longa L	Manjal	Zingiberaceae	H	Rh	F, M	0.731
Cycas circinalis L.	Kana	Cycadaceaea	T	Fr	F,M	0.925
Cyclea peltata (Lam.) Hook.f. & Thomson	Padathali	Menispermaceae	C	Rh	M	0.312
Decalepis arayalpathra Venter, (J.Joseph & V.Chandras.)	Amruthapala	Periplocaceae	S	Tr	M	0.548
Desmodium gangeticum (L.) DC.	Orila	Fabaceae	S	R	M	0.333
Dioscorea oppositifolia L	PInnan	Dioscoreaceae	C	Tr	F	0.408
Dioscorea pentaphylla L.	Nooran	Dioscoreaceae	C	Tr	F	0.886
Dioscorea tomentosa J.Koenig ex Spreng.	Nooli	Dioscoreaceae	C	Tr	F	0.894
Drynaria quercifolia (L.) J. Sm.	Panni kizhangu	Polypodiaceae	H	Tr	F, M	0.290
Entada rheedei Spreng .	Paranda	Dioscoreaceae	C	Seed	F,M	0.774
Hemidesmus indicus (L.) R. Br. ex Schult.	Narunandi	Periplocaceae	H	Tr	F M	0.527
Holostemma ada-kodien Schult.	Adapathiyan	Asclipiadaceae	C	R	M	0.172
Hydnocarpus pentandrus (Buch.-Ham.) Oken,	Marottica	Flacourtiaceae	T	Fr	M	0.312
Kaempferia galanga L.	Kacholam	Zingiberacea	H	Rh	M	0.806
Leea indica (Burm. f.) Merr.		Leeaceae	S	R,L	M	0.376
Manihot esculenta Crantz	Maracheeni	Euphorbiaceae	S	Tr	F	0.731
Maranta arundinacea L.	Koovva	Marantaceae	H	Rh	F	0.365
Nelumbo nucifera Gaertn	Thamara	Nelumbonaceae	H	Tr	F,M	0.301
Ophiorrhiza mungos L.		Rubiaceae	H	R	M	0.312
Pseudarthria viscida (L.) Wight &Arn.	Moovila	Fabaceae	S	R	M	0.494
Pterospermum rubiginosum Heyne ex Wight & Arn.	Ellooti	Sterculiaceae	T	B , R	M	0.419
Rauvolfia serpentina (L.) Benth. ex Kurz	Sarpagandhi	Apocynaceae	H	R	M	0.526
Ricinus communis L.	Aavannakku	Euphorbiaceae	S	R	M	0.559
Rubia cordifolia L.	Manjetti	Rubiaceae	C	R	M	
Sida acuta Burm.f.	Kurunthotti	Malvaceae	S	R	M	0.301
Solanum torvum Sw	Chunda	Solanaceae	S	Fr	Fd,M	
Syzygium cumini (L.) Skeels	Njara	Myrtaceae	T	Fr	F	0.591
Thottea ponmudiana Sivar.	Kodaashari	Aristolochiaceae	S	R	m	0.290
Trichopus zeylanicus subsp. travancoricus Burkill ex K.Narayanan.	Arogyapacha	Trichopodiaceae	H	Fr , L	F,M	0.924
Zingiber zerumbet (L.) J.E. Smith	kolinchi	Zingiberaceae	H	Rh	M	0.494
Zingiber neesanum (J.Graham)	Kattukolinchi	Zingiberaceae	H	Rh	M	0.634

Ramamoorthy *Zingiber officinale* Roscoe	Enji	Zingiberaceae	H	Rh	F, M	0.709

B- Bark,Fr- Fruit, R- Root, Rh- Rhizome, L-Leaf, F- Food, Tr- Tuber, Wp- whole plant, W-wood, M-Medicinal, S-Shrub, H-Herb, C-Climber, T- Tree.

Table 2. Informant Consensus Factor (ICF)

Sl. No	Category of ailment	Number of use report	Number of species	ICF value
1	Coughs and colds	19	7	0.667
2	Dermatological :Allergy, ringworm, itch	17	6	0.687
3	Diabetics ,blood purifiers	3	2	0.500
4	Digestive system disorders: Gastritis, Diarrhea, ulcers, constipation, piles.	32	8	0.774
5	Kidney disorders: Irregular urination, kidney stone	14	5	0.692
6	Microbial infection: Jaundice, fungal infection	29	4	0.892
7	Pain : Abdominal pain, toothache, headache, migraine, knee pain, body pain , stomach pain.	62	8	0.885
8	Snake bites, dog and insect bites	71	6	0.928
9	Swellings, cut, wounds	84	12	0.867
10	Cancer	22	9	0.619

Conclusion

The study also collected a broad spectrum of information concerning wild edible plants used by Kani tribe. It showed that wild edible plants are still essential to Kani tribal peoples basic healthcare. The ethnomedical information is beneficial for initiating medicinal and novel food research activities. The knowledge acquired from the tribes is helpful for further research in the domains of ethnobotany, taxonomy, and pharmacology. This study provides a framework for investigating the link between plants and people in the context of traditional medicine. We could lose this wealth of knowledge in the near future because younger tribal people aren't interested and because they tend to move to cities for better jobs. The Kani healers are dying of old age, and with them their traditions Based on the results of this study, it seems likely that the current healers in the Peppara tribal settlements are the last of their kind. So, it's important to get this traditional system of medicine and keep it alive through proper documentation and identifying specimens. Traditional medicines could also be used to make pharmaceutical drugs to treat a wide range of illnesses and the loss of these potentially valuable genetic resources ultimately affects the whole society.

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Chapter 13

FORMULATION OF FISH FEEDS CONTAINING MANGROVE LEAVES AS FEED ADDITIVE AND THEIR EFFECT ON BIOCHEMICAL COMPOSITION OF *ETROPLUS SURATENSIS* (BLOCH,1790)

Jisha S, Usha S

Abstract Biochemical composition different body parts of of pearlspot, Etroplus suratensis was studied by giving different formulated feeds containing selected mangrove leaf powder as feed additive. Four different feeds, TD1 (containing *Rhizophora apiculata*), TD2 (*Lumnitzera recemosa*), TD3 (*Aegiceras corniculatum*) and CF (without any mangrove powder) which served as control were prepared and fed the fishes for 90 days. Crude protein, lipid, carbohydrate, moisture and ash content of muscle, gills and gut of each treatment find out in order to evaluate the effect of mangroves. TD1 showed better performance in terms of proximate composition followed by TD2 and TD3 when compared with CF. All these results were statistically found significant ($P<0.05$). This study showed a clear influence of these mangrove derived diets on enhancing the level of biochemical constituents of fishes.

Key words: *Etroplus suratensis, Rhizophora apiculata , Lumnitzera recemosa, Aegiceras corniculatum, proximate composition.*

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Introduction

Fishes have been widely used as one of the most important sources of animal protein and other nutrients for human health all over the world (Berket *et al.*, 2018). Successful aquaculture needs maximum weight gain in the shortest duration with minimum mortality. In aquaculture, feed represents a predominant portion of production cost. Nutritionally balanced feed is one of the key factors in aquaculture (Bharathi *et al.*, 2019). Feed ingredients are a mixture of both organic and inorganic components. Feed additives are added during feed preparation to improve the quality of the feed and health performance, feeding efficiency of the fishes. Functional feed additives include phytogenic compounds, organic acids, immune – stimulants, yeast products, probiotics, prebiotics and enzymes (Alemayehu *et al.*,2018). Due to their active principal natures, such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils, natural plant products have been reported to have a variety of activities, including anti-stress, growth promotion, appetising tonic, immunostimulation, aphrodisiac, and antimicrobials in the finfish and shrimp larviculture. (Citarasu *et al.*, 1998; Sivaram *et al.*, 2004; Dhas *et al.*, 2017; Belua *et al.*, 2020).

The development of high-quality feeds is principally responsible for this expansion of aquaculture. Any good quality feed is prepared from proper and essential feed additives. Aqua farmers are seeing promising harvests from recent advancements in functional feed additives. These useful feed additives come from several sources. The effect of dietary inclusion of plant derived products on growth performance and nutritional quality of fishes have been widely studied (Fagnon *et al.*, 2020; Dhas *et al.*, 2017; Elham *et al.*, 2017). According to Chakraborty *et al.*, (2014) phytochemicals also act as endocrine modulators in

fish culture. Shanmugaarasu *et al*., (2018), reported that integrating mangroves with fish farming is beneficial for better seabass fish survival and growth. It was attributed to the better water quality of integrated mangrove-aquaculture system. Nagarajan *et al*., (2013) had reported the antibacterial potential of mangroves for controlling the infections of marine ornamental fish caused by Pseudomonas fluorescens, Pseudomonas aeruginosa, Vibrio parahaemolyticus and Vibrio anguillarum.

Around 210 million people live in low elevation areas within 10 km of mangroves and many of these benefits from mangrove-associated fisheries (Hutchison *et al*., 2014). *Etroplus*, mugil, lates, hilsa, setipinna are some of the most common fishes in Indian mangroves. The main physical factors which support the fisheries at mangroves are the shallow water levels, warm water temperature, slow waterflow, entangled roots, which make it an ideal place for growing algae and for spawning fish and marine animals. In addition the falling litter of mangroves also becomes a part of their food. The pearlspot, *E. suratensis* is one of the most important indigenous edible Cichlids of the Indian subcontinent exhibiting restricted distribution in the estuaries of peninsular India and Sri Lanka. It thrives well both in fresh and brakish waters. (Sebastian *et al*., 2020; Devaraj *et al*., 1975; Keshava and Mohan, 1988). The present study looks into the effects of mangroves as feed additives on biochemical composition of muscle, gill and gut of *E. suratensis*.

Materials and methods

Fish and experimental setup

About 2 months old *E. suratensis*, weighing 4-4.5g were collected from the same farm. They were acclimatized for one week at the laboratory. The fishes were divided into four groups, each group containing 10 fishes. After grouping, the weight and length of each fish were recorded and the quantity of feed needed for each group has been determined. The experiments were done in triplicates.

Experimental diet preparation

In the present investigation, four feeds were prepared. Based on the square method, the feed composition was done. The feed containing powdered leaves of mangrove species Rhizophora apiculata was designated as TD1, those with *Lumnitera recemosa* as TD2 and those with *Aegiceras corniculatum* as TD3. The feed without the inclusion of any mangrove leaf powder was taken as control feed (CF). The fish were fed with the formulated feeds for 90 days in pelleted form. The fish were fed twice daily at 3% of their body weight. All the fish were weighed every two weeks, and the feed ration was adjusted accordingly. The Proximate composition of experimental feeds were provided in table 1.

Determination of the proximate composition of fish and fish feed

After 90 days the fishes were sacrificed, recorded weight and length and used for biochemical assay. The protein, fat, ash, carbohydrates and moisture content of muscle, gills and the gut of fishes and the experimental feeds were determined (AOAC, 21st edition), NFE (Castell.1979), Gross energy content (Jobling, 1983), metabolizable energy (Lee and Lawrence,1997) and ME/DE coefficient (Noblet *et al*., 1993) were calculated using the following equations.

NFE (%) = 100 - (%moisture+%crude protein+%crude lipid+%ash+% fibre)

Gross energy content (Kj/g) = (Energy contributed by protein + Energy contributed by carbohydrate + Energy contributed by lipid)÷100

Metabolizable energy (Kj/g) = 0.173× protein (%) +0.356 × fat (%)+ 0.125 ×carbohydrate (%)

ME/DE coefficient (%)= 100.3-0.021 × crude protein

Statistical analysis

The results of the proximate analysis of experimental fishes were presented as mean ± standard deviation acquired from the statistical analysis. Statistical analysis was carried out using SPSS (Statistical Package for Social Science. Version 16.0). The significance of each feed was calculated using paired t-test.

Table 1. Proximate analysis of experimental feeds

	Total protein (%)	Total fat (%)	Ash (%)	Moisture (%)	Fibre (%)	Nfe (%)	Gross energy (kj/g)	Me:de coefficient (%)	Metabolizable energy (kj/g)
CF	33.50	9.60	11.00	9.80	8.20	27.90	4.29	99.50	13.72
TD1	32.50	8.20	10.20	9.30	9.30	30.50	4.25	99.60	13.51
TD2	34.20	10.00	11.50	9.10	8.80	26.40	4.33	99.50	13.87
TD3	31.60	8.10	10.00	10.20	9.40	30.70	4.20	99.60	13.36

Result

In the present study, we observed significant changes in the concentration of biochemical constituents of fishes fed with experimental diets TD1, TD2 and TD3 than the fishes fed with the control diet (Table 2). The protein content of the muscle of fishes fed with the control diet was 25.73±0.21. There occurred a significant (P<0.05) increase in the level of muscle protein when fed with formulated feeds containing leaf powder of *Rhizophora apiculata* (27.07± 0.08) and *Lumnitzera recemosa* (26.85±0.15). But the fishes who were fed

Table 2. Proximate analysis of *Etroplus suratensis* fed with experimental feeds CF, TD1, TD2 and TD3

PARAMETER	Body part	CF	TD1	TD2	TD3
Protein (g/100g)	Muscle Gill Gut	25.73±0.21 24.77±0.15 23.13±0.06	27.07±0.08 25.67±0.12 25.07±0.67	26.85±0.15 25.93±0.15 24.93±0.15	25.32±0.19 24.03±0.12 23.07±0.06
Fat (g/100g)	Muscle Gill Gut	1.57±0.06 1.27±0.06 1.20±0.10	3.16±0.04 1.83±0.06 1.67±0.06	2.07±0.26 1.43±0.06 1.93±0.15	2.00±0.02 1.81±0.15 2.07±0.15
Carbohydrate (g/100g)	Muscle Gill Gut	0.23±0.06 0.13±0.06 0.17±0.06	0.70±0.17 0.57±0.06 0.60±0.28	0.67±0.12 0.33±0.06 0.53±0.06	0.13±0.06 0.10±0.00 0.10±0.00
Ash (g/100g)	Muscle Gill Gut	0.93±0.15 6.83±0.12 0.73±0.06	1.02±0.01 8.00±0.17 0.87±0.06	1.27±0.15 7.97±0.12 1±0.17.00	1.15±0.22 6.83±0.06 0.94±0.33
Moisture (g/100g)	Muscle Gill Gut	81.23±0.21 75.47±0.12 87.63±0.06	81.27±1.10 73.90±0.20 88.30±0.26	81.80±0.36 75.60±0.53 88.07±0.45	79.03±1.01 75.33±0.23 84.90±0.72

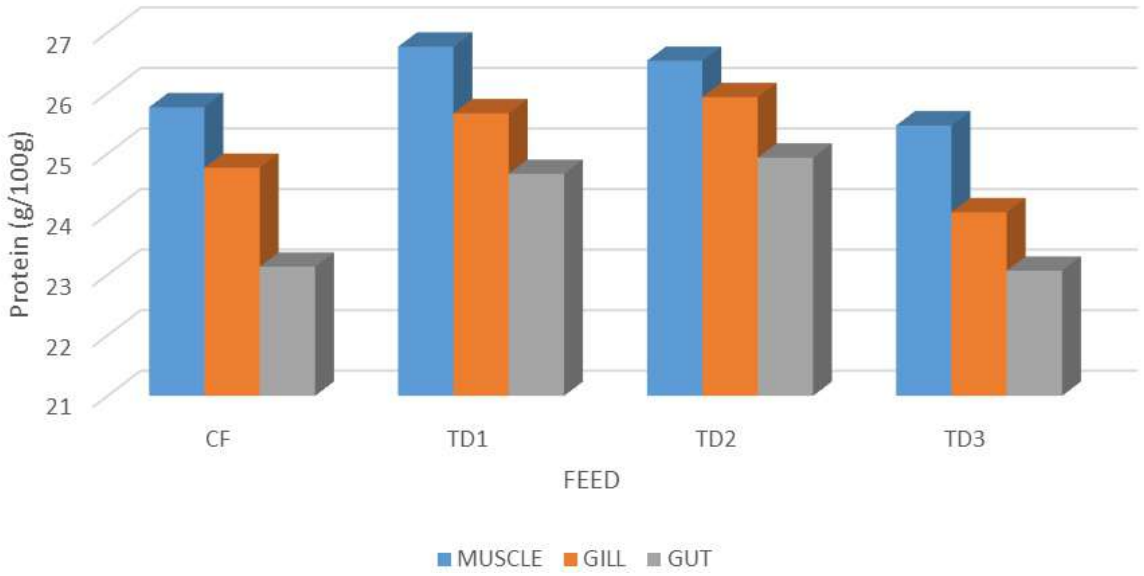

Fig. 1. Total protein in the muscle, gills and gut of *Etroplus suratensis* fed with experimental feeds

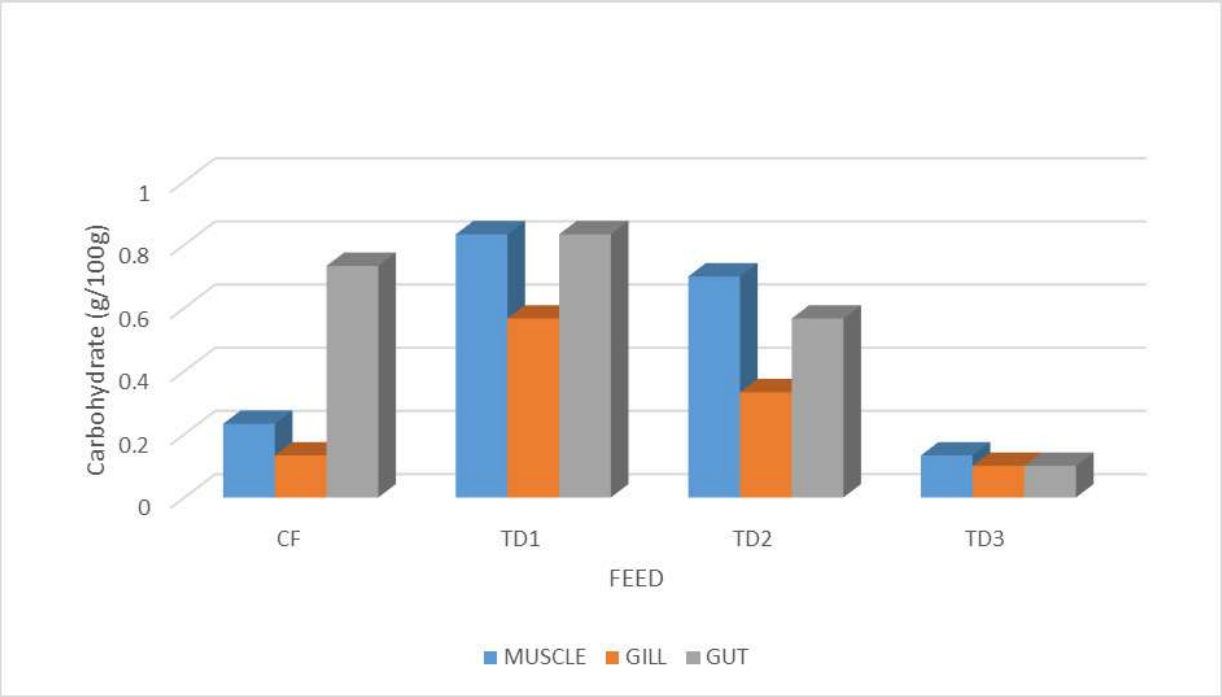

Fig. 2. Total fat in the muscle, gills and gut of *Etroplus suratensis* fed with experimental feeds

with the leaf powder of *Aegiceras corniculatum* could not produce any significant change in protein level (25.32±0.19), its protein concentration was almost similar to that of the control diet. The concentration of proteins in gills of fishes who were fed with CF, TD1, TD2 and TD3 were 24.77±0.15, 25.67±0.12, 25.93±0.15 and 24.03±0.12 respectively. Protein concentration in the gills of fishes, was remarkably higher (P<0.05) in TD2 and TD3. The amount of protein in the gut were 23.13±0.06, 25.07±0.67, 24.93±0.15 and 23.07±0.06, when fed with CF, TD1, TD2 and TD3. The increase in gut protein level when fed with TD1 and TD2 were found significant (Fig.1).

The total fat in the muscle (3.16±0.04) and gills (1.83±0.06) was significantly higher (P<0.05) in fishes fed with TD1. The total muscle, gill and gut fat estimated in CF and TD3 were 1.57± 0.06, 1.27±0.06, 1.2±0.1 and 2 ± 0.02, 1.81±0.15, 2.07±0.15, respectively. The amount of fat in the

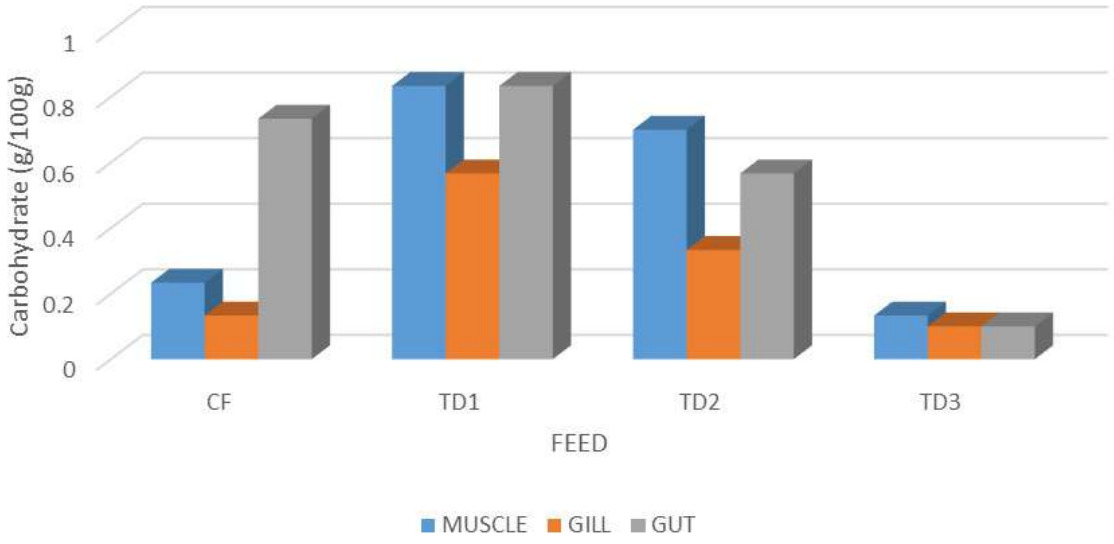

Fig. 3. Total carbohydate in the muscle, gills and gut of *Etroplus suratensis* fed with experimental feeds

muscle (2.07 ± 0.26), gills (1.83±0.06) and gut(1.93±0.15) of the fishes fed with TD2 also followed a significant increase (P<0.05) than the control (Fig.2). There is no significant variations in carbohydrate content among the fishes fed with Control Feed and experimental feeds. The carbohydrate content present in the muscle, gills and gut of fishes fed with CF were 0.23±0.06, 0.13±0.06 and 0.17±0.06 respectively (Fig.3).

Total ash content (Fig. 4) in the muscle, gill and gut of fishes fed with CF were 0.93±0.15, 6.83±0.12 and 0.73±0.06. Fishes fed with TD1 showed a significant increase (P<0.05) in total ash content in their muscle (1.02±0.01) and gills (8±0.017). Total ash in the gills (7.97±0.12) of fishes who were fed with TD2 also marked a significant increase in ash content than the CF. TD3 fed fishes could not produce any significant change

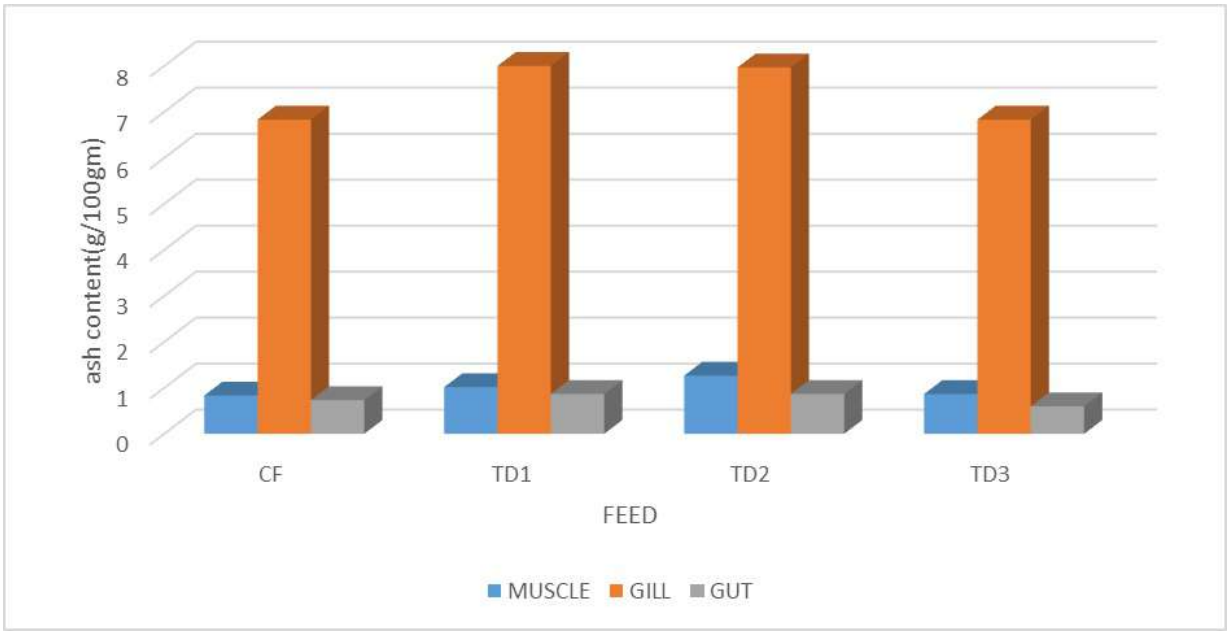

Fig. 4. Total ash in the muscle, gills and gut of *Etroplus suratensis* fed with experimental feeds

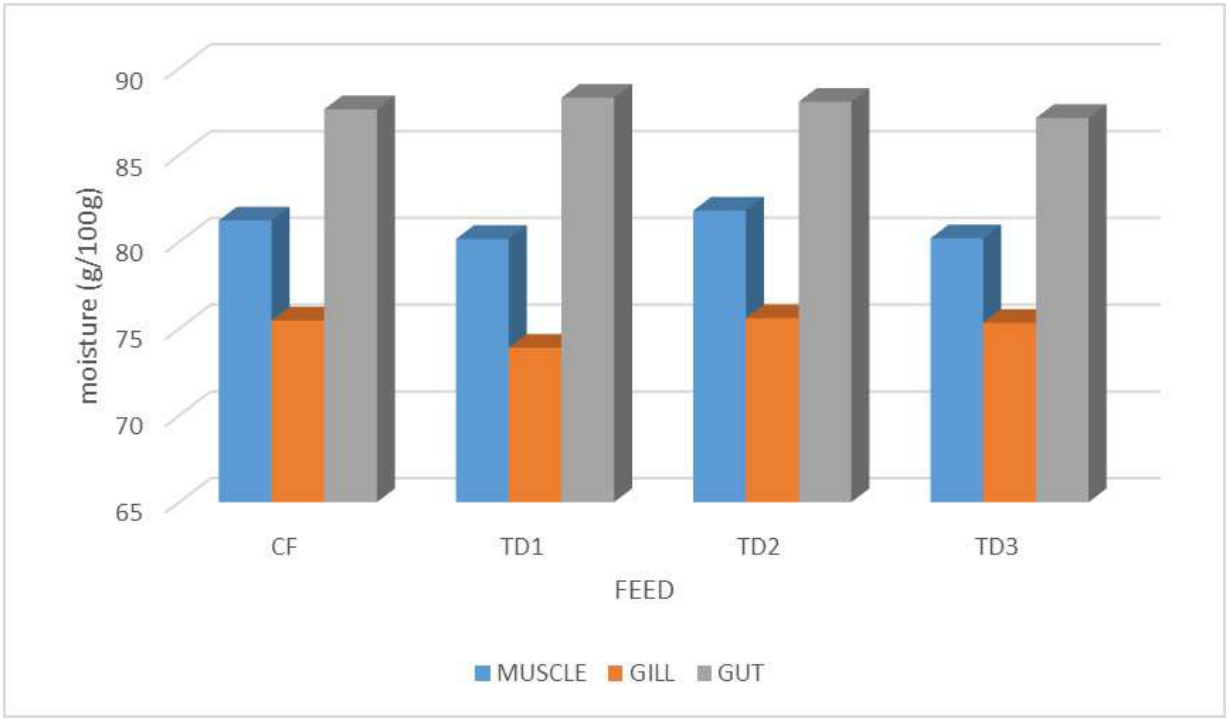

Fig. 5. Moisture content in the muscle, gills and gut of *Etroplus suratensis* fed with experimental feeds

in the ash content in its muscle (1.15±0.22), gills (6.83±0.06) or gut (0.94±0.33). The total moisture content (Fig.5) of TD1 (81.27±1.1,) TD2 (81.8±0.36) and TD3 (79.03±1.0) were significantly lower ($p>0.05$) than the CF (81.23±0.21). The moisture content of gills and gut of experimental fishes were 75.47±0.1 & 87.63±0.06 (CF), 73.9±0.2 &88.3±0.26 (TD1),75.6±0.53 & 88.07±0.45 (TD2) and 75.33±0.23 & 84.9±0.72 (TD3).

Discussion

The amount of protein and fat in fish feeds mostly defines it. All fish require relatively high levels of protein as a source of amino acids for protein synthesis. The protein in the feed is primarily needed for the build-up of (muscle) tissues and the fat is a major source of energy and for accretion of fat tissue. Fish, particularly carnivorous fish, have a limited ability to digest carbohydrates, hence the amount of carbohydrates in fish feeds is often low. As a consequence, the energy in the diet has to be derived from fat and it has a higher energy density than carbohydrates. For that reason, fish feeds are more concentrated and have thus also a higher protein level (up to about 40 – 45%) and energy density than feeds for terrestrial farm animals (Antonius, 2015).

According to Wilson, (1986) juvenile fishes require higher levels of protein for growth. Sumitra Vijayaraghavan *et al.*, (1978) observed that *E. suratensis* fed with 60- 87% protein had high food conversion efficiency. Results from the study of Palavesam *et al.*,(2008) clearly demonstrated that a diet having 35% protein supplemented with 0.5% L-lysine was found to be the optimum for maximizing the growth responses of *E. suratensis*. Earlier studies of Pillai and Ali (1997), Sarkar *et al.*, (2019) showed that, the fish fed on 25 and 32% protein containing diets had significantly lower carcass moisture, highest carcass protein and lipid as well as energy concentration. Aneykutty *et al.*, (1994) recommended an *Azolla* feed of 36.93% protein for the optimum growth of *E. suratensis*. The previous studies by Lekshmi & Prasad, (2014) found that 30% crude protein gives the highest growth and feed utilization. The essential fatty acids, for normal growth and survival of the fishes are provided by lipids .Cowey & Smith (1979), stated that 10%-20% of lipids in feed is beneficial to attain better growth and body composition in fishes. According to Ali (2004), 8.6% of lipid in fish feed improves the growth of *E. suratensis*.

The study of Raguram *et al.*,(2022) indicate that juvenile pearlspot can efficiently utilize starch as an energy source in their diet. There findings indicate that 35% dietary starch was more efficiently utilized than other carbohydrate sources by pearlspot.The amount of moisture is an index of economic value, stability and quality of feed (park 1990). A very small change in the moisture content can exert a large influence on the stability of the feed during storage (Jain1998). Fish feed contains usually 7 – 12% moisture. The moisture content should not be too high since a high moisture content can result in moulding and shorten the shelf life. Fish feeds have in usually a high ash level (about 6 – 10%) and this is due to the fish meal in the diet. Fish meal is produced from whole fish or from fish offal and contains thus also the skeleton and the bones. The skeleton and the bones are important sources of ash and minerals (e.g. calcium and phosphor) and fish feeds with a high fish meal levels have thus also a high ash level. The ash content in the feed is thus more or less an indication of the amount of fish meal in the diet. In contrast to terrestrial animals, are fish also able to absorb minerals and trace elements from the water through the gills and even through the skin. Marine fish, in contrast to fresh water fish, drink continuously and can also absorb minerals and trace elements this way.

Carnivorous fish do not have a need for dietary fiber but can tolerate some dietary fiber (e.g., <10% and at least 20% in the diets of rainbow trout and largemouth bass, respectively; Davies (2019). However, dietary fiber (usually 7% in compound aquafeeds) is essential for the health of many species of fish (particularly herbivores and omnivores) and may be beneficial for intestinal motility and health in some carnivorous fish (Davies 1985;)What is left after the determination of the moisture, protein, fat, ash and fibre is called the nitrogen free extract (NFE). Crude fibre and NFE are only present in ingredients derived from plant

sources.The fats, proteins and carbohydrates (the NFE fraction) are the major sources of energy in a feed or food. The energy densities of these three compounds are different and the amount of energy in a feed or food is related to the amount of fat, carbohydrates and proteins in the feed. The energy in a feed can be expressed as gross, digestible and metabolizable energy.

Based on earlier feeding experiments of Cowey and Smith (1979), Aneykutty *et al*., (1994), Pillai and Ali (1997), Ali (2004), Palavesam *et al*.,(2008), Lekshmi and Prasad, (2014) and Sarkar *et al*., (2019), in *E. suratensis*, we prepared four different types of feeds such as CF, TD1, TD2 and TD3 containing approximately 30-35% protein, 8-10% lipids, 10-12% ash and 9-11% moisture fulfilling the nutritional requirements of *E, suratensis*. The carbohydrates in the feed can be subdivided into crude fibre and NFE. Higher gross energy obtained in TD2 (4.33) and the lowest in TD3. Test diet 1 and 3 yielded the higher ME:DE Coefficient(99.6), while CF and TD2 yielded the lower coefficient of 99.5. Metabolizable energy of test diet 2 was higher followed by CF (13.72), TD1 (13.51) and TD3 (13.36).

According to Jacquot, 1961; Love, 1970 and Antony, 2015, fish body comprises 66-84% water, 15-24% protein, 0.1-22% lipid, 0.8-2% ash and usually negligible quantity (0.3%) of carbohydrate. The present study also showed the same range of proximate composition in experimental fishes. Growth response and feed utilization of fish fed the experimental diets were influenced by the mangrove feed additives (Table 2). In this study we observed a significant increase in total proein component in muscles of fishes when fed with TD1 and TD2. In earlier work of Sankar *et al*., (2011), it was stated that plant extract plays a vital role in growth, survival and disease resistance of the animal throughout the culture period .It was also supported by the earlier works of Citarasu (2000) and Dhas *et al*., (2017). Citarasu, stated that due to herbal maturation diet enriched Artemia feeding, the protein level in Penaeus spp get increased. Dhas *et al*., 2017, showed an increase in protein concentration in *E. suratensis* due to the herbal maturation diet administration. Similar observation msade by Md. Iftakharul *et al*., (2022), who showed that mixed mangrove leaf litter of *Sonneratia apetala*, *Avicennia officinali*s, and *Heritiera fome*s leaf litter had a positive effect on shrimp *Penaeus monodon* post larva performance. Mangrove leaves of *Rhizophora apiculata* has potential as animal feed because of their high protein content (Sari *et al*., 2022). A study by Yahaya and George (2013) showed that white mangrove leaves can be used as an additive in rabbits production at the inclusion level of 90g/kg of feed without causing any health challenge in the rabbits.

Fats, the biochemical composition, particularly lipids, may change the nutritional and sensorial quality of fish flesh (Arzel *et al*.,1994; Regost *et al*., 2001). The present study also showed variations in fat concentration when treated with formulated feeds. Fishes, treated with TD1, TD2 and TD3 showed better lipid concentration than those treated with CF. Eventhough those fishes with TD1 possessed the highest lipid content compared to the other ones. Muscles, gills and gut also showed the same gradation in lipid levels in all experimental setup. Dhas *et al*., (2017), from his study confirmed that the administration of the herbal maturation diet enhanced the level of the triglyceride, total protein, cholesterol and carbohydrate which can in turn leads to quick maturation and reproductive success in *E. suratensis*. Wulansari *et al*., (2020) proved that mangrove *Avicenia rumphiana* leaves flour supplementation in feed gave the lipid retention as same influence as vitamin C. There is no significant variations in carbohydrate content among the fishes fed with Control Feed and experimental feed. Comparatively low values of carbohydrates have been reported in all the previous studies. Carbohydrates formed a minor percentage of the total biochemical composition of the fish.

Moisture content in fishes greatly influence its physical appearance, taste, weight, freshness and moreover its shelf-life. The quantities of water in fish flesh are widely different but in most cases the range lies between 70-90% (Williams, 2018; Bashir *et al*., 2021). It has been evident from the earlier works of Pillai and Ali; (1997), Palavesam *et al*., (2008),

Antony; (2015) and Shilta *et al*., (2016) that the moisture content found in *E. suratensis* is in the range of 68- 88 %. The moisture content of the fish fed with TD1 and TD2 showed a slight variation in the amount of water from the control fish. Those one who fed with TD3 doesn't show any significant change in this regard. The moisture content in gut is higher than muscles and gills in all the experimental fishes. Ash content shows the total mineral content in the tissue. We observed a slight increase in ash content in those fishes fed with TD1,TD2 and TD3. The high value of ash in the fish species is an indication of its high mineral content like magnesium, calcium, potassium, and zinc. Interestingly gills mineral concentration goes up to 8.

It can be concluded that, all these growth promoting effects of selected mangroves may be due to the presence biologically active secondary metabolites present in it. That compounds may directly or indirectly alter the anabolic activity by boosting the activity of digestive enzymes (Cowey *et al*., 1981), or by acting as endocrine modulators (Chakraborty, *et al*., 2014) or by changing the anatomy of absorbing surface of their gut (Toutou *et al*.,2019). The beneficial effect of mangroves as feed additive may also be attributed to an increase in feed intake and high feed conversion efficiency. Further research is needed for the isolation and characterization of specific active component in order to validate the conclusion.

Summary

The results of the present study shows that *E, suratensis* perfomed better in terms of carcass composition, when fed with formulated feed containing leaf powder of selected mangrove species. Three different mangrove species *Rhizophora apiculata*, *Lumnitzera recemosa* and *Aegiceras corniculatum* were used as feed additive. Of these three species *Rhizophora apiculata* yield the best results, regarding the proximate composition of different parts of the fish. The least effective mangrove species was *Aegiceras corniculatum*. Findings from this study confirmed the beneficial effects of specific mangrove as a feed additive. However, further studies are needed in order to find out the specific component in this plant that may either increasing the efficiency of digestive enzymes, or enhancing the nutritive absorption through the intestine. These findings may help fish nutritionists to conduct further studies regarding growth performance and ingredient selection, thus lead to commercial feed formulation by incorporating this species in near future.

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Chapter 14

AN *IN VITRO* COMPARATIVE STUDY OF A SYNTHETIC INSECTICIDE METHYL PARATHION AND A BIOINSECTICIDE FROM *STREBLUS ASPER* (BISA) ON ANTIOXIDANT ENZYMES IN HUMAN LYMPHOCYTES

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Abstract Insecticide contamination is a serious health and environmental challenge that people of the world face today. Insecticides, particularly synthetic compounds, should be replaced with compensating compounds that pose less of a risk to the environment. Plants have biosynthesized a number of secondary metabolites to serve as defense chemicals against insect attacks. Such defensive chemicals are far superior to synthetic insecticides. Evidences are available that the oxygen free radical formation can be a factor in the toxicity of organophosphates. With this background, the present study was undertaken to evaluate the difference in response 'lymphocyte oxidative stress parameters' to a synthetic insecticide - 'methyl parathion' and a bioinsecticide isolated from *Streblus asper* (BISA). Dimethyl sulfoxide was used as a vehicle for dissolving the insecticides. The polyphenolic compound isolated from *S. asper* was found to have noteworthy insecticidal activity on *Dysdercus cingulatus* (Red cotton bug). The objective of this study was to compare the effect of synthetic insecticide, and a bioinsecticide on antioxidant enzymes in human lymphocytes in vitro, as it pertains to the role of free radicals. The effect of insecticides on antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase etc. and acetylcholinesterase have been intensively studied because of their functional importance, and their involvement in defense mechanism. The present study demonstrated that there was a significant decrease in activities of antioxidant enzyme in both insecticide treated samples. However, the present study proved that the magnitude of augmentation was higher in methyl parathion treated samples in comparison to bioinsecticide treated samples.

Key words: *Methyl parathion, Streblus asper, Bioinsecticide, Catalase, Glutathione peroxidase, Glutathione reductase, Superoxide dismutase*

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Introduction

Insecticides are chemicals useful in various agricultural practices. So, the farmers have relied heavily on the use of chemical insecticides to improve their crop production, which is now paying a huge toll on human health and the environment (Tripathi *et al.*, 2020). Due to indiscriminate use of insecticides, they found widespread in various parts of the environment and enter human body, where they persist and during the course of metabolism release several powerful free radicals that suppress the antioxidant mechanism (Singh *et al.*, 2018). Oxygen free radicals formation can be a factor in toxicity of organophosphates (Akhgari *et al.*, 2003; Pearson and Patel., 2016). Antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase etc. have been intensively studied, because of their functional importance, and their involvement in defense mechanism. Available literature so far conversed that the active enzymes associated with antioxidant defense mechanism are altered under the influence of insecticides both *in vivo* and *in vitro* (Dinu *et al.*, 2010).

Methyl parathion is an organophosphorus insecticide (OPI) with insecticidal properties. They

are highly effective, rapid in action and relatively economical. Due to their heterogeneous toxicity, they are used in plant protection, public health programmes, household sprays and for fumigation of storage go downs. Its indiscriminate usage causes environmental contamination, a wide spread ecological problem (Marina *et al*., 1997). Methyl parathion, became a serious health problem when illegally sprayed in private homes. It is stable and does not degrade to non-toxic substances (Kramer and Ho, 2002). Organophosphorus insecticides have been shown to produce oxidative stress due to generation of free radicals and alter antoxidant defense system in RBC (John *et al.* 2001). "Insecticides induced oxidative stress", a mechanism of toxicity has been a focus of toxicological research for the last decade. Oxidative stress results from an oxidant / antioxidant imbalance, an excess of oxidants or depletion of antioxidants (Salvemini and Cuzzocrea, 2002).

Plants appear to produce a wide variety of secondary metabolites as defensive weapons. Some of them function as bioinsecticides. They are popularized in order to achieve effective pest control and curb possible adverse effects on the environment. The advantages of botanical insecticides over synthetic insecticides are that they are safe to prepare and apply, safe on non-target organisms and to the environment. The use of botanical insecticides is now emerging as one of the prime means to protect crops and their products and the environment from insecticide pollution. Botanicals degrade more rapidly than most chemical insecticides and are, therefore, considered relatively environment friendly and less likely to kill beneficial pests than synthetic insecticides with longer environmental retention (Guleria and Tiku, 2009). Insecticidal activity of a bioinsecticide from stem bark of *S. asper* on red cotton bug (*Dysdercus cingulatus*) was reported earlier (Anila and Hashim, 2022).

Here an attempt is made to examine the difference between two unlike insecticide formulations on antioxidant enzymes in lymphocytes *in vitro*, as it pertains to the role of free radicals. Humans and other animals are exposed to myriad of chemicals, either concurrently or sequentially, throughout their life time. Often upon the metabolism of these chemicals, the formation of unstable free radical intermediates or reactive oxygen species can result. With this background, the present study was undertaken to evaluate the difference in response 'lymphocyte oxidative stress parameters' to a synthetic insecticide - 'methyl parathion (MP)' and a 'bioinsecticide from *Streblus asper* (BISA)'.

Materials and methods

Chemicals

All chemicals, including organic solvents, were purchased from Merk Groups, chemical company and all biochemicals, including Histopague 1077, RPMI-1640 medium, Fetal calf serum and biochemicals like Phenazine methosulfate, - NADPH, Nitroblue Tetrazolium chloride, Oxidised and reduced form of Glutathione were purchased from Sigma Chemical Company St Louis, USA.

Insecticide samples

Sample 1- Formulation of methyl parathion sample: Metacid 50 (MP 50% EC), a product of Bayer's was obtained from local sources.

Sample 2- Bioinsecticide from *S. asper*: The stem bark of the plant, *S. asper* was collected from Nagarcoil Forest (Tamil Nadu, India) and was authentically identified by a qualified botanist. Voucher specimens are deposited in the herbarium of Department of Botany, University of Kerala (Voucher No: KUBH 3902). Bioinsecticides were extracted from S. asper according to the procedure reported earlier (Anila and Hashim, 2022).

Isolation of lymphocytes

Human lymphocytes were isolated from fresh whole blood from healthy volunteers (25–35 years old) using Histopaque 1077. Briefly, anticoagulated blood was diluted with an equal volume of RPMI 1640 containing 10% FCS on ice for 30 min, underlaying it with Histopaque 1077 and centrifuging at 200g for 3 min at 4^{o}C. Lymphocytes were separated as a pink layer at the top of the Histopaque (Noroozi *et al*., 1998). Cells were washed with PBS and centrifuging at 200g for 3 min at 4°C (two times). Cells were rinsed with PBS and diluted to 5×10^{6} cells/ml.

Experimental design

Lymphocytes were divided into five sample groups and each sample contained 5×106 cells/ml.

Group I	Control	- 2 µl DMSO per 1 ml lymphocyte.
Group II	BISA 20	- (20 µg bioinsecticide from Streblus asper + 2µl DMSO) per 1ml lymphocyte.
Group III	MP 20	- (20 µg methyl parathion + 2µl DMSO) per 1ml lymphocyte.
Group IV	BISA 40	- (40 µg bioinsecticide from Streblus asper + 2µl DMSO) per 1ml lymphocyte.
Group V	MP 40	- (40 µg methyl parathion + 2µl DMSO) per 1ml lymphocyte.

Insecticide pretreatment

Dimethyl sulfoxide was used as a vehicle for dissolving the insecticides. Stock solutions of insecticides were prepared in dimethyl sulphoxide (DMSO) and diluted in phosphate buffered saline (PBS). The final concentration of DMSO was always 0.2% (v/v) (Lyman *et al*., 1976).

Cells were incubated with different concentrations (20 and 40 g) of insecticides for 30 minutes at 370C together with untreated control sample, which contains 2µl DMSO. These samples were then centrifuged at 200 × g for 3 minutes at 4°C. After treatment cells were centrifuged and washed twice with PBS (0.01 mol) at 200 × g for 3 minutes at 4°C and used for various biochemical analysis.

Biochemical assays

The biochemical assays included the determination of activities of enzymes involved in the antioxidant mechanism. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assessed on the basis of colour intensity of the chromogen produced by the reduction of nitro blue tetrazolium on addition of NADH, measured at 560 nm (Kakkar *et al*., 1984). Catalase (EC 1.11.1.6) was assayed by noting the decrease in extinction at 240 nm, followed by the decomposition of H_2O_2 (Maehly and Chance., 1954). Activities of glutathione peroxidase (Gpx, EC 1.11.1.9) (Paglia and Valentine., 1967) and glutathione reductase (GR, EC 1.6.4.2) (Paglia and Valentine 1967) were measured by following the decrease in the absorbance due to oxidation of NADPH. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was assayed by measuring the increment of absorbance at 340 nm due to the formation of 2,4-dinitrophenyl-S-glutathione from 1-chloro 2,4-dinitrobenzene (CDNB) and GSH (Habig et a., 1974). In addition, concentrations of thiobarbituric acid-reacting substances (TBARS) and reduced glutathione were estimated in human lymphocytes. Malondialdehyde was identified as the product of lipid peroxidation that reacts with thiobarbituric acid, forming a red-coloured compound absorbing at 535 nm (Haraguchi *et al.* 1995). Glutathione reacts with an excess of 5,5-dithiobis (2-nitrobenzoic acid), DNTB to produce a substance which has an absorption spectrum maximum at 305 nm (Anderson 1985). Protein content was estimated in samples after TCA precipitation. Protein reacts with Folin–Ciocalteau reagent to give a coloured complex which can be measured at 660 nm (Lowry, 1951).

Statistical analysis

The statistical analyses were performed with the software SPSS/Windows (SPSS 10.0. LNK). The results were expressed as the means ± SEM to show variations in a group. Differences were considered significant at $p \leq 0.05$.

Results

Effect of insecticides on the activity of Superoxide dismutase and Catalase in human lymphocytes

Significant reduction in the activities of superoxide dismutase and catalase were observed in all the insecticide treatments when compared to the control (Figure 1). When compared to low dose of BISA (20µg/ml) all other insecticide treated samples showed a significant decrease in the activities of superoxide dismutase and catalase. Compared to the

other treated samples the sample containing high dose of methyl parathion (40µg/ml) showed the most significant decrease. The inhibitory effect was dose dependent.

Effect of insecticides on the activity of Glutathione peroxidase & Glutathione reductase in human lymphocytes

A statistically significant decrement was observed in the specific activities of glutathione peroxidase and glutathione reductase with respect to the control groups (Figure 1). The samples containing high dose of insecticides showed a significant decrease in enzyme activity when compared to the samples containing the low dose of insecticides. 40µg concentration of BISA and methyl parathion exposure has brought about a profound reduction in enzyme activity when compared to the samples containing 20µg concentration of insecticide. Methyl parathion brought about a significant decline in the enzyme activity compared to BISA.

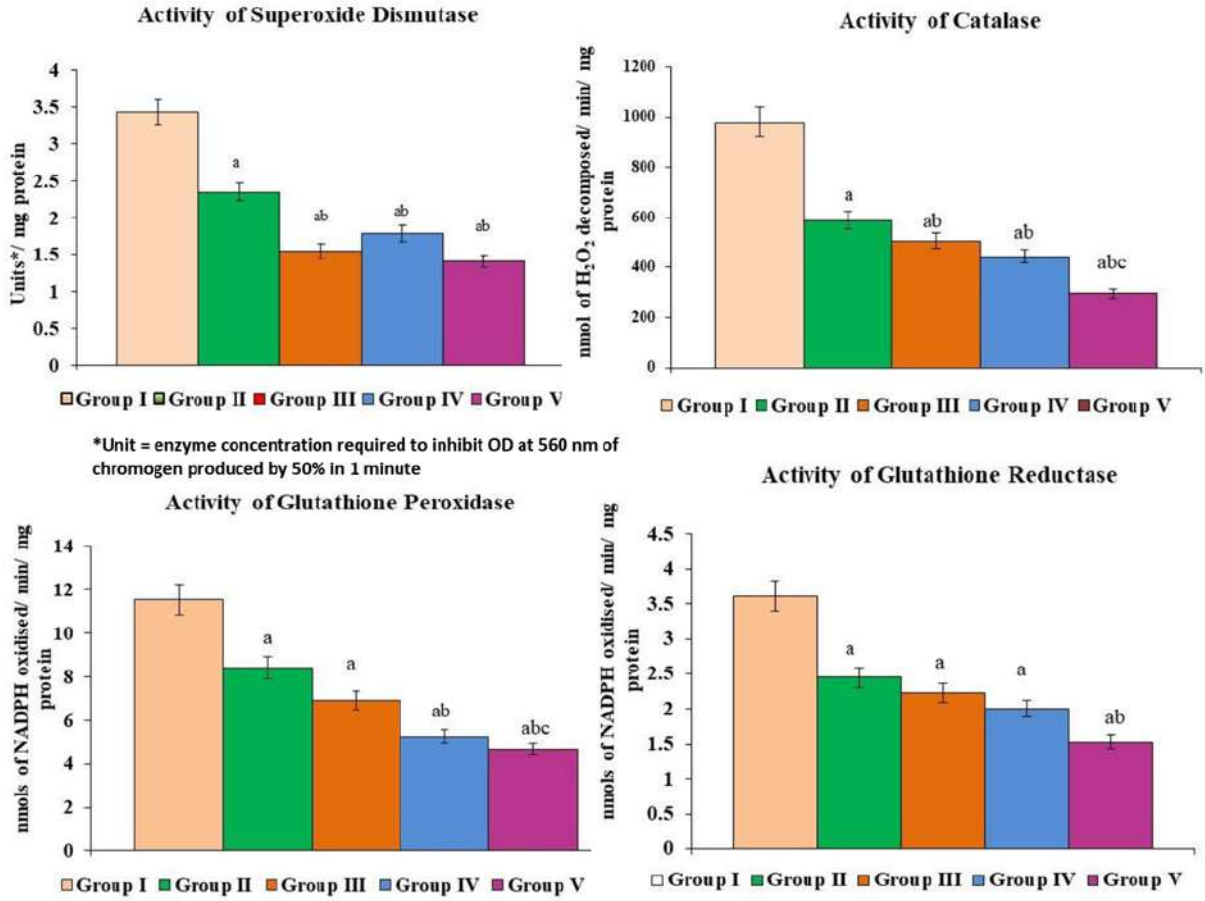

Fig. 1. Effect of insecticides on activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Values expressed as mean ± SEM, for n= 6, a: Groups II to V are compared to group I at $p \leq 0.05$, b: Groups III to V are compared to group II at $p \leq 0.05$, c: Groups IV and V are compared to group III at $p \leq 0.05$.

Effect of insecticides on activity of glutathione-S-transferase and the levels of reduced glutathione and TBARS

Figure 2. represents the effect of insecticides in the activity of glutathione S-transferase. With respect to the control sample all the treated samples showed a decline in enzyme activity. The sample containing low dose of BISA exhibited a lesser decline in enzyme activity compared to the other treated samples. A significant variation was observed in glutathione level in insecticides treated lymphocytes when compared to control group. With respect to the sample containing low dose of BISA, all the other treated samples exhibited a significant decrease in glutathione content. The samples containing the high dose of methyl parathion and

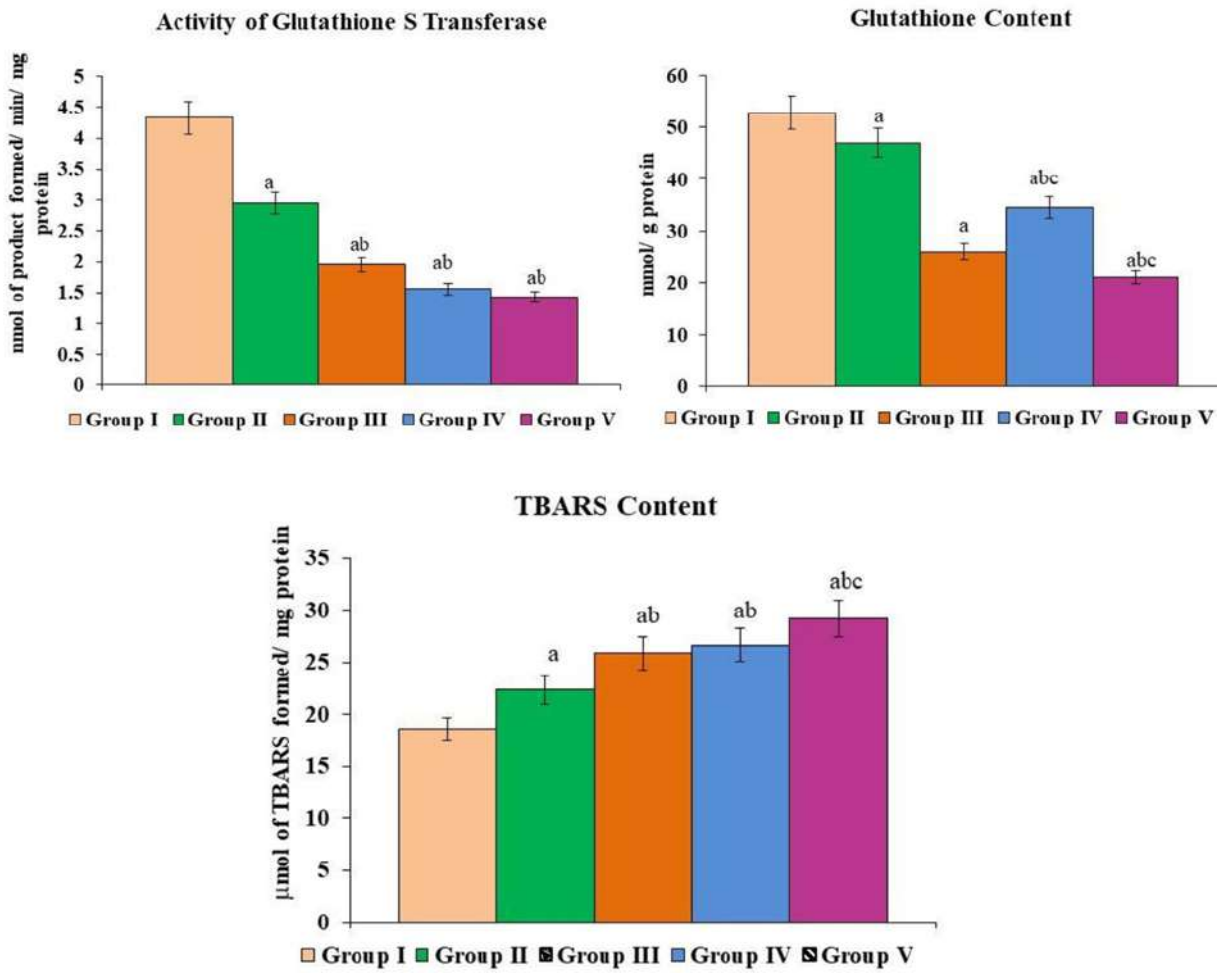

Fig. 2. Effect of insecticides on activity of glutathione-S-transferase and the levels of reduced glutathione and TBARS. Values expressed as mean ± SEM, for n= 6, a: Groups II to V are compared to group I at $p \leq 0.05$, b: Groups III to V are compared to group II at $p \leq 0.05$, c: Groups IV and V are compared to group III at $p \leq 0.05$

BISA showed the most significant decline. Methyl parathion was found to decrease the level of glutathione content than the BISA treated group. The decrease in glutathione content was found to be dose dependent.

The Figure 2. describes the enhanced effect in MDA content in lymphocytes of each insecticide treated sample, with respect to the control sample. A statistically significant variation was observed. Methyl parathion and BISA significantly increased TBARS level in a dose dependent manner. With respect to the control samples all the treated samples showed an elevation in the lipid peroxide content. A high level of TBARS was noted in the sample containing high dose of methyl parathion.

Discussion

The antioxidant system plays an effective role in protecting biological tissues below a critical threshold of reactive oxygen species those preventing oxidative stress (Sharma *et al.*, 2012). One of the important aspects of antioxidant enzymes is their synergestic functioning. Any impairment in one member of the system will influence the activities of the other enzymes (Lei *et al.*, 2016). Methyl parathion and BISA administration significantly lowers the activities of superoxide dismutase, glutathione content and its related enzymes viz. glutathione peroxidase, glutathione reductase and glutathione transferase. Catalase activities of lymphocytes are also found to decrease. While the level of MDA is found to increase significantly.

Studies on many organophosphorous insecticides has proved that they induce oxidative stress which leads to alterations at the cellular level (Sule *et al.*, 2022). Methyl parathion is classified by

WHO under the category of technical products as being hazardous that cannot be used indoors (Jaga and Dharmani., 2006). In a country like India, where plants are diverse, there is an urgent need to identify new bioinsecticides that can be used for pest management (Tripathi *et al*., 2020). Bioinsecticides are gaining popularity as non-target alternatives to traditional insecticides, providing a better track record of safety and sustainability in pest management techniques. The advantage of bioinsecticides over conventional insecticides is that they reduce or eliminate adverse, unintended targets (Seiber *et al*., 2018).

Often antioxidant enzyme levels are used as indicators of oxidative stress. This study examined the levels of antioxidant enzymes in cells exposed to the insecticides methyl parathion and a bioinsecticide isolated from *S. asper* (BISA) at two different concentrations. There was a significant decrease observed on the specific activity of antioxidant enzymes. A previous study of human serum samples from individuals poisoned with lindane or malathion indicated increased levels of superoxide dismutase and catalase following exposure (Igbedioh. 1991). A subchronic study on rats treated with moderate doses of lindane had increased SOD (Bebe and Panemangalore, 2003). In summary, it is obvious that the species, dose and length of exposure determine the extent to influence SOD and catalase activity. The findings in this study do provide insight into antioxidant enzyme's response to insecticide exposure in human lymphocytes.

A significant decrease in the level of catalase is noted on methyl parathion and BISA exposure. (Kono and Fridovich., 1982) reported that HCH administration decreases superoxide dismutase activities in rat testis which facilitates accumulation of superoxide radicals that inhibits catalase, causing augmentation of H_2O_2 generation. It is possible that a similar effect works here. The elevated H_2O_2 generation in response to the insecticide could be involved in the signal for catalase induction (Rushmore *et al*., 1991). Other studies have indicated that addition of H_2O_2 in murine cells increased the level of cell death in that culture, implicating the increase in intracellular ROS as a possible inducer of cell death (Valencia and Morán, 2004). Normally, H_2O_2 is inactivated by catalase or by glutathione peroxidase in a reaction in which GSH is used as a co substrate. Because catalase is compartmentalized into peroxisomes the detoxification of cytosolic and mitochondrial peroxides depends predominantly on glutathione peroxidase. The selenium containing enzyme glutathione peroxidase acts on GSH and H_2O_2 to produce oxidized glutathione (GSSG) and H_2O_2 (Prohaska, 1980). Ahmed *et al*., 2022 reported that initial exposure of dieldrin and phosphamidon also elicited catalase to decrease DNA damage induced and glutathine peroxidase to protect against the damage. Simultaneous exposure led to reactive oxygen species generation and oxidative stress.

The decreased GSH was due to increased demands of tripeptide for metabolism of lipid hydroperoxides by glutathione peroxidase or interaction of GSH with free radicals. Glutathione is oxidized by H_2O_2 to glutathione disulfide by the selenium containing enzyme, glutathione peroxidase, and also by other enzymes that may use lipid peroxides rather than H_2O_2 peroxide as the oxidant. Glutathione disulfide is subsequently reduced by gluthione reductase, using NADPH as the reductant (Nazıroğlu, 2009). Under oxidative conditions, the concentration of glutathione can be considerably diminished through conjugation to xenobiotics (Kasperczyk *et al*., 2004). Initial administration of methyl parathion under low concentrations, increased level of glutathione reductase activity under methyl parathion induced stress could be an adaptive mechanism to maintain intracellular GSH concentration (Mishra and Srivastava, 2015). The increased formation of lipid peroxide content in liver under methyl parathion stress could be due to decreased availability of cofactor GSH. Malathion induced impairment in glutathione status as well as the activities of related enzymes resulted in increased production of hydroperoxides as indicated by thiobarbituric acid reaction i.e. a higher lipid peroxide content. The alteration in thiol level might produce a major change in redox status of glutathione compounds thereby evoking serious defects in antioxidant defenses (Mathews and Devi 1994).

Glutathione S- transferase makes glutathione a more reactive nucleophile by binding glutathione in such a way that the sulfur is induced to ionize more completely and binding a second molecule close by so that reaction can be facilitated (Netto *et al.*, 2016). Methyl parathion bind to the active site of glutathione S-transferase which catalyses conjugation of OPI to GSH in larvae of yellow meal worm (Abel *et al.*, 2004). All these reports give ground to the fact that GST utilizes reduced glutathione for the detoxification of insecticides and fall in GST is suggestive of peroxidative damage. BISA is also found to restrain the activities of SOD, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase with the concomitant increase in thiobarbituric acid reactive substances (index of lipid peroxidation). All these were accompanied by reduction in glutathione content. Antioxidant defense mechanism which plays a pivotal role in protecting tissues from harmful effects of elevated reactive oxidant species generation is suppressed. Overall, this study provides a foundation for in vitro studies on immune cells, chemical exposure and enzyme analysis. Increased ROS generation was detected and was more enhanced for the organophosphorous insecticide than the plant based insecticide BISA. This clearly indicates the modulation of the GSH enzyme system and the potential onset of oxidative stress following exposure to these insecticides, particularly the organophosphorous insecticide. Thus it is apparent from the above discussion that methyl parathion induces reactive oxygen species and decreases the level of antioxidant enzymes significantly compared to BISA which brings about similar effects at a smaller degree.

Conclusion

Exposure to insecticides causes a disturbance in prooxidant/antioxidant systems resulting in a myriad of different oxidative challenges. Oxidative stress may be initiated by a decline in the antioxidative defense system or by factors that may decrease the concentrations of antioxidants. Oxidative stress describes a condition in which cellular antioxidant defenses are insufficient to keep the levels of reactive oxygen species below a toxic threshold. The role of reactive oxygen species in insecticide toxicity was examined by assessing the levels of oxidative enzymes in lymphocytes treated with two different insecticide constituents – BISA and methyl parathion. Often when cells or organisms are exposed to chemicals, reactive oxygen species are generated and as a result, enzyme induction occurs to offer cellular protection against these reactive oxygen species. Thus oxidative enzymes are often used as indicators of oxidative stress of cells. The enzymes of interest are superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase. Quantitation of each enzyme was monitored using spectrophotometric techniques.

The most robust and significant alteration in antioxidant system is decrease in GSH concentration; as a result of release of free radicals from detoxification of insecticides. The antioxidant enzymes stabilize the unstable free radicals normally produced in the system by giving them the electron to 'calm down' and get consumed in the process of detoxification. When oxidative stress occurs, certain by-products are left behind that are excreted by the body like malondialdehyde from damaged lipids and proteins. The higher the levels of these markers, the greater will be the chance of forming oxidative stress. It can be concluded from the study that both methyl parathion and BISA at a concentration of 20 g/ml and 40 g/ml induced oxidative stress in human lymphocytes in a concentration dependent manner as evidenced by the significant decrease in activities of superoxide dismutase, catalase glutathione peroxidase, glutathione reductase, and glutathione S- transferase. Insecticides induced oxidative stress by increasing pro-oxidants and decreasing some of the major antioxidants in the human lymphocytic cells. Organophosphorus insecticide methyl parathion induced a greater level of oxidative stress compared to the BISA as evidenced by the significant alteration in antioxidant enzyme activity. Because environmental safety is a global concern, we must raise awareness among farmers, manufacturers, government agencies, policymakers, and the general public about the benefits of using bioinsecticides for pest

management. More research is needed for the development of cost effective biopesticides in the developing countries so as to to bring sustainability to global agriculture for food and feed security.

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Chapter 15

STANDARDIZATION OF MICRO-MACROSCOPIC AND PHYSICOCHEMICAL PARAMETERS OF AN ETHNOBOTANICAL PLANT; *ALANGIUM SALVIIFOLIUM* (L.F.) WANGERIN LEAF

Parvathy A P, Saranya Murali, Vilash V

Abstract *Alangium salviifolium* is commonly known as sage leaved alangium, stone mango or hill sack tree. The plant has been used to treat various ailments in traditional and classical medicine. The species *A. salviifolium* is a very scarcely studied group as far as its pharmacognostical point of view. In the present study, micro-macroscopic characterization, powder analysis, fluorescence analysis, and physicochemical characterization of *A. salviifolium* leaves were carried out. Microscopic characterization of *A. salviifolium* leaf includes anatomical studies and quantitative leaf microscopy. Analysis of *A. salviifolium* leaf powder revealed the presence of vessels, tracheids, calcium oxalate crystals and starch grains. Chemical tests of *A. salviifolium* leaf powder was conducted with different reagents and observed under visible light and UV light. The extractive values of the drug were; alcohol extractive value 0.66%, petroleum ether extractive value 3.86 % and water extractive value 4.60%. The extractive values may help in identifying the presence of several types of adulterations. All the data obtained from the present study will provide immense help in authenticating the plant materials even in dried, crushed or powdered form. The present investigation put forward important standardization parameters for a meagerly studied medicinal herb *A. salviifolium*.

Key words: *Alangium salviifolium, micro-macroscopic characterization, powder analysis.*

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Introduction

Alangium salviifolium (L.f.) Wangerin of Cornaceae family is an important medicinal plant with a wide spectrum of biological activity. The leaves of *A. salviifolium* are used as astringent, laxative, refrigerant. It is also used for the treatment of rheumatism, leprosy, gastric ulcers, wound healing, epilepsy, scabies, gonorrhea, jaundice, hepatitis, diabetes, syphilis and asthma (Kijima *et al.*, 1992). In Ayurveda, the roots and the fruits are used for the treatment of rheumatism, burning sensation and haemorrhages. The root barks of *A. salvifolium* were used externally as an antidote against snake/ scorpion, rabbit, rat, dog bites. In Philippines, the roots and the fruits are used for treatment of rheumatism and hemorrhoid externally (Rajamanickam *et al.*, 2009). Root bark is an antidote for several poisons (Warrier *et al.*, 1993). Fruits are sweet, refrigerant, emetic and antiphlegmatic agent which is used as a poultice for treating burning sensation and also used as an antidote for scorpion stings. In traditional medicine, the roots and fruits of this plant are externally used for the treatment of bites by rabbits, rats and dogs. Seeds are anticancerous, diuretic, antimicrobial, and antiepileptic (Kumar *et al.*, 2010).

Materials and methods

Materials

The leaf of *A. salviifolium* were selected based on its medicinal importance in curing various aliments mentioned in classical and folk medicines

Chemicals

All the chemicals used in this study were obtained from different sources as stated below

Name of Chemical	Company
Saffranin	SD Fine Chem Ltd., Mumbai
NaOH	SD Fine Chem Ltd., Mumbai
Methanol	SD Fine Chem Ltd., Mumbai
Toluene	Ranbaxy Laboratories Ltd.,
HCL	SD Fine Chem Ltd., Mumbai
HNO3	SD Fine Chem Ltd., Mumbai
FeCL3	SD Fine Chem Ltd., Mumbai
Grams Iodine	SD Fine Chem Ltd., Mumba
Glycerine	SD Fine Chem Ltd., Mumbai
H2SO4	SD Fine Chem Ltd., Mumbai
K2Cr2O7	Ranbaxy Laboratories Ltd., Mumbai
Petroleum ether	SD Fine Chem Ltd., Mumbai

Instruments

Name of the Instrument/Equipment	Company and make
pH Meter	Eutech-ION 2700, Singapore
Microscope with camera attachment	Carl Zeiss, Germany
Weighing balance	Shimadzu, Japan

Other minor equipments used for this study were the Bunsen burner, Gooch crucible, micropipettes, needle, etc.

Methods

1. Collection and authentication of plant material

A. salviifolium, leaf was collected from Kallar, Thiruvananthapuram district of Kerala, India and authenticated by the plant taxonomist of the department.

2. Macroscopic and organoleptic characterization

Fresh leaves of *A. salviifolium* were collected and analyzed for both qualitative and quantitative traits. The following macroscopic and organoleptic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste were evaluated (Trease and Eavans, 2002).

3. Microscopic characterization

3.1. Anatomical studies of the leaf

Freehand transverse sections of fresh leaves lamina and midrib were prepared, stained with safranin, mounted on glass slides using glycerin, observed under a light microscope with camera attachment and photomicrographs were taken (Trease and Evans, 2002).

3.2. Quantitative leaf microscopy

The following quantitative microscopic studies of *A. salviifolium* leaf were carried out according to standard procedures.

3.2.1 Determination of stomatal number and stomatal index

A piece of leaf was cleared by boiling with sodium hypochlorite solution. The upper and lower epidermis was peeled separately. The peeled epidermis was placed on the slide and mounted with glycerine. The average number of stomata per square mm of the epidermis of the leaf is calculated from the microphotographs taken using camera attached microscope. Values of the upper and lower epidermis were determined separately (Anonymous, 2011) using the equation:

Stomatal index (SI) = S X 100/E + S.

Where, S= the number of stomata per unit area and E= the number of epidermal cells in the same unit area of leaf.

3.2.2. Determination of vein- islet number and vein-let termination number

The vein- islet number is the average number of vein- islet per square mm of a leaf surface midway between midrib and margin and the average number of terminated vein-let islets per square mm of a leaf was taken as vein-let termination (Trease and Evans, 2002)

3.3. Powder analysis

Fresh leaves were separated from the collected plant, thoroughly washed with fresh water, shade dried and powdered. The leaf powder is boiled with chloral hydrate for 5-10 min, and then stained with phloroglucinol, safranine, glycerine and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains (Kokate, 2003, Khandelwal, 2002).

4. Fluorescence analysis

The fluorescence character of the plant powders (40 mesh size) was studied both in day light and UV light (254 and 366nm) after treatment with reagents like sodium hydroxide, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride, sulphuric acid, potassium dichromate etc. The colour changes were noted (Chase and Pratt, 1949, Kokoshi *et al*., 1058)

5. Physicochemical analysis

Different physicochemical parameters of *A. salviifolium* leaf powder was determined according to the quality control methods for medicinal plant materials (Anonymous, 2011).

5.1. Determination of pH

pH 1% solution: Dissolved 1 g of the leaf powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

pH 10% solution: Dissolved 10 g of the leaf powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode (Vilash *et al*., 2016).

5.2. Determination of alcohol soluble extractive

Macerated 5 g of air dried drug coarsely powdered with 100 mL of ethanol of specified strength in a closed flask for 24 h, shaking frequently for 6 h and allowed to stand for 18 h. The filtrate is evaporated to dryness in a tared flat bottom shallow dish, dried at 105°C and weighed. The percentage of alcohol-soluble extractives is calculated with reference to the air dried drug.

5.3. Determination of water soluble extractive

Followed as directed for the determination of alcohol soluble extractive using water instead of ethanol.

5.4. Determination of petroleum ether soluble (40-60° C) extractive

Proceeded as directed for the determination of alcohol soluble extractive, using petroleum ether (40 – 60° C) instead of ethanol.

5.5. Loss on drying

About 2-3 g of powder is accurately weighed in a China dish and kept in a hot air oven maintained at 105° C for 5 h. After cooling in a desiccator, the loss in weight was recorded. Loss on drying (%) LOD = Loss in weight of the drug in g x 100.

5.6. Swelling Index

One gram of drug powder was taken in a measuring cylinder (25 mL) and suspended in 25 mL distilled water for 1h by thoroughly mixing every 10 min. After 3h, the volume in mL occupied by the plant material including any sticky mucilage was measured. The experiment was repeated three times for accuracy and the swelling index was calculated.

5.7. Foaming index

Finely divided crude drug (1g) was transferred into a 500 mL flask containing 100 mL of boiling water. The mixture was maintained at moderate boiling for 30 min. It was cooled and filtered into a 100 mL volumetric flask and added sufficient water to the filtrate to dilute the volume. The prepared decoction was poured into 10 stoppered test tubes each 1mL, 2mL…10mL. The volume of the liquid in each tube was adjusted (10 mL) with water. The tubes were duly stoppered and shaken then in a lengthwise motion for 15 sec (two shakes per second) and allowed to stand for 15 min. The foam height in each tube was measured. Foaming index=1000/a, a is the volume of the plant decoction for forming foam of height 1cm.

5.8. Determination of total ash

About 2-3g weighed crude drug powder in a tared silica dish was ignited and weighed. Scattered the powder drug on the bottom of the dish and incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, cool and weighed. The % w/w of total ash with

reference to the air–dried drug was calculated.

5.9. Determination of acid insoluble ash

Boil the ash for 5-10 min with 25 ml of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible, washed with hot water, ignited and weighted. The percentage of acid insoluble ash is calculated with reference to the air-dried drug. The %w/w of acid insoluble ash with reference to the air-dried drug was calculated.

5.10. Determination of water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5-10 min. Collect the insoluble matter in Gooch crucible, wash it with hot water and ignite it in a crucible for 15 min at a temperature not exceeding 450°C. Subtract the weight of insoluble matter from the weight of ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash is calculated with reference to the air dried drug. The % w/w of water- soluble ash with reference to the air dried drug was calculated.

6. Statistical Analysis

All the data were expressed as mean + standard deviation (SD)

Results

Macroscopic and organoleptic characterization

Macroscopic and organoleptic characters of the fresh leaves were noted and the results were presented in the Plate 1 and Table 1.

Table 1. Macroscopic and organoleptic characters of *A. salviifolium* leaf

Macroscopic parameters		Observations
Phyllotaxy		Alternate
Type		Simple
Lamina size	Length	10 ~16 cm
	Width	2 ~ 3.5 cm
Shape		Oblong - lanceolate
Apex		Attenuate or subacute
Margin		Entire
Venation		Reticulate
Base		Cuneate
Petiole		1± 0.45 cm
Surface		Glabrous and glossy above, glabrescent or puberulous below
Odour		Characteristic smell
Taste		Tasteless
Colour	Upper	Dark green
	Lower	Light green

Results are out of 25 observations ± SD

Microscopic characterization of *A. salviifolium* leaf

Anatomical studies of leaf

Microscopic characters of *A. salviifolium* was represented in Plate 2. The leaf of *A. salviifolium* is a typical dorsi ventral dicot leaf with a prominently projecting abaxial midrib and a thin lamina. The midrib of the leaf is ~350µm thick and ~250µm wide. It is flat on the adaxial side; thick and semicircular on the abaxial side. The transverse section of the midrib region consists of a single layered, vertically elongated epidermal cells (Plate 2a). Adaxial epidermal cells of the midrib region consist of smaller squarish cells. On the abaxial semicircular part, the midrib epidermis is thin. The ground tissue in the midrib region consists of compactly arranged thin walled angular parenchyma cells (Plate 2a). The adaxial foliar epidermis is imperforate and is composed of pentahexagonal thick walled cells. It is profusely perforated with

Plate 1. *Alangium salviifolium* (L. f.) Wangerina. a. Habitb. b. Leaf upper surfacec. c. Leaf lower surface

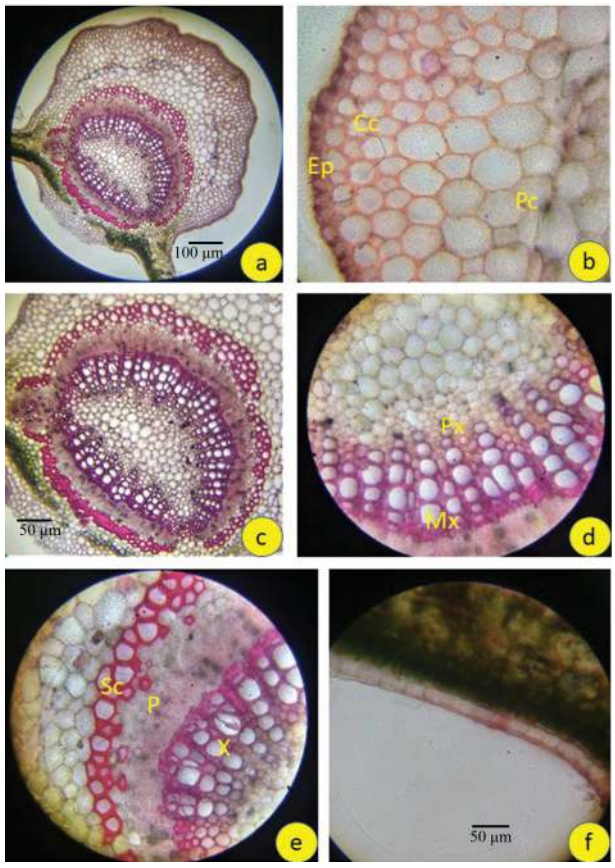

Plate 2. Microscopic characterization of *A. salviifolium* leaf a. T.S of Midrib b. Epidermal region c. Vascular bundles d. Linearly arranged xylem (Px-Proto xylem, Mx- metaxylem) e. Sc- Sclerenchymatus bundle shaeth f. Leaf blade

anamocytic stomata.

The mesophyll tissue is differentiated in to adaxial layer of cylindrical compact palisade cells and 4-5 layers of mesophyll cells. The vascular bundle of the midrib is single, prominent and collateral and consists of short, continuous strict parallel lines of xylem elements and thick phloem located on the lower part of the xylem. The vascular bundle is surrounded by a clearly remarkable sheath of sclerenchyma cells (Plate 2c). The lamina is 110µm thick. It is smooth and even on both surfaces. The adaxial epidermis consists of very thick, squarish or rectangular thick walled cells which are 20- 30 µm thick. The abaxial epidermis is thin, cylindrical and contains stomata.

Quantitative leaf microscopy

Quantitative leaf characteristics were observed and the result were shown in the Table 2.

The leaf showed densely reticulate venation with thick primary veins and gradual thinning secondary and tertiary veins. Polygonal vein islets with thick and straight marginal veins. Leaf showed unbranched, thick and straight simple vein terminations together with branched, dichotomously complex terminations. Stomata are present only on the lower surface of the leaf.

Powder Analysis

The powder as such is green in colour (Plate 3). Analysis of *A. salviifolium* leaf powder revealed the presence of vessels, tracheids, calcium oxalate crystals and starch grains (Plate 3).

Fluorescence analysis

A chemical test of *A. salviifolium* leaf powder was conducted with different reagents and observed under visible light and UV light (Plate 4). The results were compared with their respective observations in visible light and they were represented in the Table 3. and Plate 4.

Physicochemical Analysis

Physicochemical parameters of *A. salviifolium* leaf powder were evaluated and the observations are present in Table 4

Plate. 3 Powder analysis of *A. salviifolium* leaf
a. Leaf powder b. Xylem fibre c. Tracheid d. Xylem vessels e. Prismatic crystals f. Tracheid g. Calcium oxalate crystals

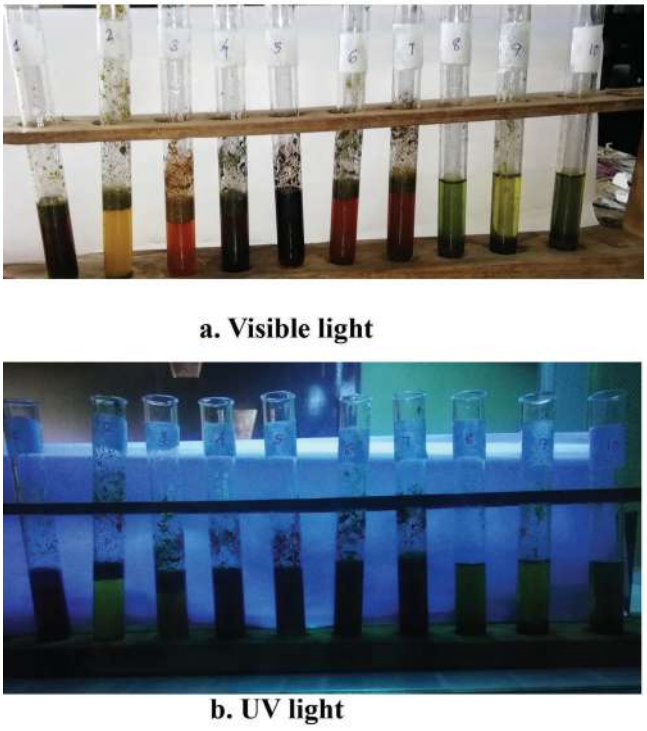

Plate 4. Fluorescence analysis of A. salviifolium leaf powder
a. Colour developed under visible light
b. Fluorescent Colour developed under UV light (2, 8 and 9)

Table 2. Quantitative leaf microscopy of A. salviifolium leaf

Parameters	Mean ± SD
Stomatal number – upper surface	0
Stomatal number – lower surface	68.50 ± 5.70 /sq mm
Stomatal index– upper surface	0
Stomatal index – lower surface	51.54 ± 2.25 / sq mm
Vein islet number	12.25 ± 2.32 /sq mm
Vein let number	10.50 ± 4.52 / sq mm

Table 3. Observations of *A. salviifolium* leaf powder under visible and UV light after reacting with different reagents.

Treatments	Visible light	UV light
1. Powder +5ml NaOH	Dark green PMS 3435	Black PMS 433 2X
2. Powder+5ml FeCl3(5%)	Yellow PMS 129	LightgreenPMS36 6*
3. Powder+ 5ml HCl (0.1 N)	Red PMS 165	BlackPMS433 2X
4. Powder+5ml HNO3(40%)	Green PMS 5605	BlackPMS433 2X
5. Powder+5ml H2SO4(98.8%)	Brown PMS4975	BlackPMS433 2X
6. Powder+5ml Gram's iodine	Orange PMS151	BlackPMS433 2X
7. Powder +5ml K2Cr2O7	Dark orange PMS1535	BlackPMS433 2X
8. Powder+5ml Ethanol (100%)	Green PMS370	GreenPMS366*
9. Powder+5ml Methanol (80%)	Light greenPMS 382	GreenPMS374*
10.Powder+5mlToluene(92.14 %)	Green PMS378	GreenPMS383

Pantone matching system *Presence of florescence

Table 4. Physicochemical parameters of *A. salviifolium* leaf powder

pH of water solution	1% w/w	6.08±0.34
	10% w/w	6.34±0.32
Alcohol soluble extractive		0.66±0.12%
Water- soluble extractive		4.6±0.43%
Petroleum ether soluble extractive		3.86±0.12%
Loss on drying (LOD)		0.10±0.03
Swelling index		1.00±0.12
Foaming index		Less than 100
Total ash		53.45±3.54%
Water- soluble ash		4.09±0,23%
Acid insoluble ash		2.15±0.12%

Mean of 6 observations ± SD

Discussion

The species *A. salviifolium* is a very scarcely studied group as far as its pharmacognostical point of view. Regarding the macroscopic characters (Table 1), the leaf colour is typically dark green and has a characteristic smell. Microscopic characterization of *A. salviifolium* leaves such as the compactly arranged thin walled angular parenchyma cells of ground tissue in the midrib region, short, presence of anamocytic stomata in the lower epidermis, continuous strict parallel lines of xylem elements and thick phloem (Plate 2) in the vascular bundle with an outer remarkable sheath of sclerenchyma cells (Plate 2) make the midrib a key identifying region of the leaf.

The undifferentiated mesophyll cells are found in the laminar region. Quantitative leaf characteristics such as polygonal vein islets with thick and straight marginal veins, unbranched, thick and straight simple vein terminations together with branched, dichotomously complex terminations etc. were helps in identifying the plant even in crushed form. The powder analysis study of *A. salviifolium* shows the presence of vessels, tracheids and calcium oxalate crystals. The plant powder exhibited fluorescence phenomenon primarily due to the peculiarities of its chemical constituents. Through these analytical techniques, the quality of the crude drug can be assessed which ultimately helps people to identify the genuineness of the drug and this parameter can be used as a fingerprint for the identification of plant material (Reddy and Chathurvedi, 2010). Fluorescence analysis is important in distinguishing the drug from its

adulterants in its powdered form (Ansari, 2006; Gatade *et al.*, 2015). In the present study a comparative account of the fluorescence analysis treated with different chemical reagents and observed at visible light and UV light has also been observed.

Evaluation of the physicochemical parameters aids in the formation of pharmacopoeial standards. Various physicochemical characteristics of the drug are depicted in (Table 4). Extraction of the drug from plant powder with different solvent give a particular amount yield of crude drugs; alcohol is 0.66%, petroleum ether 3.86 % and water 4.6%. This may also help in identifying the presence of several types of adulteration and exhausted materials.

A great bulk of information on identifying the purity and quality of plant material can be obtained from its macroscopy, microscopy, powder characters and physicochemical parameters. The present investigation put forward important standardization parameters for a meagerly studied medicinal herb *A. salviifolium*. All the data obtained from the present study will provide immense help in authenticating the plant materials even in dried, crushed or powdered form.

Conclusion

The present investigation put forward important standardization parameters for the

important medicinal herb *A. salviifolium* which may help as a tool for identifying the authenticity of plant material. The pharmacognostical evaluation of *A. salviifolium* revealed the presence of characteristic features like macroscopic characters, microscopic features like the presence of anamocytic stomata in the lower epidermis, parallel lines of xylem elements, remarkable sclerenchyma bundle sheath cell, powder studies, fluorescence analysis, physicochemical parameters etc. will be extremely beneficial to identify the authenticity of *A. salviifolium* leaf even from the crushed or powdered form.

Acknowledgment

The authors are grateful to the Principal, Sree Narayana College, Kollam for providing the facility for carrying out the study. We thank the 'Fund for Improvement of S & T Infrastructure (FIST)' of the Department of Science & Technology, Government of India for the instruments we used throughout the study were purchased under DST FIST scheme of Government of India.

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Chapter 16

IN VITRO REGENERATION AND CALLUS INDUCTION IN *HEMIDESMUS INDICUS* (L.) R. BR., AN IMPORTANT ETHNOBOTANICAL MEDICINAL PLANT

Lakshmi A P, Athira Madhav Surya, Chithra Vijayan

Abstract *Hemidesmus indicus* (L.) R. Br., commonly known as Indian sarsaparilla, is an ethnopharmacologically important plant belonging to the family Apocynaceae. The plant is facing an imminent danger of threat because of habitat destruction and illegal collection due to its high market demand. Conventional methods for the propagation of *H. indicus* have a lot of limitations. The present study evaluates the effect of different explants and growth regulators to establish an efficient micropropagation protocol for the rapid multiplication of *H. indicus*. Nodal and internodal segments were used as explants for the study. The highest rate of shoot induction was observed in medium fortified with IBA (0.5mg/l) and Kinetin(1mg/l)while minimum shoot induction was reported with NAA (1mg/l). The combination of NAA and 2,4-D (0.5mg/l+1mg/l) produced white callus. The callus developed in medium supplemented with 2,4-D and BAP (1mg/l +1mg/l) was green in colour. The developed plants were acclimatized in pot successfully and also maintained in normal environment. The present in vitro procedure can be used in conservation and mass propagation of this ethnobotanically important plant.

Keywords: Hemidesmus indicus, micropropagation, explant, nodal segments, internodal segments, medicinal plant, in vitro regeneration, MS medium.

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Introduction

The increasing demand of medicinal plants necessitates their large-scale propagations and to cope with it, *in situ* and *ex situ* conservation techniques are used which helps in protecting and conserving important medicinal plants (Paunescu, 2009). Tissue culture technique for plant propagation is essential for conservation as it supplements seed banking as well as other *ex situ* techniques (Thorpe, 2007). *In vitro* propagation methods offer powerful tool for conservation of germplasm and mass-multiplication of threatened plant species (Murch *et al*., 2000).

Hemidesmus indicus (L.) R. Br is popularly known as 'Indian sarsaparilla' formerly belonging to family Apocynaceae. It is one of the most widely used medicinal plant in India, well known for its medicinal values. It has a high degree of effectiveness against gout, dyspepsia, boils, leprosy and is reported to suppress both humoral and cell mediated immunity (Gupta, 1981; Khory and Katrak, 1981). The present investigations were undertaken to evaluate, the effect of different growth regulators and explants thereby establishing an efficient *in vitro* propagation protocol for plant production to fulfil the demand in pharmaceutical industries.

Materials and methods

Plant material

The material for the present study was collected in and around Thiruvananthapuram district and was maintained in the Botanical Garden, Department of Botany, Sree Narayana College Kollam. Nodal and inter-nodal segments were used as explants for the

present study.

Culture medium

MS (Murashige and Skoog, 1962) medium was used in all the experiments. Stock solutions of micro and macro nutrients and vitamins were prepared separately and stored in 4±°C. Hormones were added to the medium either alone or in combination and pH was adjusted to 5.8 before autoclaving at 120°C for 15 minutes. The culture was maintained at a temperature of 25°C with a photoperiod of 16h/day under 50 Mmol /m light intensity provided by fluorescent lamps.

Explant sterilization

Explants were collected from healthy plants and were immersed in 1%(w/v) Labolene (Qualigenes, India) for 10 minutes and kept under running tap water for 30 minutes. Surface sterilisation was carried out using 0.1% Bavistin(w/v) and 0.1% mercuric chloride (1 min).

Shoot induction

Under aseptic conditions nodal explants were inoculated on MS medium supplemented with BAP (0.5-1.00 mg/l),2,4-D (0.5-1.00mg/l) and NAA (0.5-1.00MG/l) alone and in combinations.

Callus induction

For callus induction internodes were inoculated on MS medium supplemented with 2,4-D (0.5-1.00mg/l) and NAA (0.5-1.00mg/l) alone and in combinations.

Results

In the present study nodes and inter-nodes of *H. indicus* were used as explants. Various concentration of mercuric chloride was tested for surface sterilisation of explants and 0.1%(w/v) aqueous mercuric chloride was found most effective. The MS medium supplemented with various concentration of plant growth regulators responded variously in different time intervals.

Shoot formation was stimulated by the media with different auxins. Minimum induction of shoot (20%), from nodal explants, with average number of 2±0.6 were found in MS medium fortified with 1 mg/l NAA. The shoot was formed after 8 days (Fig. 1). As concentration of NAA decreased from 1mg/l, it resulted in a downfall in the rate of shoot generation ability and responded variously by forming callus.

The plant nodal segments when treated with 2,4-D responded varyingly. Average induction of shoot (50%) with average number of shoot (4±0.1) was found in MS medium supplemented with 0.25 mg/l of 2,4-D. The shoot was formed after 9 days (Fig. 2). As the concentration of 2,4-D increased from 0.25 mg/l to 1mg/l, it resulted in decrease in the rate of shoot generation ability and resulted in the formation of callus. Maximum shoot induction (80%) with mean number of shoot 6.8+-0.10(mean length 6.4±0.13) was observed in the medium containing 0.5mg/l IBA. The shoot induction started after 7 days (Fig. 3)

Effect of different cytokinin on in vitro shoot formation

Nodal explants were aseptically cultured on MS medium containing different kinds of cytokinin, BAP and Kin, to evaluate their effect on *in vitro* shoot regeneration.

Shoot formation was stimulated by the media with various cytokinin combination. Average induction of shoot (45%) with average number (4 ± 0.14) was found in MS medium supplemented with 0.5mg/l BAP. Shoot induction appeared within 5 to 6 days (Fig. 4). MS medium supplemented with kin (1mg/l) produced shoot within 6 days (Fig. 5). The combination of kin (0.5mg/l) and BAP (0.5mg/l) also promoted shoot induction within 7 days (Fig. 6)

Effect of growth hormones on callus induction on internodal explants of *H.indicus*

1. Effect of auxins on callus induction

Callus formation was stimulated using various auxin concentrations either singly or in combination. Average induction of callus (45%) was found in MS medium supplemented with 0.5 mg/l NAA within 10 days. The callus was green in colour. Maximum induction of callus (80%) was found in MS medium supplemented with 1mg/l 2,4-D. The callus was formed after 10 days and was green in colour. The average induction of callus (50%) was found in MS medium supplemented NAA (0.5mg/l) along with 2,4-D (1mg/l) after 12 days. The callus was white in colour(Fig. 7).

Fig. 1. NAA (1mg/L)

Fig. 2. 2,4-D (0.25mg/L)

Fig. 3. BAP (0.5 mg/L)

Fig. 4. BAP (0.5 mg/L)

Fig. 5. Kinetin (1mg/L)

Fig. 6. BAP (0.5mg/L) + Kinetin (0.5mg/L)

Fig. 7. 2,4 D (1mg/L) + NAA 0.5mg/L)

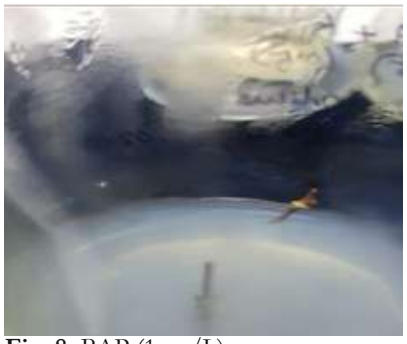

Fig. 8. BAP (1mg/L)

Fig. 9. BAP (1mg./L)+ 2-4 D (1mg/L)

2. Effect of cytokinin on callus induction

The average induction of callus (50%) was found in MS medium supplemented with BAP (0.5mg/l) after 11 days when internal segments were used as explants. The callus was white in colour (Fig. 8)

3. Effect of auxin - cytokinin combination on callus induction

Minimum induction of callus (20%) was found in MS medium supplemented with 2,4-D (1mg/l) along with BAP (1mg/l). The callus was green in colour (Fig. 9). MS medium supplemented with NAA (0.5mg/l) produced callus within short time (7days).MS medium supplemented with NAA (0.5mg/l) along with 2,4-D (1mg/l) produced callus after 12 days.

Acclimatization of plantlets in greenhouse and field transfer

The *in vitro* rooted plantlets were shifted to the greenhouse for hardening and such hardened plantlets were finally transferred to the nursery poly-bags containing potting mixture. To support further growth and acclimatization, these plantlets were maintained in the greenhouse for 6–7 week. Eco-friendly paper cups and transparent polythene cups played an important role in ex vitro rooting and hardening process of *H. indicus*, which overcame the drawbacks of traditional methods of hardening and acclimatization. The hardened plantlets were transferred to the pots and then to field after

another 3–4 week. Normal growth and morphology was observed after field transfer.

Discussion

Malathy and Pai (1998) have reported the *in vitro* propagation of *H. indicus*. Micropropagation was achieved in Murashige and Skoog's basal Medium (MS) supplemented with benzyladenine (3 mg/L). Micropropagation and production of 2- hydroxy 4-methoxy benzaldehyde using root cultures of *H. indicus* was reported by Sreekumar *et al.* (1998, 2000). Ramulu *et al.* (2003) have reported the regeneration of plants from root segments derived from aseptic seedlings. Improvement in clonal propagation of *H. indicus* through adenine sulphate has been reported by Neetha *et al.* (2003). Somatic embryogenesis and plant regeneration from leaf cultures of *H. indicus* have been reported by Swaroopa and Dixit (2006).

The sterilized cultures of *H. indicus* were established from the juvenile nodal and inter-nodal explants and the response of the cultures was found better in case of nodal explants . The physiological status, season of collection, size and quality of the explants are some of the important factors which impart major role in the induction of shoots and establishment of cultures in many plant systems (Smith 2000). Nodal shoot segments were reported the best explant type for regeneration of shoots *in vitro* in many plant species (Phulwaria *et al.* 2013; Patel *et al.*, 2014). The obtained results also coincide with the reported works. Of the different concentrations of plant growth regulators studied, MS medium supplemented with NAA (1 mg/l) the shoot was formed after 8 days.2,4-D also take part in the formation of shoot. MS medium supplemented with 2,4-D (0.25mg/l) produce shoot after 9 days. IBA (0.5mg/l) produce shoot within 7 days.0.5mg/l BAP produce shoot within 5-6 days. Kinetin is another cytokinin used for shoot induction 1mg/l kinetin produce shoot within 5-6 days. The combination of kinetin and BAP also promote shoot induction.MS medium supplemented with BAP (0.5 mg/l) along with kinetin (0.5mg/l) produce shoot within 7 days. Continuous and subsequent subculture on the same concentration of medium at regular intervals increased the rate of shoot multiplication. The explants when inoculated in medium fortified with 0.5 mg/l of BAP produced white coloured callus within 11 days while 1mg/l 2,4-D produced green coloured callus within 10 days. Similarly, 0.5 mg/l NAA produced green coloured callus within 7 days. When combination of NAA and 2,4-D (0.5mg+1mg/l) was used, the inter-nodal explant responded by producing white callus while combination of BAP and NAA (0.5mg/l+0.5mg/l) formed white callus. Also, the combination of 2,4-D and BAP (1mg/l+1mg/l) produced callus with green colour. In this study BAP showed superior results as compared to kinetin. The efficacy of BAP over Kin in shoot proliferation has also been well reported in number of plant species (Rathore *et al.*, 2008; Phulwaria *et al.*, 2013).

The results are in agreement with the earlier reports in *Lawsonia inermis* (Ram and Shekhawat, 2011), *Celastrus paniculatus* (Phulwaria *et al.*, 2013) and *Morinda coreia* (Shekhawat *et al.* 2015a) etc. The higher concentrations of cytokinins than auxins in the culture medium could induce shoot organogenesis (Sharma and Singh 1997; Rout and Das 1997). The efficacy of IBA over other auxins for root induction has been acknowledged in many reports in different plant species (Arora *et al.*, 2010; Shekhawat and Shekhawat 2011; Rathore *et al.*, 2013; Patel *et al.*, 2014).

Conclusion

In the present study a rapid and improved micro-propagation protocol has been developed for *H. indicus*, which could enable mass scale propagation of this endangered plant species to overcome difference in demand and supply in the market. Since, very good rate of root induction has been achieved through *ex vitro* rooting method, it reduced time, energy and cost of production of micro-propagated plantlets and increased the chances of survival of *H. indicus* in the field condition. The protocol could also be used for further studies in mass production of roots through adventitious root cultures in liquid medium, since roots are mostly used by the pharmaceutical industries to get bioactive compounds.

Acknowledgment

We thank the 'Fund for Improvement of S & T Infrastructure (FIST)' of the Department of Science & Technology, Government of India for the instruments we used throughout the study were purchased under DST FIST scheme of Government of India.

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Vilash V, Ratheesh N, Latha S (eds.). Biodiversity Challenges and Threats; Current Scenario 2023 *ISBN 978-81-958369-2-5*

Chapter 17

ETHNOBOTANICAL STUDY OF SELECTED WILD EDIBLE PLANTS OF MUTHUVAN TRIBES IN NOORANKARA SETTLEMENT, IDUKKI DISTRICT, KERALA

Ajinsha J S, Rajkumar G

Abstract Ethnobotany is the scientific study of the interrelationship between traditional knowledge about plants and their effective use by the indigenous community. From the beginning, humans have been depending on nature and natural resources for all their basic needs. Humans learned to distinguish edible plants from poisonous plants by observing animals. Muthuvans are the earliest inhabitants of the Western Ghats, and they live away from the mainstream of the population deep inside the evergreen forest of the Idukki district. The study area was frequently visited, and ethnobotanical data was collected through semi-structured questionnaires in their local language and discussion with the help of village heads (Kani), local villagers, and tribal practitioners around the settlement area. A detailed ethnobotanical survey revealed that the Noorankara settlement in the Valara forest range of Adimali grama panchayathu of the Idukki district relies on 34 wild edible plant species belonging to 28 genera and 20 families. These plants consisted of 12 shrubs, 9 trees, 8 herbs, and 5 climbers. Among these 35.29% are shrubs and Malvaceae, Phyllanthaceae occupies the higher number of species used. Apart from the edible uses, 12 species have therapeutic and practical uses and also function as vitamin supplements. All information related to the species is listed in alphabetical order, followed by their plant name, local name, family, habit, part used, and uses.

Keywords: Muthuvan, Ethnobotany, Wild, Edible, Plants, Documentation

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Introduction

Ethnobotany is the scientific study about the interrelationship between traditional knowledge about plants and their effective use by the indigenous community. The term ethnobotany derives from "Ethnos" a Greek word, meaning "nation". Ethnos again derives from "Ethnic" which means a group having common origin, Culture or language. Ethnobotany was first used by Hashberger(1985). The science of ethnobotany is concerned with the relationships between man and vegetation involving man's dependence upon vegetation as well as the tremendous influence man has had on vegetation (King, 1974). Plant biodiversity being destroyed today as a result of various human activities. The loss of biodiversity threatens the world's future food supply. The search for food substitutes and proper utilization of natural resources are now global concerns (Manandhar, 1993).

The various tribal sects are repositories of rich knowledge on various uses of plant genetic resources, which have hitherto remained unknown (Khoshoo, 1991). Muthuvans, one of the earliest inhabitants of the Western Ghats. They are believed to have migrated from Tamil Nadu and settled in the evergreen forests of the Western Ghats a thousand years ago. Traditionally, the Muthuvans are nomadic agriculturists, hunters, and trappers.

Wild edible plants play an important role in human life since time immemorial. The inhabitants of rural communities depend on these plants for

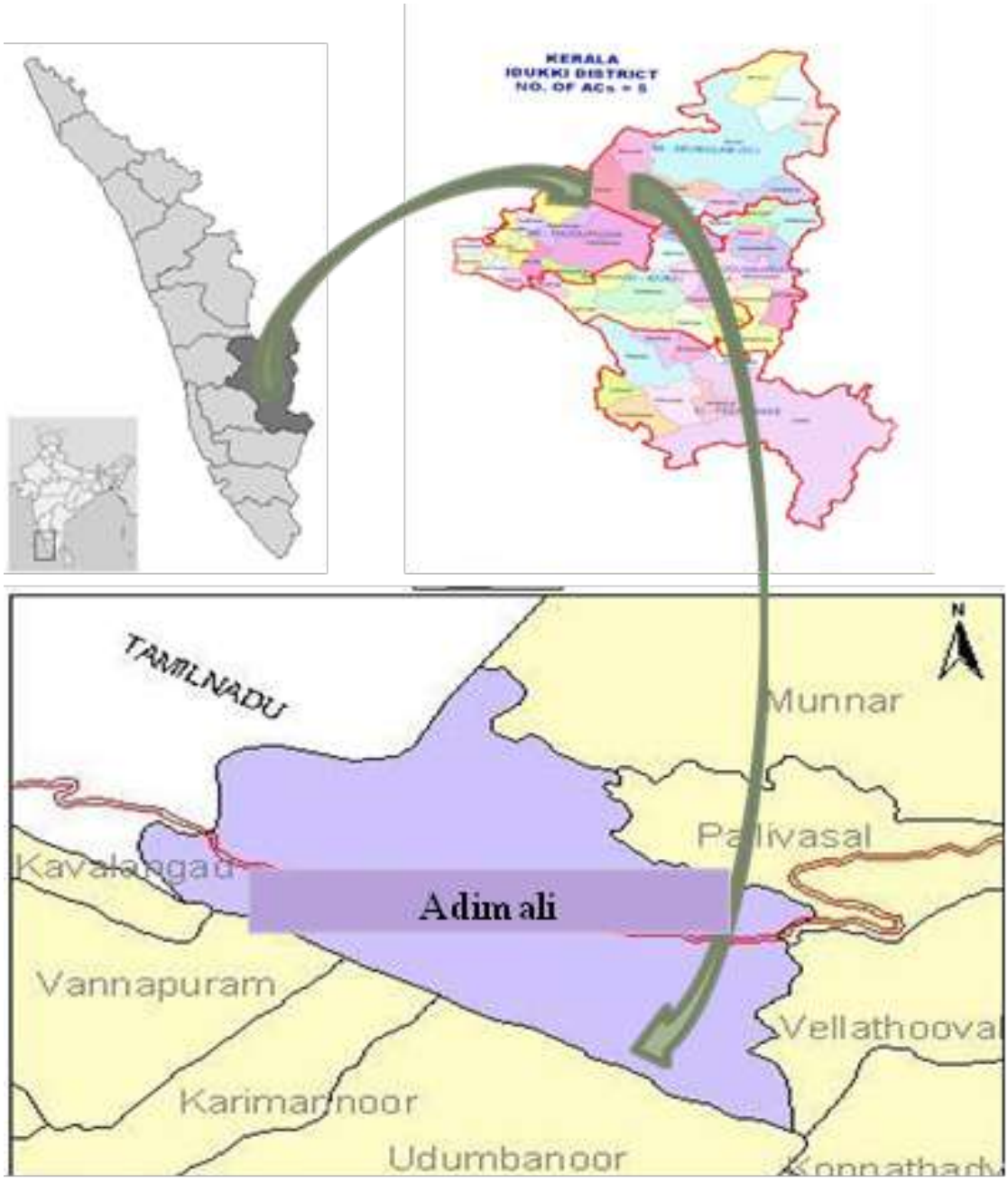
daily needs e.g., food, vegetables, spices, beverages and medicine. Lulekal *et al.* (2011) have stated that wild edible (e.g., those yielding edible leaves, flowers, fruits, stems, seeds or starchy underground parts, including fungi) are those plant species, which are neither cultivated nor domesticated, but are accessible from their natural habitats and used for consumption.

Wild edible plants are existing in the forests, protected areas, wetlands and grasslands, which can be used as food by appropriate means of collection, preparation and preservation (Kallas, 1996). Our ancestors collected wild grains, vegetables, fruits, tubers etc. for food and learned to eliminate the toxic substances from them, if any, by various means to make them palatable and then settled such plants on their farms (Singh and Singh, 1981).

Materials and methods

The study was conducted during the year 2022. The aim of the study was to explore, collect, Identify and preserve the wild edible plants used by the muthuvan tribes of noorankara kudi settlement. Noorankara kudi settlement is belongs to Adimali Grama Panchayath, Idukki Dist Kerala.

Idukki is the second largest district both in area wise as well as in tribal population This Panchayath



is situated in the phytogeographic region of the Western Ghats and covers a total area of 271 53 sq km The land area is surrrounded by, in north Mankulam Grama Panchayath, in south Periyar river, in west Kuttampuzha Grama Panchayath and in east Vellathuval and Pallivasal Grama Panchayats Administratively this panchayath divided in to 21 wards and also divided into 13 Muthuvan settlements

The Muthuvans or Mudugars are a tribe of hill cultivators in Coimbatore and Madura. In Kerala, they are found in the Adimali and Devikulam forest regions of Idukki district and in the adjoining Western Ghats of the Palakkad and Thrissur districts. They are the most ethnic community in idukki.

The study area was frequently visited and ethnobotanical data (local name, useful part, uses, mode of preparation, etc.) was collected through semi-structured questionnaires in their local language and discussion with the help of village heads (Kani), local villagers, and tribal practitioners around the settlement area. The continuous field survey and interviews conducted in the study area and repeatedly covering the tribal settlements in different seasons to record the utilization of plant species. Identification of plants will be made by regional and local floras. Herbarium specimens were prepared as per standard procedures and deposited in TBGT.

Result

A detailed ethnobotanical survey revealed that the Noorankara settlement in the valara forest range of Adimali grama panchayathu of Idukki district relies on 34 wild edible plant species belonging to 28 genera and 20 families.

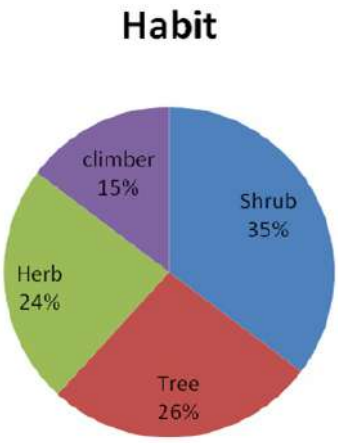


Fig. 1. Percentage distributions of habit classes

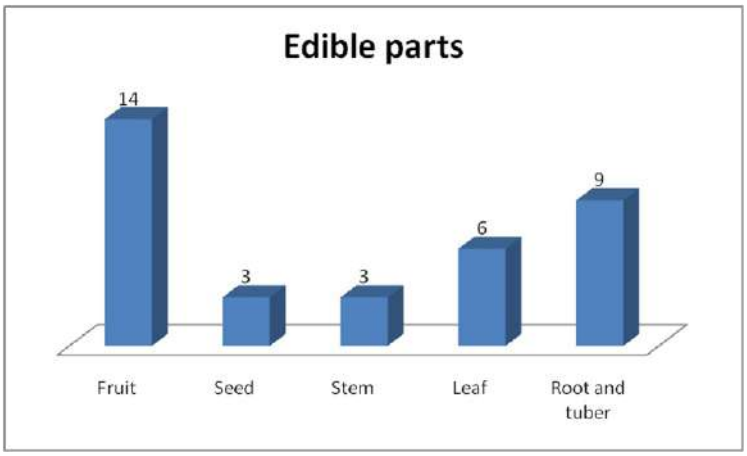


Fig. 2. Percentage distribution of wild edible parts

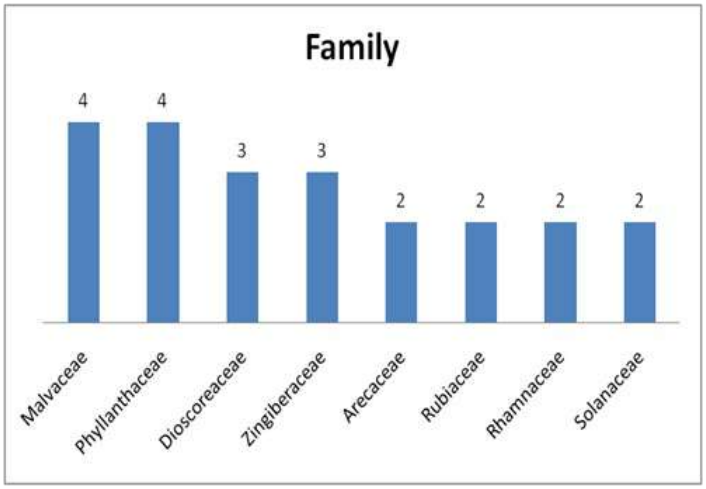


Fig. 3. Familywise distribution

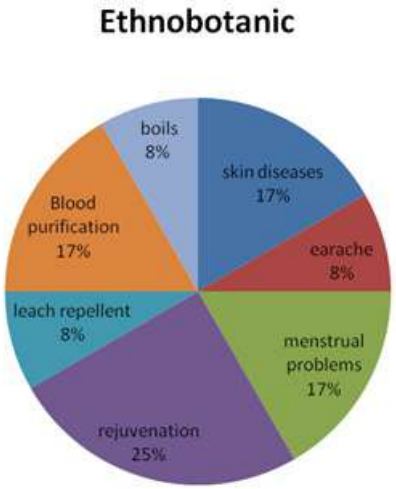


Fig. 4. Percentage distribution of Ethnobotanic data

Sl. No.	Botanical Name	Local Name	Family	Habit	Parts Used	Edible use	Medicinal use
1	*Alternanthera brasiliana* (L.) Kuntze	Cheera	Amaranthaceae	Herb	Leaf	Cooked like vegetable	Used for Blood purification and increase the blood count
2	*Aporosa cardiosperma* (Gaertn.) Merr.	Vetti, Ponvetti	Phyllanthaceae	Tree	Fruit, aril	Eaten as Raw	
3	Arenga wightii Griff.	Ayathengu, Aazhuthumpana	Arecaceae	Tree	Fruit(endosperm), tender shoot	Endosperm after processing, tender shoot eaten directly or making some dishes with rice flour	
4	*Baccaurea courtallensis* (Wight) Müll.Arg.	Mootikaya, Mootilpazham, Mootippuli	Phyllanthaceae	Tree	Fruit, aril	Eaten as Raw, or pickled.	
5	*Boerhavia diffusa* L.	Thazhuthama	Nyctaginaceae	Herb	Leaf	Cooked like vegetable	
6	*Breynia androgyna* (L.) Chakrab. & N.P.Balakr.	Velicheera	Phyllanthaceae	Shrub	Leaf	Cooked like vegetable	
7	*Caryota urens* L.	Olattipana	Arecaceae	Tree	tender shoot	tender shoot eaten directly or making some dishes with rice flour	
8	*Cullenia exarillata* A. Robyns	Karani	Malvaceae	Tree	Fruit	They eat the Fruit of Cullenia exarillata mixed with jaggery	For rejuvenation
9	*Curcuma angustifolia* Roxb.	Vellakoova	Zingiberaceae	Herb	Rhizome	Eat the rhizome after processing	Rhizome is leach repellent
10	*Curcuma species*	Chomalakoova	Zingiberaceae	Herb	Rhizome	Eat the rhizome after processing	
11	*Curcuma zanthorrhiza* Roxb.	Manjakoova	Zingiberaceae	Herb	Rhizome	Eat the rhizome after processing	
12	*Colocasia esculenta* (L.) Schott	Thalu	Araceae	Herb	Leaf	Cooked like vegetable	
13	*Cycas circinalis* L.	Chananga, Kalanga	Cycadaceae	Tree	Fruit(endosperm)	Eat after processing and cooked.	
14	*Cymbopogon citratus* (DC.) Stapf	Ey pullu	Poaceae	Herb	Whole plant	Whole plant boiled with Persicaria chinensis and drink	During the time of eclipse they boil the Cymbopogon citrates and Persicaria chinensis and drink that water.
15	*Dioscorea oppositiflora* Griseb.	Thettakizhangu	Dioscoreaceae	Climber	Roots and tubers	Eaten after cooking	
16	*Dioscorea pentaphylla* L.	Noorakizhangu	Dioscoreaceae	Climber	Roots and tubers	Eaten after cooking	
17	*Dioscorea species*	Nooran Neduvan	Dioscoreaceae	Climber	Roots and tubers	Eaten after cooking	
18	*Ensete superbum* (Roxb.) Cheesman	Kalluvazha, Malavazha	Musaceae	Shrub	Fruit, seed, flower stalk	Eaten as Raw	
19	*Entada rheedei* Spreng.	Makkanga Panal,	Fabaceae	Shrub	Endosperm	Eat After processing	

20	*Glycosmis pentaphylla* (Retz.) DC.	Panal, Panchi	Rutaceae	Shrub	Fruit	Eaten as Raw	Root is used to make decoctions and oil preparation.
21	*Hemidesmus indicus* (L.) R.Br.	Naruneendi	Apocynaceae	Herb	Root	Root boiled in water and drink	skin diseases,
22	*Hibiscus hispidissimus* Griff.	Panachampuli	Malvaceae	Shrub	Leaf	Used to make chutneys.	Leaf is used to cure boils
23	*Hibiscus sabdariffa* L.	Muppuli Chethi,	Malvaceae	Shrub	Fruit	Used to make chutneys	
24	*Ixora brachiata Roxb. Dicot*	Thechi, Thetti	Rubiaceae	Small trees	Fruit	Eaten as Raw	
25	*Mussaenda frondosa* L.	Amma karumbi makal velumbi	Rubiaceae	Shrub	Leaf	Cooked with egg	
26	*Opuntia elatior* Mill.	Chappathikalli	Cactaceae	Shrub	Fruit, Young pods	Eaten as Raw	
27	*Persicaria chinensis* (L.) H.Gross	Odutha	Polygonaceae	Shrub	shoot	Chew the stem and drink the sap on water scarce condition	Overcome menstrual problems
28	*Phyllanthus emblica L.*	Nelli	Phyllanthaceae	Tree	Fruit	Eaten as Raw or pickled, processed	Increase blood count.
29	*Physalis angulata* L.	Njodinjotta, Njottanjodian Pottichedi	Solanaceae	Herb	Fruit	Eaten as Raw	
30	*Sida acuta* Burm.f.	Kurunnotty	Malvaceae	Shrub	Root	Root boiled in milk and drink	For rejuvenation
31	*Solanum betaceum* Cav.	Marathakkali	Solanaceae	Shrub	Fruit	Eaten as Raw	
32	*Spondias pinnata* (L.f.) Kurz	Ambazham, Kattambazham	Anacardiaceae	Tree	Fruit	Eaten as Raw or pickled	Earache
33	*Ziziphus oenopolia* (L.) Mill.	Cheruthudali, Kottaipazham, Thodalli	Rhamnaceae	Shrub	Fruit	Eaten as Raw	Stomachache Cure
34	*Ziziphus rugosa* Lam.	Kottamullu, Malamthudali, Thodali	Rhamnaceae	Shrub	Fruit	Eaten as Raw	Menstrual problems

Their habit wise analysis indicates that shrubs (35%) made the highest proportion of the edible plant species followed by trees (26%), herbs (24%) and climbers (15%) in descending order (Fig.1).

The plant parts used were Roots and Tubers, Leaves, Fruit, Seed, Shoot for food supplement. Analysis indicates fruits of 14 (41%), Roots and tubers of 9 (26 %) leaves of 6 (17%) Seed and Shoot of 3(8%) plant species as most preferred edible plant parts (Fig. 2).

In terms of number of species used, Malvaceae (4) and Phyllathaceae (4) was the most dominant family followed by Dioscoreaceae (3) and Zingiberaceae (3), Arecaceae, Rubiaceae, Rhamnaceae, Solanaceae have 2 species each, Twelve families were represented by a single species each (Fig. 3).

Among the 34 species of wild edible plants, 122 species used for medicinal purpose. Among these 12 medicinally promising plants 3 (25%) are used for rejuvenation and 2 (17%) species are used to cure skin diseases, menstrual problems and Blood purification. The remaining 5 species cures single diseases (8%) in each (Fig. 4).

Conclusion

Forest is a common habitat for collection of these plants. They provide food and nutrients to local people and could also be a source of cash income. However, both WEPs and their associated indigenous knowledge are facing various threats. Thus, conservation and sustainable utilization of these plants in this area are of the utmost importance. Documentation of these species may provide basic information for conservation. During the present ethnobotanical field study it was observed that the local people of the study area

Pictures of extensive field exploration

fulfil the deficiency in food needs by supplementing with wild edible plants in their daily diet. Thus, the WEPs are used as common household foods and make a substantial contribution to food security of the people of the study area. Therefore, steps are needed to undertake extensive education about their importance and assess their nutritional values to serve as a direct or indirect source of food to local inhabitants as well as tribal people. Many of the WEPs may not be freely available in future due to over exploitation, habitat destruction and invasion of exotic plant species. Therefore, efforts can be made to bring some of them under cultivation in order to maintain a continuous supply and help in their conservation. Moreover the tradition of using wild palatable plants is still alive in the rural populations, but is vanishing. Consequently, the recording, preserving, and infusing of this traditional knowledge to upcoming generations is pressing and vital.

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Chapter 18

PHYSICOCHEMICAL EVALUATIONS AND PHARMACOGNOSTICAL STANDARDISATION OF *STROBILANTHES ALTERNATA* (BURM.F.) MOYLAN EX J.R.I WOOD

Nandu Krishnan R, Ammu S K, Vilash V

Abstract *Strobilanthes alternata* (Burm.f.) Moylan ex J.R.I. Wood (Acanthaceae), is a tropical low-creeping medicinal herb. In Kerala, the plant is popularly known as 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. It has been used in the traditional system of medicine in India for treating various ailments like cut wounds, haemorrhage, venereal disease, excess mensuration etc. In the present study, macroscopic and organoleptic characterization, quantitative leaf microscopy, , powder analysis, fluorescence analysis and physicochemical analysis of *S. alternata* plant were carried out. The result showed that, the young stem is scrabid in nature due to the presence of a large number of uniseriate trichomes. Leaves are ovate, simple and pubescent with a light green colour on the upper and purple on the lower surface. The stem T.S is irregular in outline with single layered epidermis having a uniseriate trichome followed by 8-10 layers of collenchymatous outer cortex, a single layer of pigment producing layer, 6-9 layers of polygonal parenchymatous cells with starch grains in the inner cortex. The secondary xylem has arranged as a broken ring with a broad pith. The polygonal inner cortical cells are filled with plenty of starch grains. In the mature stem, the xylem is stellate in appearance with outer semicircular strips of continuous phloem, the outer cortex has uniseriate pigment producing cells. The microscopic studies on powder analysis showed the presence of characteristic features such as druses. The powder analysis of the dried plant powder showed the presence of pitted vessels, xylem fibers, scalariform tracheid, fragmented tracheids, xylem vessels with spiral thickening, xylem vessels and rays and calcium oxalate crystals in the form of druses etc. All the results from the present study will help to identify the authenticity of the plant even from the crushed or powdered form.

Keywords: *Pharmacognosy, Strobilanthes alternata, Ethnomedicine, Physicochemical analysis*

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Introduction

The interrelationship of man and medicinal plants is as old as human civilization. In India, about 65% of the population in rural areas depends on Ayurveda and medicinal plants to meet their primary health care requirements (Anonymous, 2013). Now a day, there is a growing interest in drugs of natural origin because of their easy availability, economic and less or no side effects. One of the drawbacks of the therapeutic efficacy of medicinal plants is their adulteration. Standardization and authentication of natural drugs are the only way to identify the adulteration and confirm purity of raw drug, and is done only through pharmacogenetic studies (Silja *et al.*, 2008) *Strobilanthes alternata* (Burm.f.) Moylan ex J.R.I Wood is a medicinal plant of the family Acanthaceae. It is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. It is grown as an indoor or outdoor plant. In Kerala, the plant is

popularly known as 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. It has been used in the Indian traditional system of medicine treating various ailments like cut wounds (Edwin and Nair., 2011) dysentery (Akhil and Prabhu, 2003), haemorrhage, verneral disease, excess mensuration etc. (Asha *et al.*, 2014).

Pharmacognostic characterization including physiochemical evaluation is meant for identification, authentication, detection of adulteration and compilation of quality control of the raw drug material. Since the plant, *S. alternata* is used in traditional medicinal system of Kerala, it is necessary to standardize its physicochemical and pharmacognostical parameters. *S. alternata* is a glorious medicinal plant used by ethnic groups from time immemorial. A proper phytochemical and pharmacogenetic studies are crucial for clinical experimentation and the development of new drug formulations based on this plant. Various pharmacogenetic parameters evaluated in the present study include the macro and microscopic characterization of the stem, powder analysis, fluorescence analysis and physicochemical parameters such as pH, total ash value, extractive value, swelling index, foaming index, etc.

Materials and Methods

1 Collection of plant material

S. alternata plants were collected from Kollam district, Kerala, India. Herbarium specimens were prepared and identified by the taxonomists of the department. The fresh plants were collected for morphological and anatomical studies and the whole plant was shade dried and powdered for further study purposes.

2. Macroscopic and organoleptic characterization

The following macroscopic and organoleptic characteristics for the fresh leaves were noted: phyllotaxy, size, shape, colour, venation, presence or absence of petiole, apex, margin, base, lamina, texture, surface, odour and taste (Trease and Evans, 2002; Wallis, 1985).

3. Microscopic characterization

3.1 Anatomical studies

Free hand transverse sections of stem, leaf lamina and midrib were prepared, stained with safranin, mounted on glass slides using glycerine and observed under light microscope with camera attachment and photomicrographs were taken (Trease and Evans, 2002).

3.2 Quantitative leaf microscopy stomatal number and stomatal index

A small piece of leaf was cleared by boiling it with sodium hypochlorite solution. The upper and lower epidermis was peeled separately. The peeled epidermis was placed on a slide and mounted with glycerine water. The average number of stomata per mm2 of the epidermis of the leaf (stomatal number) is calculated from microphotographs taken using camera attached microscope (Anonymous, 2011). Values for the upper and lower epidermis were determined separately using the equation.

Stomatal index (SI) = S × 100 /E+S.

Where, S= the number of stomata per unit area and E = the number of epidermal cells in the same unit area of the leaf.

4. Determination of vein-islet number and vein-let termination number

The veinislet number is the average number of veinislets per mm2 of a leaf surface midway between midrib and margin and the average number of terminated veinlet islets per mm2 of a leaf was taken as veinlet termination (Trease and Evans, 2002).

5. Powder analysis

The fresh plant was collected, thoroughly washed with fresh water, sliced, shade dried and powdered. The plant powder was boiled with chloral hydrate for 5 to 10 min, and then stained with safranin to determine the presence of lignified cells, calcium oxalate crystals and iodine solution was used to detect starch grains (Khandelwal, 2002).

6. Fluorescence analysis

Fluorescence analysis helps to find the fluorescence character of the plant powder (40 mesh) was studied both in daylight and the UV light (254 and 366 nm) after treatment with different reagents like sodium hydroxide, picric acid, Acetic acid, hydrochloric acid, nitric acid, iodine, Ferric chloride etc (Chase and Pratt, 1949; Kokoshi *et al.*, 1958). Colour changes were noted using the

pantone colour chart.

7. Physicochemical analysis

Different physicochemical parameters of *S. alternata* leaf powder were determined according to the quality control methods for medicinal plant materials (Anonymous, 2011).

7.1. Determination of pH

1% solution was prepared by dissolving 1 g of the plant powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode. 10% solution was prepared by dissolving 10 g of the plant powder in 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

7.2. Determination of alcohol soluble extractive

Take 5 g of the air dried powder in 100 mL of ethanol in a closed flask, shaken frequently during 6 h and allowed to stand for 18 h. Filtrate is collected and evaporated to dryness in a tared flat bottom shallow dish, dried at 105˚C and weighed. The percentage of alcohol soluble extractive is calculated with reference to the air dried drug.

7.3. Determination of chloroform soluble extractive

Methodology followed as directed for the determination of alcohol soluble extractive using water instead of ethanol.

7.4. Determination of hexane soluble extractive

Methodology proceeded as directed for the determination of alcohol soluble extractive, using Petroleum ether instead of ethanol.

7.5. Loss on Drying (LOD)

About 23 g of plant powder is accurately weighed in a China dish and kept in a hot air oven maintained at 105˚C for 5 h. After cooling in A desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

7.6. Swelling index

Plant powder (1 g) was taken in a measuring cylinder (25 mL) and suspended in 25 mL distilled water for 1 h by thorough mixing every 10 Min. After 3 h, volume in mL occupied by the plant material including any sticky mucilage was measured. The experiment was repeated three times for accuracy and the swelling index was calculated.

7.7. Foaming index

Finely divided (sieve No. 1250) plant powder in 1 g was kept in to a 500 mL flask containing 100mL of boiling water for 30 min then cooled and filtered into a 100 mL volumetric flask and added sufficient water to make up the volume. The prepared decoction was poured in to 10 stoppered test tubes each 1 mL, 2 mL up to 10 mL. The volume of the liquid in each tube was adjusted to 10 mL with water. The tubes were duly stoppered and shaken in a lengthwise motion for 15 sec (two shakes per second) and allowed to stand for 15 min. The foam height in each tube was measured.

Foaming index =1000/a

'a' is the volume of the plant decoction for foaming foam of height 1cm.

7.8. Determination of total ash

About 23 g weighed crude drug powder in a tared silica dish was ignited and weighed. Scatter the powder drug on the bottom of the dish and incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, Cooled and weighed. The % w/w of total ash with reference to the air-dried drug was calculated.

7.9. Determination of acid insoluble ash

Boiled the ash for 510 min with 25 mL of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible, washed with hot water, ignited and weighed. Percentage of acid insoluble ash is calculated with reference to the aired dried drug. The % w/w of acid insoluble ash with reference to the air-dried drug was calculated.

7.10. Determination of water-soluble ash

To the crucible containing the total ash, add 25 mL of water and boil for 510 min. Collect the insoluble matter in a Gooch crucible, wash with hot water and ignite in a crucible for 15 min at a temperature not exceeding 450°C. Subtract the weight of insoluble matter from the weight of the ash. The difference in weight represents the water soluble ash. Percentage of water soluble ash is calculated with reference to the air dried drug. The % w/w of water-soluble ash with reference to the air-dried drug was calculated.

7.11. Percentage extractive and characteristics of fractions

S. alternata powder was first extracted with hexane using Soxhlet apparatus, powder was then dried and again extracted with chloroform and finally with ethanol to get; 1. Hexane fraction, 2. Chloroform fraction and 3. Ethanolic fraction. Yield in g/100 g leaf powder of extracts were calculated. Consistency, colour and odour were noted.

9. Statistical analysis

All the data were expressed as mean ± standard deviation (SD).

Results

Macroscopic and organoleptic characters

Leaves are simple, ovate, acuminate with crenate margins. Leaf surface is pubescent with light green in upper and purple colour in lower surface (Plate 1). Leaves are with no characteristic odour and taste (Table 1).

Table 1. Macroscopic and organoleptic characters of *S.alternata* leaf

Character	Observation
Phyllotaxy	Opposite
Type	Simple
Lamina length	7.09 cm
Lamina width	4.31 cm
Shape	Ovate
Apex	Acuminate
Margin	Crenate
Venation	Reticulate
Base	Cordate
Surface	Pubescent
Colour -upper	Light green
Colour - lower	Purple
Odour	No characteristic odour
Taste	No characteristic taste

Quantitative leaf microscopy

Quantitative leaf characteristics were observed and the results were shown in the Table 2 and Figure 2.

Anatomical studies

The cross section of the petiole showed the presence of single layered rectangular cutinised epidermal cells with nonglandular trichomes. Epidermis is followed by 4 to 8 layers of compactly packed chlorenchyma cells. The chlorenchyma region is followed by compactly packed polygonal parenchyma cells.

T.S of lamina shows single layered cutinised upper epidermis, single layered lower epidermal cells with multi-layered unbranched epidermal hairs, two to three layered chlorenchymatous compactly packed palisade layer followed by four to six layers of loosely packed spongy tissue. The adaxial and abaxial epidermis is packed with numerous stomata.

T.S of stem is irregular in outline with single layered epidermis with uniseriate trichome followed by 8-10 layers of collenchymatous outer cortex, single layer of pigment producing layer, 6-9 layers of polygonal parenchymatous cells with starch grains in inner cortex. Secondary xylem has arranged as broken ring with broad pith (Figure 2).

Powder analysis

Under the microscope it showed the presence of pitted vessel, xylem fibers, scalariform tracheid, fragmented tracheids, xylem vessels with spiral thickening, xylem vessels and rays and calcium oxalate crystals in the form of druses etc. (Plate 3).

Fluorescence analysis

Chemical tests of *S. alternata* powder with different reagents were done and observed under visible and UV light. Under UV light, the sample 6,7,11 shows the presence of fluorescence. The results were compared with their respective

Plate 1. *Strobilanthes alternata*
a. Habit b. Leaf adaxial view c. Leaf abaxial view

Table 2. Quantitative leaf microscopy of *S.alternata*

Parameter	Range	Mean ± SD
Stomatal number -upper	860-982	940±34
Stomatal number - lower	6500-7300	7012±432
Subsidiary cell length (µm)	0.48-0.58	0.50± 0.08
Subsidiary cell width (µm)	0.16-0.24	0.19±0.05
Stomatal index- upper	8.42-9.53	9.21±0.93
Stomatal index - lower	32.84-38.43	36.42±4.32
Vein islet number	12-18	15.53±3.54
Veinlet termination number	10-13	12.43±1.23

Values are expressed as ±SD of 10 values

Table 3. Observations of *S. alternata* powder under visible and UV light after reacting with different reagents.

Sl. No	Treatment	Observations under Visible light	Observations under UV light
1	Powder + 1N NaOH	Yellow PMS 471	PMS 412 black
2	Powder + 1M NaOH + ethanol	Green PMS 388	PMS 419 black
3	Powder + dil HCl	Red PMS 032	PMS 433 black
4	Powder + 5% iodine	Light orange PMS 471	PMS 426 black
5	Powder + 5% $FeCl_2$	Black PMS 426	PMS 433 Black
6	Powder + dil NH_3	Light yellow PMS 110	PMS 110 Dark yellow
7	Powder + ethanol	Light green PMS 382	PMS yellow 109
8	Powder + H_2SO_4	Reddish Brown 1815	PMS 412 black
9	Powder + HNO_3	Red PMS 1805	PMS 419 black
10	Powder + $K_2Cr_2O_7$	Deep black PMS 433	PMS 426 black
11	Powder + Toluene	Light green PMS 374	PMS 101 light yellow
12	Powder + water	Deep orange PMS 159	PMS 433 black

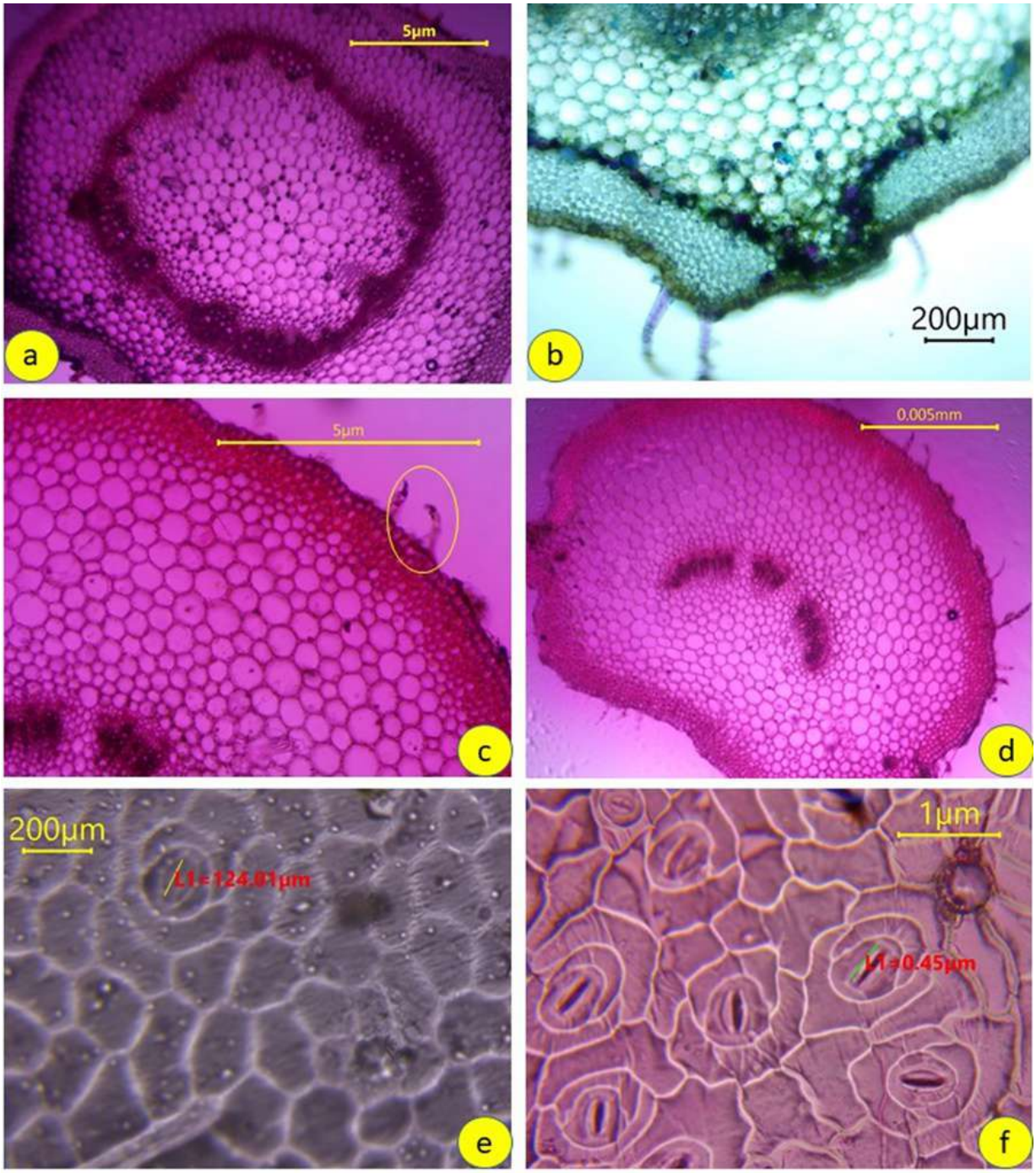


Plate 2. Microscopic characterization of *S.alternata*
a.T.S stem b. Pigment producing cortical cells c. T.S of petiole showing uniseriate epidermal hairs
d. T.S of petiole e. stomatal impressions of leaf adaxial side. f. stomatal impressions of leaf abaxial side

observations in visible light and they were represented in Table 3 and Plate 4.

Physicochemical analysis

Physicochemical parameters of *S. alternata* powder was evaluated and observations are noted and presented in Table 4.

Discussion

The source and quality of raw materials play an important role in guaranteeing the quality and stability of herbal preparations (Calixto, 2000). Thus, in recent years there has been an emphasis on standardization of tribal medicinal plants of therapeutic potential by pharmacognostical studies, as it is more reliable, accurate and inexpensive. The morphological characters observed in the present study can be used to distinguish the plant from its closely related species. The young stem is scrabid in nature due to the presence of large number of

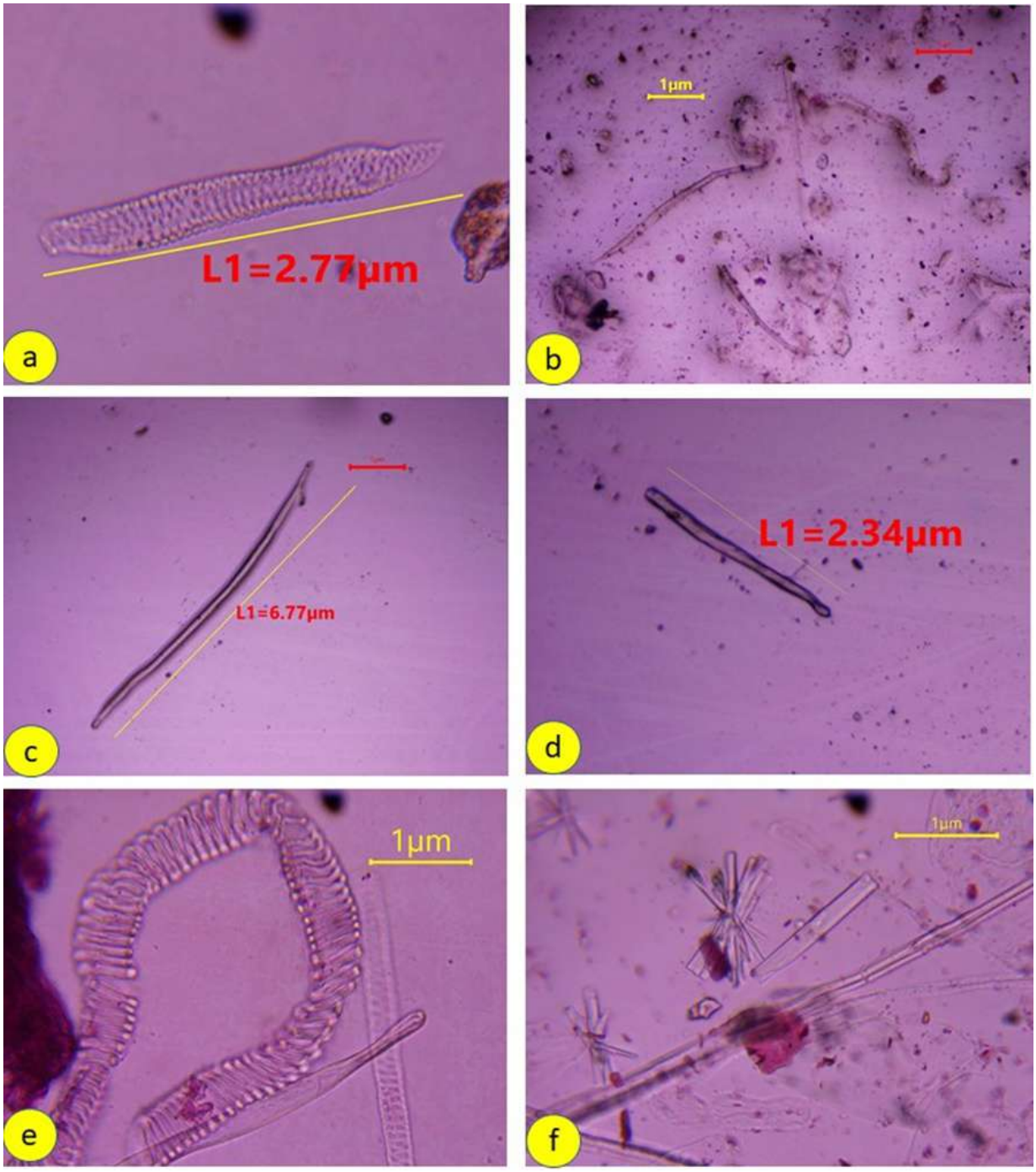
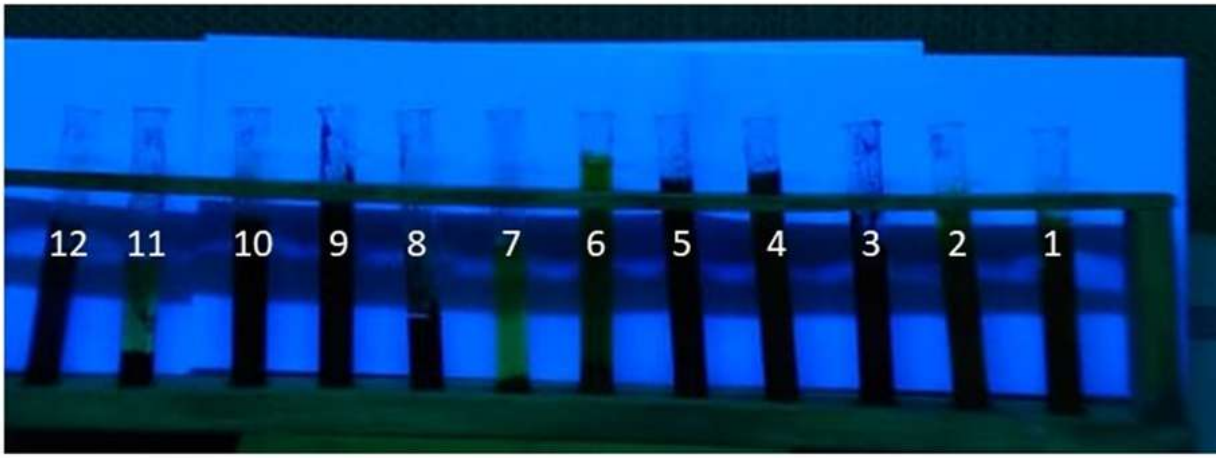


Plate 3. Powder analysis of *S. alternata*
a. Pitted vessel b. Xylem fiber c. Scalriform tracheid d. Fragmented tracheids e. Xylem vessels with spiral thickening f. Xylem vessels and rays f. Druses

uniseriate trichomes. Ovate, simple, pubescent leaves with light green in upper and purple colour on the lower surface, arranged in the opposite manner.

Anatomical features are also important in systematics for identification, placing anomalous groups in satisfactory positions in classification and for indicating patterns of relationships that may have been observed by superficial convergence in morphological features (Essiett *et al.*, 2012). In the anatomical findings, T.S of stem showed characteristic features like, single layer of pigment producing layer (Plate 2.b.), polygonal inner cortical cells with starch grains, specific arrangement of secondary xylem in broken ring with broad pith. The quantitative leaf microscopy revealed high range (6500-7300) of lower stomatal number (Plate 2).

a. Colour developed under Visible light



b. Colour developed under UV light

Plate 4. Flourescence analysis *S.alternata*
a. Colour of the plant powder observed in visible light after treatment with differentreagents
b. Colour observed in UV light after treatment with different reagents

PH of water solution	1%= 8.15 10%=7.68
Alcohol soluble extractive	4.73 ±0.52% W/W
Chloroform soluble extractive	3.65 ± 0.48%W/W
Hexane soluble extractive	2.35 ±0.24% W/W
Total ash	13.45± 2.43% W/W
Acid-insoluble ash	8.25± 0.72% W/W
Water soluble ash	5.17 ± 0.65%W/W
Swelling index	0.2 ± 0.21
Foaming index	>100

Powder microscopic analysis showed the presence of pitted vessel, xylem fibers, scalariform tracheid, fragmented tracheids, xylem vessels with spiral thickening, xylem vessels and rays and calcium oxalate crystals in the form of druses (Plate 3.f). When physicochemical methods become inadequate, the plant materials can be distinguished from their adulterants on the basis of fluorescence characterization. *S. alternata* plant powder produced characteristic fluorescence in UV light when treated with various reagents. Various physiochemical parameters evaluated in this study can be used for

adulterant resolution or improper handling of the raw material and compilation of a suitable monograph for *S. alternata*.

Ash values can be used as reliable aid for identification of the plant materials and detecting adulteration (Nayak *et al.*, 2010). It gives an idea of earthy matter or the inorganic composition and other impurities present along with drug. Based on the result obtained, the total ash value obtained was 13.45± 2.43% W/W, acid-insoluble ash was 8.25± 0.72% W/W and water soluble ash was 5.17 ± 0.65%W/W respectively. The acid insoluble ash was very low which shows that a very small amount of the inorganic component is present which is insoluble in acid and this is of diagnostic importance. The extract values give an idea about the nature of the chemical constituents present in the plant and is useful for the estimation of specific constituents soluble in that particular solvent.

Conclusion

The proper identity, purity and quality of a crude drug is a fundamental process in drug development. In the present study, a great bulk of information related to the identity, purity and quality of plant material is gained while evaluating the macroscopy, microscopy, powder characters and physicochemical parameters of the plant *S. alternata*. Thus the present investigation put forward important standardization parameters for a meagrely studied medicinal herb *S. alternata* which has a new entry as medicinal aid and also as a tool for identifying authenticity of plant material.

Various pharmacognostic parameters are evaluated in the present study it includes the macro and microscopic characterisation of the plant, powder analysis, fluorescence and physicochemical analysis etc. The results obtained suggests that *S. alternata* posses distinct features with purple colouration. The microscopic studies on powder analysis showed the presence of characteristic features such as druses. Powder analysis, fluorescence analysis and physicochemical parameters of the plant is conducted in the present study and it will be beneficial in order to identify the authentication of the plant even from the crushed or powdered form. It is hoped that this study would direct to the establishment of some new invents.

Acknowledgement

The authors are grateful to the Principal, Sree Narayana College, Kollam for providing the facility for carrying out the study. We thank the 'Fund for Improvement of S & T Infrastructure (FIST)' of the Department of Science & Technology, Government of India for the instruments we used throughout the study were purchased under DST FIST scheme of Government of India.

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Chapter 19

BIODIVERSITY OF WILD LEAFY GREENS USED BY THE TRIBAL COMMUNITIES OF MEENAGADI PANCHAYATH OF WAYANAD DISTRICT, KERALA

Asiya P, Girigan G

Abstract Wayanad is hilly terrain in the southern Western Ghats and lies at an altitude of 750 m from the mean sea level. Forest types of tropical wet green, dry evergreen, semi evergreen, moist deciduous are seen in this district. Hence the biodiversity of this district is very diverse. But nowadays due to climate change, increasing population, and environmental degradation, there is a high risk of biodiversity loss at a large scale. Under such circumstances, the knowledge and uses of nutritious, climatically adapted wild leafy vegetables will be irreversibly lost. Most of the younger generation have a perception that collecting or consuming wild leafy greens reduces social prestige. Among the tribes and the heterogenous community present in the study area, the Paniya community consumes more species of wild leafy greens than the others. The present study has identified thirty-four leafy green species from the study area. Among the 34 species identified, 23% belong to the Amaranthaceae family, 63% are herbaceous plants, and 71% are found on the roadside. Popular choices include *Alternanthera sessilis* (Ponnankanni), *Amaranthus spinosus* (Mullan cheera), *Amaranthus viridis* (Kuppacheera), and *Solanum nigrum* (Mudunga chappu) are frequently consumed. According to use value analysis, the leafy green species *Boerhavia diffusa* is considerably more important with 79 usage reports and a use value (UV) of 0.98, followed by *Amaranthus spinosus* with a UV of 0.96 and *Bacopa monnieri* with a UV of 0.81.

Key words: *Western Ghat, Leafy green, Use value*

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Introduction

Wayanad, one of Kerala's fourteen districts, is located on the southern extremity of the Deccan plateau. The location lies between the north latitudes 11026' -12000' and the east longitudes 75075' -76056'. The elevation ranges from 700 to 2,100 meters above sea level (Narayanan *et al.*, 2011). Wayanad is a biodiversity hotspot in the Western Ghats, home to a diverse range of indigenous plants and wildlife. Wayanad has the highest number of tribal (adivasi) people in Kerala. In Wayanad, tribals rely primarily on indigenous vegetables, both cultivated in kitchen gardens and wild, to supplement their food. Wild leafy vegetables (WLVs) are an important source of micronutrients for persons who follow a carbohydrate-rich diet (Misra *et al.*, 2008). Many tribal groups in Wayanad hold knowledge of a diverse range of species for culinary needs (Anilkumar *et al.*, 2008; Narayanan *et al.*, 2006)

These indigenous tribes value the cultural and spiritual relevance of leafy plant diversity, as well as its importance as food and medicine. These herbs are also preferred by tribal people as a home remedy for skin ailments and other problems (Nisha and Sivadasan, 2007; Raji and Raveendran, 2011). The knowledge related to the use of WLVs is a component of indigenous knowledge (IK) that is primarily known to tribes. Local communities around the world increase their nutrition security

and socio-cultural identity through the use of traditional food that has evolved in harmony with the natural environment (Turner, 2005). As a result, the study was carried out to identify the key WLVs used by the tribal community in Wayanad and to analyse their uses.

Methodology

The study team visited tribal hamlets located in Meenangadi Grama Panchayath for the purpose of understanding the plant diversity accessed by tribal households. Random households were selected for the study. Individual house hold questionnaire surveys and focus group discussions were conducted to obtain the data. After gaining confidence from the tribal people, the team conducted a transect walk through different landscapes to explore the diversity and usage of plants. Specimens of plants were collected for identifying their scientific names and identified with the help of a taxonomist. A total of 80 surveys and four focus group discussions were conducted and pooled the data. Use Value (UV) was also determined. According to Philips and Gentry (Philips *et al* ., 1994). UV is used to determine the relative importance of a particular species with respect to other species, and it is obtained as the sum of total use reports of a particular species divided by the total number of informants. The equation is given below,

$$UV=\Sigma U/N,$$

Where ΣU is the total number of uses and N is the total number of respondents. The high value of UV indicates that the plant is relatively important for its life 23 and a lower value shows the lesser importance of the plant species with respect to others (Musa *et al*., 2011).

Results

Most of the tribes are either landless or marginal landholders. Both men and women work outside for their survival. Since land holding is limited, they give less interest to cultivate plants. Forage of wild food plants is an important coping strategy adopted by the tribes in the study area to meet food and nutrition security. Wild food plants serve as alternatives to staple foods during time of food scarcity in households and provide valuable supplement for a nutritionally balanced diet. Women play a vital role in collecting, processing and cooking wild edibles. They access these genetic resources from a number of landscapes they interact. They access scrib jungles, wasteland, wayside, streamside, coffee plantation, rice fields and banana fields for accessing wild edibles.

The women give more priority to collecting leafy greens. Consumption of wild leafy greens was common among the tribes. By collecting leafy greens they reduce their dependence on the market for vegetables. They enjoyed a customary right to collect uncultivated plants from agricultural landscapes owned by their neighbours/landlords.

The leafy greens they consumed by cooking with salt and oil or by adding grind coconut and small onion to the above preparation. They use the same greens or a mixture of two or more greens depends up on the availability and quantity of the plants. The study identified thirty four plant species that are used as wild leafy greens but only few are widely used. *Alternanthera sessilis* (Ponnankanni), *Amaranthus spinosus* (Mullan cheera), *Amaranthus viridis* (Kuppacheera), *Solanum nigrum* (Mudunga chappu) are frequently consumed ones. The observations were shown in Table 1.

From the identified thirty four species 23.53% of them came under the family Amaranthaceae followed by the Fabaceae family with 11.78%, and 64.71 % of plants were herbs, 20.59% were shrubs and 14.71% were climbers and 28 plants were habitated in way side. The results were shown in figure 1, 2 and 3 . Apart from the leafy green use, also collected the ethnomedicinal and fodder, or other important uses of the same and calculated the use value of the same.

The use value of the leafy greens used by the tribal community from the study area was studied. The plant species Boerhavia diffusa has more use value of 0.99 followed by *Centella asiatica* and *Amaranthus spinosus* with 0.98 and 0.96 respectively.

Issues in the collection and conservation of wild leafy greens

The transformation of landscapes is treated as one of the biggest barriers to the conservation of wild leafy greens. Most of households are of the

Table. 1 The results of the leafy greens being consumed by the tribal groups from the study area

S. No	Botanical Name	Local name	Family	Habit	Habitat	Medicinal	Used by	TUR*	UV
1	*Cyathula prostrata*	Cherukadaladi	Amaranthaceae	Herb	Wayside	Eeye diseases (21), Clean cattles (1)	KUR,PAN	22	0.28
2	*Achyranthus aspera*	Valiyakadaladi	Amaranthaceae	Herb	Wayside	Gynaological problems (20), swelling in legs (24), laxative (10), cattle feed(2)	KU,KUR,PAN**	56	0.7
3	*Amaranthus caudatus*	Kaattucheera	Amaranthaceae	Herb	Wayside	Antialmenthic (15), laxative (33)	KU,KUR,PAN	48	0.6
4	*Amaranthus viridis*	Kuppacheera	Amaranthaceae	Herb	Wayside	Ulcer (5), urinary infection (19), laxative (16)	KU,KUR,PAN	40	0.5
5	*Amaranthus spinosus*	Mullancheera	Amaranthaceae	Herb	Wayside	Diarrhea, stomach disorders, ulcerated mouths, vaginal discharges, laxative (70)	KU,KUR,PAN	77	0.96
6	*Alternanthera bettzickiana*	Cherucheera	Amaranthaceae	Herb	Wayside	Purifying blood (10), laxative (30)	PAN	40	0.5
7	*Alternanthera brasiliana*	Choracheera	Amaranthaceae	Herb	Wayside	Inflammation, cough, and diarrhea(9)	PAN	9	0.11
8	*Alternanthera sessilis*	Ponnamkanni	Amaranthaceae	Herb	Paddy field	Antipyretic, anti- inflammatory(51)	KU,KUR,PAN	51	0.64
9	*Adenia hondala*	Koombichappu	Passifloraceae	climber	Wayside	Laxative (9), cattle feed(1)	PAN	10	0.13
10	*Bacopa monnieri*	Brahmi	Scrophulariaceae	Herb	Wayside	Anxiety, mental fatigue, memory eczema, psoriasis (64), veterinary medicine (1)	KU,KUR,PAN	65	0.81
11	*Boerhavia diffusa*	Thazhuthama	Nyctaginaceae	Shrub	Wayside/ Paddy field	Kidney disorders (71), cough, skin diseases (3), asthma and jaundice (4)	KU,KUR,PAN KUR,PAN	79 39	0.99 0.49
12	*Cardiospermum helicacabum*	Pokkanam thookki /	Sapindaceae	Cimber	Wayside	Asthma (32), used as feed of goat (7) Muscle pain (34)	PAN,OTH	34	0.43
13	*Capsicum fructosum*	Uzhinja	Solanaceae	Shrub	Wayside	Skin disorders (21), laxatives (33)	PAN	54	0.68
14	*Cassia tora*	Cheena paranki Thuvara	Caesalpinia ceae	Herb	Wayside	Brain power (51), pustules (22), infection in cows (5)	KU,KUR,PAN	78	0.98
15	*Centella asiatica*		Apiaceae	Herb	Paddy field	Laxative (35)	KU,KUR,PAN	35	0.44
16	*Colacasia esculenta*	Muthil	Araceae	Herb	Wayside	Constipation (19)	KU,PAN	19	0.24
17	*Colacasia antiquorun*	Kollithal	Araceae	Herb	Wet land	Laxative (1)	PAN	1	0.01
18	*Costus spinosus*	Thaal	Zingiberaceae	Herb	Wayside	Jaundice, fever, laxative (16), cattle feed (2)	PAN	18	0.23
19	*Commelina bengalensis*	Unnithand Kannisoppu	Commelinaceae	Herb	Wayside	Asthma, diarrhea, rheumatism, headache, fever, wounds, pain, and constipation (18)	PAN	18	0.23
20	*Diplazium esculantum*	Churuli	Athyriaceae	Herb	River side	Urinary tract infection (39), kidney stone (5), cattle feed (2)	KUR,PAN	46	0.58
21	*Euphorbia hirta*		Euphorbiaceae	Herb	Wayside	Laxative (9)	PAN	9	0.11
22	*Laportea interrupta*	Paalcheera	Urticaceae	Herb	Wayside	Rheumatic arthritis (7), kidney infections (22),	PAN,KU,KUR	31	0.39
23	*Hygrophylla schulli*	Choriyanum	Acanthaceae	Herb	Wetland	jaundice and oedema (2)	PAN	6	0.08
24	*Monochoria vaginalis*	Kozhimullan/ Vayalchulli	Pontederiaceae	Herb	Paddy field	Cough, asthma, toothache, stomach and liver problems (6)	KUR,PAN	46	0.58
25	*Oxalis corniculata*	Karinkoovalam Puliyarila	Oxalidaceae	Shrub	River / steam side/ Paddy field	Anti-inflammatory (37), antifungal (3), antiulcer (3) and hepatoprotective (3) Laxative (8)	PAN	8	0.1
26	*Physalis minima*		Solanaceae	Herb	Wayside	Muscle relaxant, and anti-inflammatory and diuretic (8)	PAN	8	0.1
27	*Portulaca oleracea*	Njottanjodiyan	Portuacaceae	Shrub	Wayside				
28	*Cleome viscosa*	Kozhuppacheera	Capparaceae	Herb	Wayside	Rheumatic (4) arthritis, wound healing (2)	PAN	6	0.08
29	*Vigna vexillata*	Nai kaduku Kaattuppayar	Fabaceae	Climber	Way side	Arthritis, swellings in joints (14)	PAN	14	0.18
30	*Mimosa pudica*		Mimosaceae	Climber	Way side	Urogenital disorders (2) and also applied on wounds (63)	PAN,KU,KUR	65	0.81
31	*Caesalpinia mimosoidea*	Thottavadi	Caesapiniaceae	Shrub	Way side	Ulcer, Heal wound (6)	KUR	6	0.08
32	*Mukia madaraspentata*	Koomullu	Cucurbitaceae	Climber	Wayside	Cough, abdominal disorders (9)	PAN	9	0.11
33	*Persicaria chinensis*	Mukkappeera	Polygonaceae	Shrub	Wayside	Stomach pain (13)	PAN	13	0.16
34	*Persicaria glabra*	Chorakam Pulichappu	Polygonaceae	Shrub	Wayside	Diarrhea, dyspepsia, itching skin (11), excessive menstrual bleeding (8)	KU,KUR,PAN	19	0.24

*Total use reports **KU Kuruma, KUR Krichiya, PAN Paniya

ISBN 978-81-958369-2-5

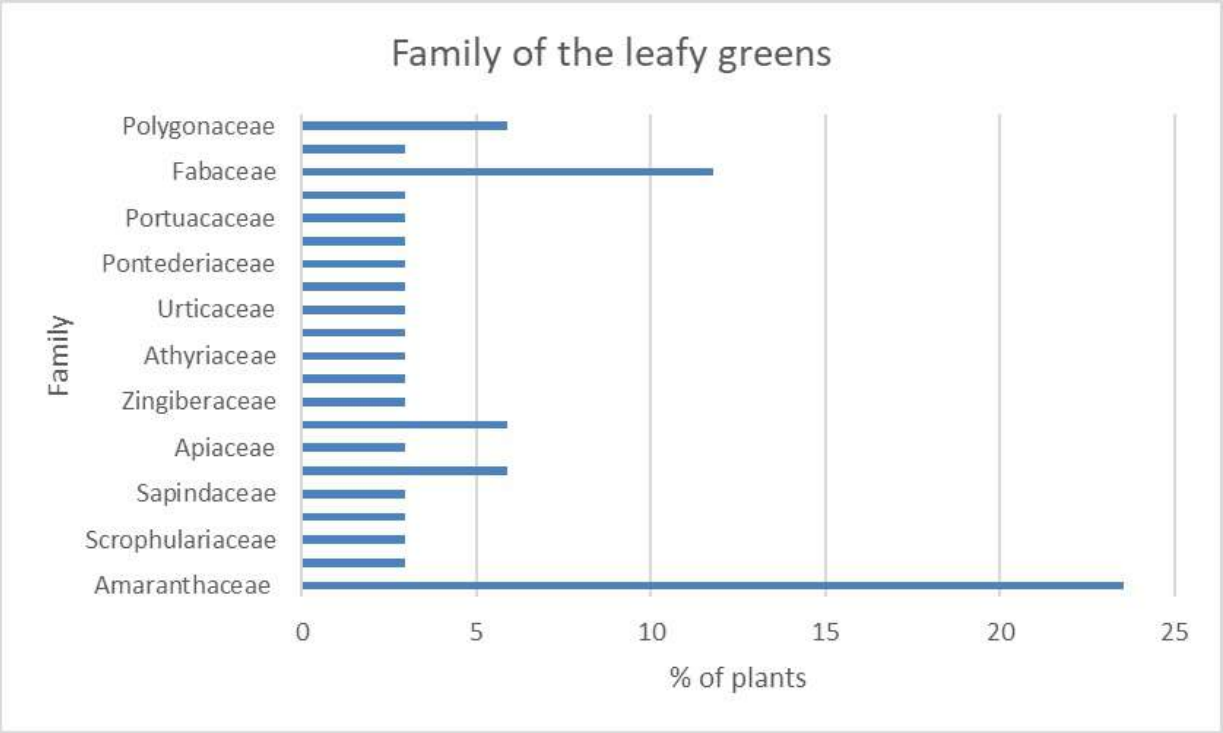

Fig. 1. The family of leafy greens being used by the tribal community from the study area

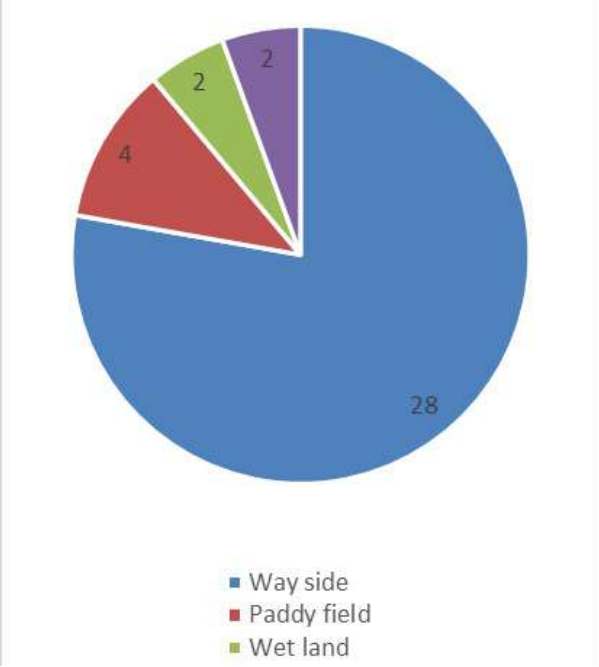

Fig. 2. Habitat of the leafy greens

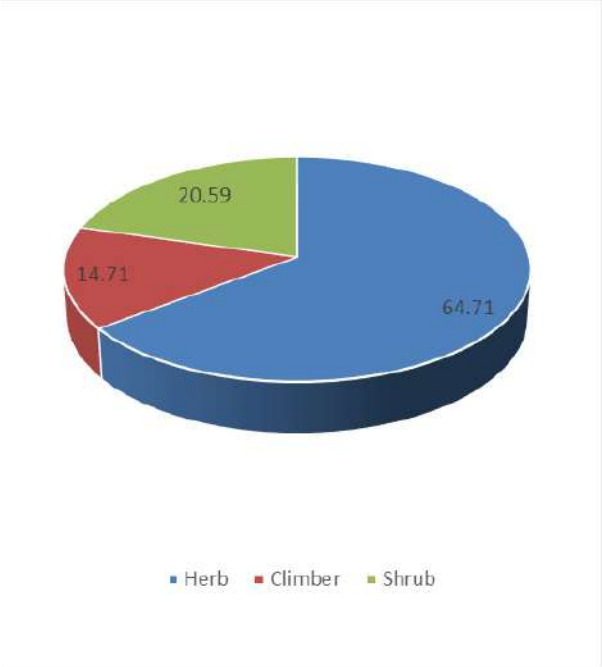

Fig. 3. Habit of the leafy greens

opinion that transformation activities like mining and quarrying have affected the availability of leafy greens. Similar to this, they also opined that changes in the cropping pattern and intensification of agriculture have also resulted in less availability of leafy greens. Intensive agriculture in the form of increased application of chemical fertilizers and pesticides restricted them from accessing wild leafy greens from banana fields. Youngsters in the tribal community pay less interest to collect or consume wild edibles because of two reasons, (1) lower social prestige associated with the collection of wild edibles and (2) lack of transfer of knowledge on the identification and processing of wild edibles.

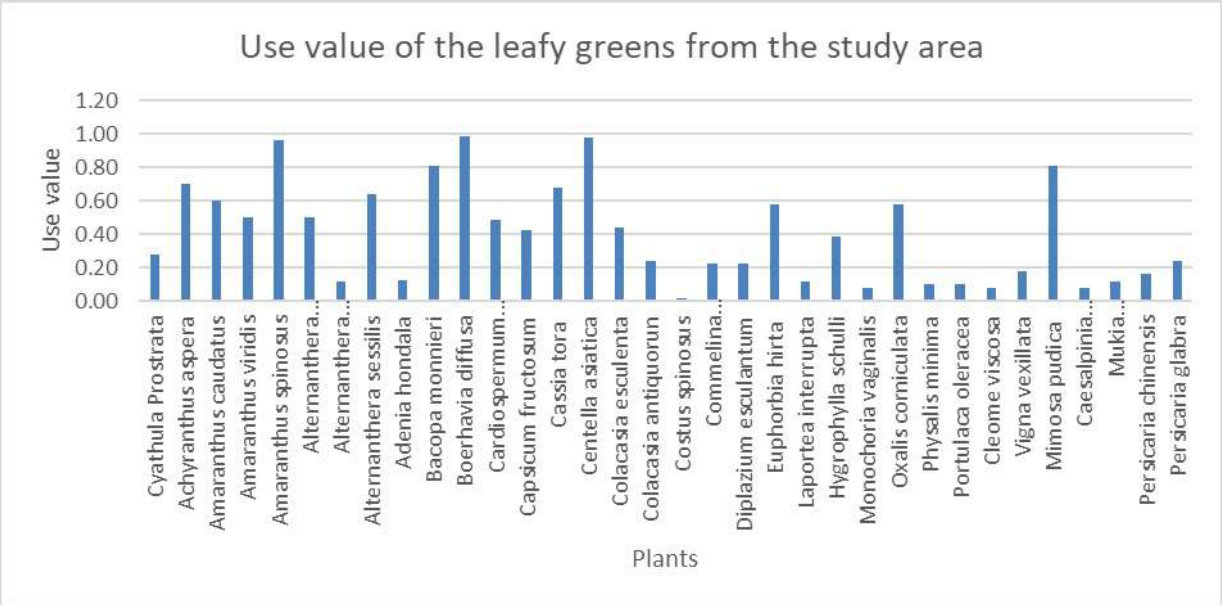

Fig. 4. Use value of the leafy greens from the study area

Discussion

In general, the collection and consumption of wild leafy greens are increasingly stigmatized as symbols of poverty and 'tribalness' (Daimari *et al.*, 2019). Since ancient times, plants and their derivatives have been traditionally used as medicine for the treatment of various diseases (Devi Prasad *et al.*, 2014). Many plants such as B*oerhavia diffusa*, *Centella asiatica*, *Amaranthus spinosus* etc., are used for the treatment of brain strengthening, kidney disorders, stomach pain, etc. (Hema *et al.*, 2006). Among the tribal communities focused in this study, the Paniya community is really successful in exploring all the available edible plant groups from all types of vegetation ranging from dense forest to even plain grazing land areas (Shyama *et al.*, 2012)

Conclusion

The study revealed that the tribes are still depending on wild leafy greens for their food and nutritional needs. Wild leafy greens are alternate to vegetables from the market. Women play a vital role in the collection and cooking of wild leafy greens and thereby ensuring household nutritional security. Many factors restrict them from accessing wild edibles compared to the past. Landscape transformation, agriculture intensification, lack of interest from the part of youngsters etc., are a major threats to the collection and conservation of wild leafy greens. Lack of awareness about the nutritional values, unscientific intervention on the part of land managers etc., also posing threats. Yet, people still access wild leafy greens. Access to diverse wild leafy greens to enrich their diet and ensure a healthy life, despite poor socio-economic background.

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Chapter 20

MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL CHARACTERIZATION OF *ALTERNANTHERA SESSILIS* (L.) R. BR. AND *ALTERNANTHERA BRASILIANA* (L.) KUNTZE

Drisya Krishna T S, Amala Ajayakumar, Nisha A P

Abstract *Alternanthera sessilis* (L.) R.Br. and *Alternanthera brasiliana* (L.) Kuntze comes under the family Amaranthaceae. Here, morphological characterization and phytochemical analysis of both plants are studied. In morphological analysis, both quantitative and qualitative analyses were carried out. The stem anatomy of both species is similar but shows slight differences between them. In principal component analysis, the first principal component accounts for the maximum variation of 31.27%, and the second principal component accounts for 16.03% variation. The highest loaded variable in PC1 is leaf colour and in PC2 is internode length. UPGMA cluster analysis showed three principal clusters. The first principal cluster consists of accessions AB1, AB2, AS4, and AS1. The second principal cluster consists of AB3, AB4, and AB5. The third principal cluster consists of AS5, AS3, and AS2. Here the accessions AS5 and AS3 are grouped to form a sub-cluster. So, *A. sessilis* showed similarities with *A. brasiliana* in certain characters. In the preliminary phytochemical screening using ethyl acetate extract of both plants, the presence of different phytochemicals such as tannin, flavonoid, and phenolics was confirmed. The presence of steroids is found only in *A. brasiliana*. The total phenolic content in the leaf extract of both species was estimated. *Alternanthera brasiliana* has greater phenol content than *A. sessilis*. Antioxidant activity of both *A. sessilis* and *A. brasiliana* was determined using DPPH radical scavenging assay and reducing power assay. The antioxidant activity is greater in *A. brasiliana*.

Keywords: *Principal component analysis, UPGMA cluster analysis, Accessions, Antioxidants, DPPH radical scavenging assay, Reducing power assay.*

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Introduction

Amaranthaceae is a family of flowering plants commonly known as the Amaranth family, about its genus Amaranthus. Different classes of chemical constituents such as flavonoids, phenolics, saponins, alkaloids, and tannins are isolated from the members of this family. Economic importance includes vegetable crops, ornamentals, and some others are considered as detrimental weeds (Mosyakin and Robertson 2003).

Alternanthera sessilis (L.) R. Br. is one of the members who come under the family Amaranthaceae. *Alternanthera* includes 134 accepted species. *A. sessilis* is also called sessilis joy weed. It can also grow in seasonal water-logged areas and areas with extremely dry conditions (Holm *et al.*, 1997). *A. sessilis* is also a weed, that is grown in fields with sorghum, millet, cotton, cassava, cereal crops, and vegetable farms (Gupta, 2014). Fan *et al*, (2013) listed it as a noxious weed in the United States (USDA-NRCS, 2014). However, the most recent study of the genera *Alternanthera* suggests that *A. sessilis* originated in South America, and from here, it was introduced to the old world (Sanchez *et*

al, 2012). It is an annual or perennial herb, 0.2-1m in height, consisting of strong tap roots. The stem is generally prostrate, often rooting at the nodes, sometimes they were floating or creeping. The stem is cylindrical and slightly hairy with numerous branches. It is propagated vegetatively by fragments and seeds. The characteristic feature is the presence of white, scarious bract, and sessile spike. Its flowers are self-pollinated.

Another species that come under the genus *Alternanthera* is *Alternanthera brasiliana* (L.) Kuntze. It is commonly called joy weed, described as perennial with a prostrate stem and branchy, presenting a circular to polygonal stem with long internodes and swollen nodes. At nodes, the leaves are attached opposite. Leaves are oval-lanceolate and red in color. The inflorescence is usually a pedicellate spike which is white in color with a slightly scarious bract. The leaf is amphistomatic described by (Dela *et al*., 2002). Both *A. brasiliana* and *A. sessilis* leaves are used like spinach and in soups. Morphological characters are the most widely used tool in the classification of higher plants. The phytochemical survey aims to detect diverse groups of naturally occurring phytochemicals in plants. Chemical constituents may be therapeutically active or inactive. The phytochemical research approach is considered effective in discovering the bioactive profile of plants of therapeutic importance. Antioxidant activity is determined to analyze the presence of substances called antioxidants that prevent or slow the damage to cells caused by free radicals. In recent years, malnutrition is an overwhelming problem in developing countries. Poor nutrition and inadequate supplements in the food thrive to be a reason for many diseases. This could be surpassed by the intake of foods rich in all vitamins, essential minerals, and trace elements. So the objective of the present study is to analyse the morphological, anatomical and phytochemical characters in *A. sessilis* and *A. brasiliana*

Materials and methods

Five accessions of both *A. sessilis* and *A. brasiliana* were collected from different locations in the Kollam district. Specific accession codes were allotted to each character (Table1).

1. Morphological characterization

Observations on ten qualitative (Table 2) and six quantitative (Table 3) characters of both species were scored in all accessions. Vegetative and floral characters are studied in all these accessions collected. The qualitative and quantitative characters selected for the study were recorded. The measurements of each selected character were taken using a standard ruler. For morphometric analysis, quantitative data were subjected to one-way ANOVA using SPSS version 16. Multivariate analysis was performed using the procedure of Principal Component Analysis (PCA). Data were subjected to cluster analysis based on the UPGMA method to find out the similarity and dissimilarities among the accessions.

2. Anatomical characterization

Anatomical features of the stems of both species were studied. For this, the thin cross sections of the stems were taken using a sharp

Table 1. Details of different accessions of *A. sessilis* and *A. brasiliana*

Sl. No.	Accession code	Place of collection	Genetic name	Status
1	AS1	Kallumthazham	*Alternanthera sessilis*	weed
2	AS2	Anchal	*Alternanthera sessilis*	Weed
3	AS3	Panayamchery	*Alternanthera sessilis*	Weed
4	AS4	Madhurappa	*Alternanthera sessilis*	Weed
5	AS5	Thadicadu	*Alternanthera sessilis*	Weed
6	AS6	Punalur	*Alternanthera brasiliana*	Weed
7	AS7	Anchal	*Alternanthera brasiliana*	Weed
8	AS8	Thevarthottam	*Alternanthera brasiliana*	Weed
9	AS9	Edayam	*Alternanthera brasiliana*	Weed
10	AS10	Madhurappa	*Alternanthera brasiliana*	Weed

Table 2. List of qualitative variables with abbreviation and description

Si. No	Character	Abbreviation	Description
1	Habit	HB	0-Herb
2	Leaf shape	LS	0-Elliptic
			1-Obovate
			2-Ovate
			3-Lanceolate
3	Leaf apex	LA	0-Acute
			1-Optuse
			2-Acuminate
4	Leaf margin	LM	0-Entire
			1-Undulate
5	Leaf surface	LS	0-Glabrous
			1-Pubescent
6	Leaf position	LP	0-Opposite
7	Bract	BR	0-Scarious
8	Braccolorur	BRC	0-White
9	Inflorescence	INF	0-sessile spike
			1-peduncled spike
10	Leaf color	LC	0-Green
			1-Red

razor. These cross-sections were stained using safranin and observed under an electron microscope. It helps to find out the anatomical similarities and dissimilarities between them.

3 Phytochemical Analysis

3.1 Preparation of the extract

The leaves of both *A. sessilis* and *A. brasiliana* were collected from healthy plants and shade dried separately and powdered in an electric mixer grinder. For the preparation of the extract, 10 gm of the powder was mixed with 50 ml of ethyl acetate and kept it for 24hrs. After 24hrs the extract was filtered and this extract was used for the following tests.

3.2 Test for alkaloids

2ml of extract mixed with one or two drops of Mayer's reagent. Cream colour precipitate indicates the presence of alkaloid.

3.3 Test for tannins

A portion of the extract is mixed with distilled water and then it is followed by filtration. The filtrate is mixed with 2 drops of ferric chloride solution. The black color indicates the presence of tannins.

3.4 Test for flavonoid

The aqueous extract is mixed with a 10% ferric chloride solution. Green precipitate indicates the presence of flavonoids.

3.5 Test for phenolics

1ml of the extract mixed with a few drops of dilute iodine solution. The appearance of transient red color indicates the presence of phenolics.

3.6. Test for terpenoids

5 ml of the extract mixed with 2 ml of chloroform and 3 ml of concentrated Sulphuric acid. A reddish-brown color layer at the interphase indicates the presence of terpenoids.

3.7. Test for saponins

Extract diluted with 20ml distilled water and agitated for 20 minutes. If it does not form foam, it indicates the absence of saponins.

3.8. Test for steroids

1 ml of the extract is mixed with 10 ml of chloroform and added an equal volume of concentrated sulphuric acid. The upper layer in the test tube turned red and the sulphuric acid layer

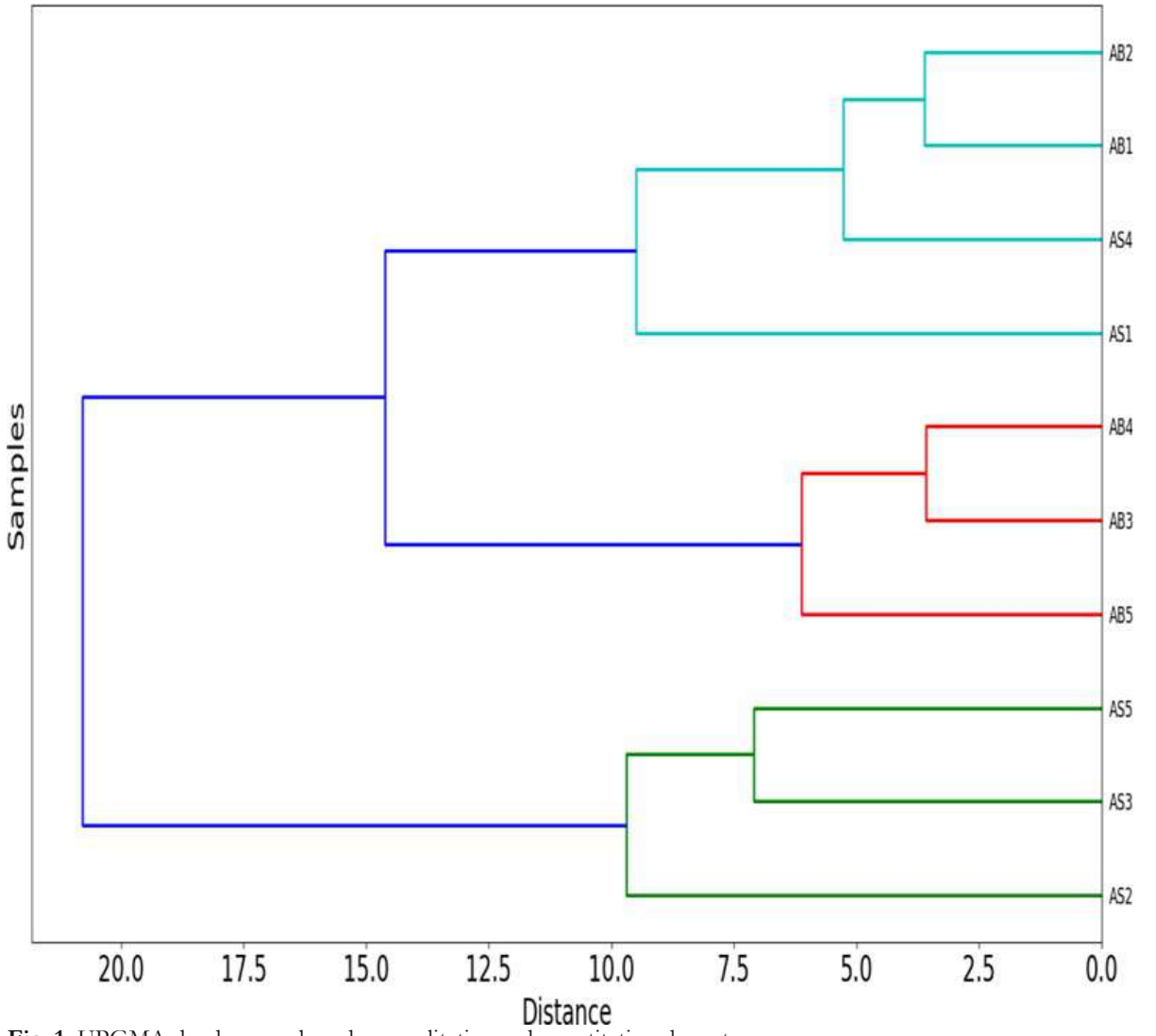

Fig. 1. UPGMA dendrogram based on qualitative and quantitative characters.

show yellow with green fluorescence.

3.9. Test for cardiac glycoside

2 ml of the extract mixed with bromine water. The yellow color indicates the presence of cardiac glycosides.

4. Determination of total phenol content and antioxidant activity

The leaves of both *A. sessilis* and *A. brasiliana* were powdered in an electric mixer grinder and 10 g of the sample was extracted with 50 ml of ethyl acetate by continuous shaking. The extract was filtered through the Whatman No:1 filter paper. The residue was re-extracted twice and filtered. The extract was dried under a vacuum (Jena *et al.*, 2002). The extracts from both plants were used for the determination of total phenol content and antioxidant activity.

4.1 Determination of total phenol content

The total phenol content in the sample was estimated by the Folin Ciocalteau method (Singleton and Rossi., 1965). Distilled water (3.9 ml) and Folin's reagent (0.5 ml) were added to 0.1 ml of the extract. The solution was incubated at room temperature for 3 minutes and 2ml of 20% sodium carbonate solution was added to the mixture. The solution was then kept in a boiling water bath for one minute, cooled, and absorbance was measured at 650 nm. catechol was used as the reference standard.

4.2 Estimation of antioxidant activity

4.2.1. DPPH radical scavenging activity

The ethyl acetate extract of *A. sessilis* and *A. brasiliana* were subjected to 2, 2- Diphenyl-1-Pieryl-hydrazyl-hydrate (DPPH) assay. Free radical scavenging activity using DPPH radical scavenging

Table 3. List of quantitative variables selected with abbreviation and description

Sl. No.	Character	Abbreviation
1	Leaf length	LL
2	Leaf number	LN
3	Leaf breadth	LB
4	Internode length	INL
5	Bract length	BL
6	Number of spikes at one node	NSPN

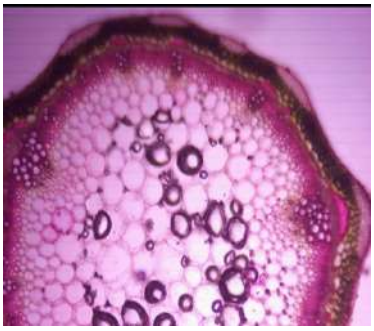

Fig. 2. *A. sessilis*

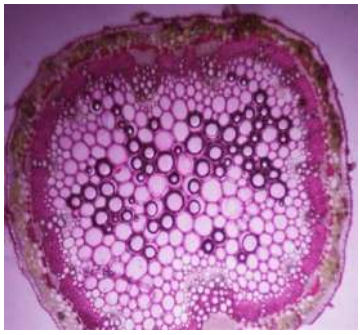

Fig. 3. *A. brasiliana*

activity of the extract was determined according to the Blois method (Blois, 1958) with some modification. Different concentrations (20, 40, 60, 80, and 100 µg) of the extract were taken in different test tubes. The volume was adjusted to 100µl by adding methanol. The methanolic solution of DPPH (1 ml of 0.1 mM) was added to these tubes and shaken vigorously. These tubes were allowed to stand for 20 minutes. The control was prepared as above without extract and methanol was used as the baseline correction. The changes in the absorbance of the sample were measured at 517 nm.

4.2.2. Reducing power assay

The reducing power was based on the Fe (3) to Fe (2) transformation in the presence of extract. Various concentrations of both plant extracts (2ml) were mixed with 2ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferric cyanide (10mg/ml). The mixture was incubated at 50°C for 20 minutes followed by the addition of 2ml of trichloroacetic acid (100mg/l). The mixture was centrifuged at 3000 rpm for 10 minutes to collect the upper layer of the solution. A 2ml supernatant from each of the mixtures was mixed with 2 ml of distilled water and 0.4 ml of 0.1% fresh ferric chloride solution. After 10 minutes the absorbance was measured at 700 nm Higher absorbance of the reaction mixture indicates higher reducing power.

Results and Discussions

1. Morphological characterization

Five accessions of both *A. sessilis* and *A. brasiliana* collected from different localities of Kollam district showed variations in their morphological characters. The easily observable qualitative morphological variation is their leaf colour. The leaf colour in *A. sessilis* (L.) R. Br. is green and in *A. brasiliana* (L.) kuntze, it is red in colour. Certain qualitative morphological characters such as habit, position of leaves, nature of bract and flower colour are same in all the 5 accession of *A. sessilis* and *A. brasiliana*. In addition to this there is sessile spike in all accessions of *A. sessilis* while *A. brasiliana* consists of both sessile and peduncled spike.

All accessions of *A. sessilis* and *A. brasiliana* are herbs. In *A. brasiliana*, three types of leaf shapes are observed which includes lanceolate, ovate and elliptic and 2 types of leaf apex such as acuminate and acute are found from the study of five accessions.

A. sessilis also showed variations in their shape of leaves and leaf apex. The observations made from five accessions showed that there are three types of leaf shapes, which include elliptic, obovate and ovate and two type of leaf apexes, acute and obtuse. There are two types of leaf margins (entire and undulate) and two types of leaf surfaces (pubescent and glabrous) are found in both plants. In all the ten accessions of two species of *Alternanthera*, leaves are opposite, scarious and flowers with white coloured bracts.

Qualitative characters observed in different accessions of *A. brasiliana* and *A. sessilis* are represented in Table 4.

Quantitative characters showed some variations (Table 5). From the quantitative characters the highest leaf length (8.16 cm) is found in one accession of *A. brasiliana* (AB1) and shortest leaf length (3.6 cm) is found in one accession of *A.*

Table 4. Qualitative characters observed in different accessions of *A.sessilis* and *A.brasiliana*

Cchara cter	AS1	AS2	AS3	AS4	AS5	AB1	AB2	AB3	AB4	AB5
HB	Herb	Herb	Herb	Herb	Herb	Herb	Herb	Herb	Herb	Herb
LS	Elliptical	Elliptical	Elliptical	Obovate	Ovate	Lanceolate	Ovate	Lanceolate	Lanceolate	Elliptical
LA	Acute	Acute	Acute	Optuse	Acute	Acuminate	Acute	Acuminate	Acuminate	Acute
LM	Entire	Entire	Undulate	Entire	Undulate	Undulate	Entire	Entire	Entire	Entire
LSR	Pubescent	Pubescent	Pubescent	Glabrous	Pubescent	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous
LP	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite
BR	Scarious	Scarious	Scarious	Scarious	Scarious	Scarious	Scarious	Scarious	Scarious	Scarious
BRC	White	White	White	White	White	White	White	White	White	White
INF	Sessile Spike	Sessilespike	Sessile spike	Sessile spike	Sessile spike	Peduncled spike	Peduncled spike	Sessile spike	Peduncled spike	Peduncled spike
LC	Green	Green	Green	Green	Green	Red	Red	Red	Red	Red

Table 5. Quantitative morphological characters observed in *A. brasiliana* and *A. sessilis*. (LL, LB, INL in cm and BL in mm)

Name of accession	NL	LL	LB	INL	BL	NSPN
AS1	28.80 ± 2.588	3.600 ± 0.9460	1.560 ± 0.2510	4.540 ± 1.1971	3.40 ± 0.548	3.00 ± 0.707
AS2	42.40 ± 6.542	3.640 ± 1.4153	1.300 ± 0.1581	7.360 ± 1.3631	2.60 ± 0.548	8.80 ± 2.280
AS3	48.80 ± 4.494	6.520 ± 1.2478	2.740 ± 0.3647	12.24 ± 1.1349	2.60 ± 0.472	6.00 ± 1.225
AS4	22.80 ± 4.494	5.200 ± 0.9747	2.060 ± 0.2074	7.260 ± 0.4722	3.20 ± 0.447	3.00 ± 0.707
AS5	51.40 ± 4.393	3.860 ± 0.6348	1.600 ± 0.2121	7.020 ± 0.5630	2.40 ± 0.548	6.00 ± 0.707
AB1	23.40 ± 8.112	8.160 ± 2.1755	3.760 ± 0.7092	7.400 ± 4.2901	4.60 ± 0.548	1.40 ± 1.342
AB2	22.20 ± 6.017	6.880 ± 2.7508	3.320 ± 1.0378	5.820 ± 1.4464	5.00 ± 0.707	1.00 ± 0.707
AB3	39.60 ± 13.164	7.980 ± 2.2152	3.900 ± 0.6422	5.060 ± 1.0310	4.40 ± 0.548	1.40 ± 1.342
AB4	37.00 ± 8.860	6.820 ± 1.3572	3.900 ± 0.6205	4.100 ± 1.3820	4.00 ± 0.000	2.20 ± 0.837
AB5	34.00 ± 4.743	7.840 ± 1.7994	3.440 ± 0.8444	4.040 ± 0.9711	4.80 ± 0.447	1.80 ± 1.304

sessilis (AS1). Different sizes of leaves are found in the same plant. The highest leaf bredth (3.9 cm) is found in two accessions of *A. brasiliana* (AB4) and shorter leaf bredth (1.3 cm) is found in accessions of A.sessilis (AS2).The highest internode length (12.24cm) was found in the accession of *A. sessilis* (AS3) and shorter internode length (4.04 cm) was found in the accession of *A. brasiliana* (AB5).

The bracts of all accessions of both *A. brasiliana* and *A. sessilis* is represented in Fig 5 & 6 respectively. The bracts are scarious and white in colour in all accessions of both species. Highest bract length (5 mm) and lowest number of spikes at one node (1 spike) were found in the accession of *A. brasiliana* (AB2). The shortest bract length was observed in one accession of *A. sessilis* (2.4 mm) (AS5). Highest number of flowers per spike is found in the accession of *A. sessilis* (AS2). Kumar *et al.* (2011) reported similar morphological characters of *A. brasiliana*

2. Morphometric analysis

2.1. Analysis of variance

Analysis of variance carried out in different quantitative characters showed significant variations ($P < 0.5$).

2.2. Principal Component Analysis

The principal component analysis indicates that there are ten principal components in the data set.

2.3. Loadings for principal components

In principal component analysis, the first principal component accounted for the maximum variation of 31.27% (Table 6). The first principal component had traits with highest loadings are leaf length, leaf breadth, bract length, number of spikes per node and leaf colour. The second principal component accounted for 16.03% variation with highest loading characters are internode length, leaf shape, leaf margin. The highest loaded variable in PC1 and PC2 are leaf colour and nature of leaf margin respectively. So these characters showed maximum variations in *Alternanthera*.

3. Cluster Analysis

UPGMA cluster analysis showed three principal clusters (Fig. 1). The three principal clusters are cluster 1, cluster 2, and cluster 3. The first principal cluster consists of accessions AB1, AB2, AS4 and AS1. Of these AB1 and AB2 are grouped together to form a sub cluster. The accession AS4 and AS1 exists independently. The second principal cluster consists of AB3, AB4 and AB5. Of these the accessions AB3 and AB4 together form a subcluster. The accession AB5 occur independently. The third principal cluster consists of AS5, AS3 and AS2. Here the accessions AS5 and AS3 are grouped to form a sub cluster. From the dendrogram, it was clear that some accessions of *A. sessilis* showed similarities with *A. brasiliana* in certain characters. Cluster Analysis has been employed to assess similarities among genotype in plant breeding programmes (Rakonjac *et al.*, 2014).

4. Anatomical characterization

The transverse section of the stem of *A. sessilis*

is wavy in outline with an outer single layered epidermis that consists of polygonal cells (Fig. 2). Trichomes are present. The epidermis is followed by 5-6 layered cortex and is followed by 2-3 layers of chlorenchymatous cells. Below that, the vascular bundles are arranged in a ring that is conjoint and collateral. Each vascular bundle is encapped by a patch of pericyclic fibers. A large parenchymatous pith is found at the centre region.

In *A. brasiliana*, the T.S. of the stem is circular in outline (Fig. 3). It consists of a uniseriate layer of epidermis made up of polygonal cells. Below that, there are 2- 3 layers of chlorenchyma followed by collenchyma, and vascular bundles are arranged in a ring-like manner. The cambium forms phloem centrifugally and xylem centripetally. A large parenchymatous pith is found.

5. Preliminary Phytochemical Analysis

In the present study, eight phytochemical screening tests have been carried out. The result obtained in the study was presented in Table 7. When the extract is treated with Mayer's reagent, no yellow colour precipitate is formed. It indicates the absence of alkaloids. The green precipitate formed after treating with 10% ferric chloride indicates the presence of flavonoid. The formation of black colour when treated with ferric chloride indicates the presence of tannins. When extract treated with dilute iodine solution, a transient red colour was appeared due to the presence of phenolics. Terpenoids and saponins are absent. A red upper layer with yellow fluorescence on treating with sulphuric acid and chloroform indicates the presence of steroid (Fig 12d). The test for cardiac glycoside shows its absence.

6. Total phenolic content

The total phenolic content of ethyl acetate extract of both *A. sessilis* and *A. brasiliana* calculated (Table 8). The total phenol content in *A. brasiliana* is higher (2374.45mg/100g) than *A. sessilis*. Shaharuddin *et al*, (2019) also reported higher phenolic content in *A. brasiliana*

Table 6. Principal Component analysis and the percentage of variation accounted for by the first two PC .

Variables	PC1	PC2
NL	0.2005	0.1838
LL	**-0.3137**	0.2491
LB	**-0.3314**	0.2372
INL	0.1952	**0.4191**
BL	**-0.34514**	-0.1509
NSPN	**0.3263**	0.119
HB	0	0
LS	-0.2345	**0.3093**
LA	-0.2288	**0.2564**
LM	0.0943	**0.5761**
LSR	**-0.2655**	0.1208
LP	0	0
BR	0	0
BRC	0	0
INF	**-0.2869**	-0.0108
LC	**-0.3515**	0.057
Percentage of variation	31.27	**16.03**

Significant principal component values indicated in boldface.

Table 7. Preliminary phytochemical screening of ethyl acetate extract of *A. sessilis* and *A. brasiliana*.

Phytochemical constituent	Test	*A. sessilis*	*A. brasiliana*
Alkaloids	Mayer's reagent test	-	-
Tannins	Ferric chloride test	+	+
Flavanoids	Ferric chloride test	+	+
Phenolics	Iodine test	+	+
Terpenoids	Reaction with chloroform and concentrated sulphuric acid	-	-
Saponins	Foam formation test	-	-
Steroids	Reaction with chloroform and concentrated sulphuric acid	-	+
Cardiac glycosides	Bromine water test	-	-

Table 8. Total phenol content of *A. sessilis* and *A. brasiliana*.

Plant	Total phenol content (mg/ 100g equivalent of standard)
Alternanthera sessilis	1060.475
Alternanthera brasiliana	2374.45

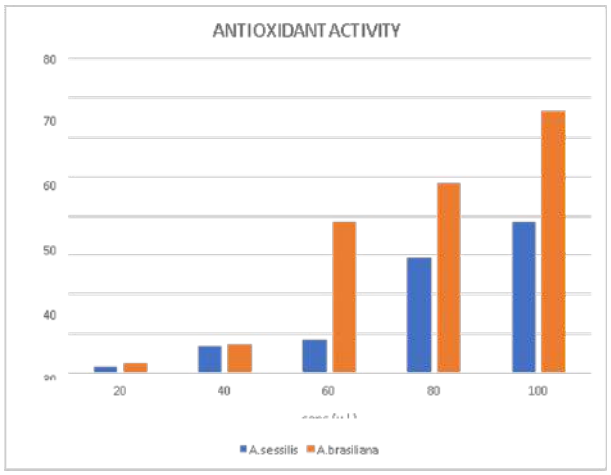

Fig. 4. DPPH radical scavenging activity of *A. sessilis* and *A. brasiliana*

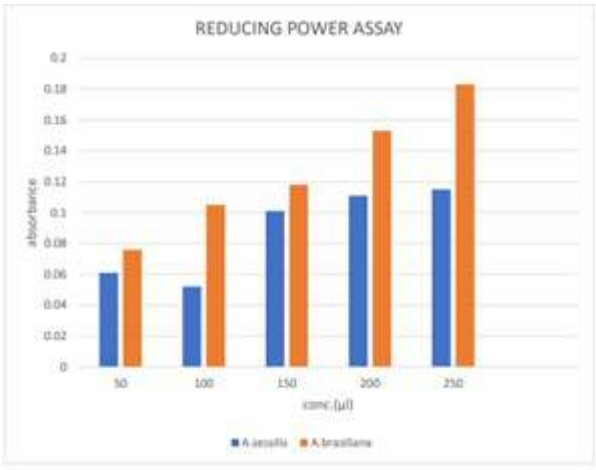

Fig. 5. Reducing power activity in *A. sessilis* and *A. brasiliana.*

7. Antioxidant activity

7.1. DPPH Radical Scavenging Assay

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between 33 antioxidant molecules and radical progress, results in the scavenging of the radical by hydrogen donation. Both *A. sessilis* and *A. brasiliana* exhibited a comparable antioxidant activity at varying concentrations tested (20, 40, 60, 80 & 100 µg/ml). Among the two species studied, *A. brasiliana* showed highest antioxidant activity. There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested (Fig 4). Kasthuri and Ramesh (2018) also conducted DPPH radical scavenging assays in *Alternanthera* species. They also reported a dose dependent radical scavenging activity in *Alternanthera* species.

7.2. Reducing Power Assay

In the present study, the ethyl acetate extract of *A. sessilis* and *A. brasiliana* showed a dose dependent reducing power activity (Fig 5). In this assay also *A. brasiliana* showed highest reducing power than *A. sessilis*. Reducing power is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the extract that possesses reducing property. Compounds with reducing power indicated that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants. The antioxidants, present in the plant extracts reduce the ferric cyanide complex (Fe^{3+}) to ferrous cyanide and form Fe^{2+} (Kasthuri and Ramesh, 2018).

Summary

A. sessilis and *A. brasiliana* comes under the family Amaranthaceae. Here, morphological characterization and phytochemical analysis of both plants are studied. In morphological analysis, both quantitative and qualitative analysis were carried out. The stem anatomy of both species is similar but there are some slight differences between them. In principal component analysis, the first principal component showed a maximum variation of 31.27%. The second principal component accounted for 16.03% variation. UPGMA cluster analysis showed three principal clusters. The first principal cluster consists of accessions AB1, AB2, AS4, and AS1. So, *A. sessilis* showed similarities with *A. brasiliana* in certain characters. In the preliminary phytochemical screening using ethyl acetate extract of both plants, the presence of different phytochemicals such as tannin, flavonoid, and phenolics was confirmed. The presence of steroids is found only in *A. brasiliana*. The total phenolic content in the leaf extract was estimated. *A. brasiliana* has greater phenol content. Antioxidant activity was determined using DPPH radical scavenging assay and reducing power assay. The

antioxidant activity is greater in *A. brasiliana*.

Acknowledgement

We express our sincere gratitude to the FIST scheme of Department of Science and technology, Government of India, STAR college scheme of the Department of Biotechnology, Government of India and the Principal, Sree Narayana college, Kollam for providing the facility.

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Chapter 21

RELEVANCE OF AGROECOLOGY WITH RESPECT TO *EX-SITU* CONSERVATION OF *TRICHOPUS ZEYLANICUS* SUBSP. *TRAVANCORICUS* (BEDD.) BURKILL EX. K NARAYANAN

Angala M, Anto M, Anilkumar C

Abstract *Trichopus zeylanicus* subsp. *travancoricus* is a small perennial ethnomedicinal rhizomatous herb of the family Dioscoreaceae commonly known as 'Arogyapacha". Restricted distributions were reported in the Peppara, Shendurney and Neyyar Wildlife Sanctuaries, Agasthyamala Biosphere of the southern Western Ghats. The present paper deals with ecological principles simulatable for the propagation and *ex- situ* conservation of Arogyapacha. Detailed niche specific factors of abiotic and biotic interactions with selected populations, in which microclimatic and soil mycorrhiza were observed. Analysis of edaphological factors such as the low pH and phosphorous availability and higher content of zinc with the control feral soil may also influence proactive species growth factors. From these observations, trials on various agroecological combinations were carried out using mature seeds and vegetative propagation with different nursery conditions.

Key Words: *Arogyapacha, Perennial herb, ex-situ conservation, Propagation.*

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Introduction

Trichopus zeylanicus subsp. *travancoricus* (Bedd.) Burkill ex. K Narayanan belongs to the family Dioscoreaceae and is commonly known as Arogyapacha or Sathankalanga. In India, this sub-species is mainly distributed in the Peppara, Shendurney and Neyyar Wildlife Sanctuaries, Kerala and Kalakkad – Mundanthurai Tiger Reserve, Tamil Nadu of Agasthyamala Biosphere Reserve. The Kani tribes inhabiting in the Agastyarhills of the Western Ghats, Kerala traditionally uses unripe fruits of the plant as an ant-fatigue and stamina boosting herbal drug. Phytochemical and pharmacological properties of the plant is resembles *Panax ginseng*, hence the procumbent plant is referred as 'Kerala Ginseng' (Pushpangadan *et al.*, 1988 and Anilkumar *et al.*, 2002).

The therapeutic value of the plant was scientifically validated and developed as a product named 'JEEVANI' by Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram. Scientific evaluation of the tribal therapeutic claims on this plant has several properties like adaptogenic, antifatigue, immuno enhancing, cardioprotective, anticancer, antidiabetic, hepatoprotective, antinociceptive and anti-inflammatory. A chemical investigation of the plant revealed the presence of glycolipids, flavonoids, chromones *etc.* from the aerial parts. Taxonomical, pharmacognostical, phytochemical, pharmacological and tissue cultural studies were completed on this plant. Nevertheless, the cultivation aspects still remain obscure. In this background, studies on the agroecology of *T. zeylanicus* subsp. *travancoricus* for its sustainable use becomes relevant.

Materials and Methods

Candidate species- *T. zeylanicus* subsp. *travancoricus*

T. zeylanicus subsp. *travancoricus* is an ethnomedicinal perennial herb belongs to the family Dioscoreaceae. In India, confined only in the Agasthyamala Biosphere Reserve (ABR) of the south Western Ghats.

Field trips

Frequent field trips were conducted during 2017-2022 to the ABR areas to decipher existing population diversity as well as niche specific survival strategies of *T. zeylanicus* subsp. *travancoricus*. Population structure, dynamism, phenological aspects and seed biological studies were done and recorded during the trips. Photographs of samples were taken by Canon DS126251 (EOS7D) and herbarium specimens were examined and voucher specimens were deposited in TBGT, the Herbarium at Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram (Voucher numbers: 99207, 99208, 99209, 99210, 99211, 99212)

Climatological factors

The climatological factors (2019-2022) of *T. zeylanicus* subsp. *travancoricus* populations were documented in three major seasons such as summer (February to May), monsoon (June to October) and winter (November to January). The parameters recorded for the present study were atmospheric temperature (°C), relative humidity (RH, %) and sunlight intensity (Lux). Those parameters were directly measured by thermometer (Barigo), Moisture meter (Barigo) and light meter (lutron LX – 1102) respectively. The data collected during the field trips were averaged to get the mean value.

Edaphological factors

Abiotic parameters: Soil temperature (°C) and moisture (%) were recorded using digital soil thermometer (Dimples model) and moisture meter (Lutron PMS - 713) respectively. For soil analysis, five soil samples each were collected as profiles of 0–30 cm depth from six populations of *T. zeylanicus* subsp. *travancoricus* (in situ) and one from home yard soil (*ex situ*). The samples were mixed and homogenized. After eliminating recognizable stones, plant and animal debris, these composite samples were air-dried and filtered through a 2 mm mesh sieve and stored at 40C in sealed containers (Carney and Matson, 2005). The samples were further analysed for pH, electrical conductivity, available and exchangeable micro and macro nutrients (phosphorus, potassium, carbon, sodium, calcium, magnesium, boron, sulfur, iron, manganese, zinc, copper, lead, cadmium, nickel and chromium), percentage of sand, silt, clay, water holding capacity and porosity, exchangeable acidity, bulk density and particle density at Central Soil Analytical Laboratory, Thiruvananthapuram.

Biotic parameters: Mycorrhizal associations: Mycorrhizal association of *T. zeylanicus* subsp. *travancoricus* was studied with randomly selected 10 plant roots and soil samples (rhizosphere region) each from all populations. The roots were cut into small pieces and boiled with 10% KOH solution for 1-2 hours in a boiling tube. Treated roots were carefully rinsed with distilled water and immersed in 2% HCl for 5 minutes. Trypan blue was added tothe tube and retained for 3 minutes. Finally, the root segments of 1 cm in length were mounted on slides using lacto phenol and examined in a compound phase contrast microscope (Phillips and Hayman, 1970).

The mycorrhizal colonization percentage in roots was calculated as per the following formula

Mycorrhizal colonization in roots (%)= (Number of mycorrhizal root segments)/(Total number of root segments observed) × 100

Mycorrhizal spores present in rhizosphere soil were isolated following the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). Soil samples of 100 g were suspended in water and allowed to settle down for 5-10 minutes. The suspension was passed through a series of sieves with pore size of 250, 206, 90 and 40 µm. The spores were collected from the soil suspension and placed on filter paper. The spores were analysed under a Binocular microscope and transferred to a sterile slide and mounted in lacto phenol. Microscopic analysis was made under high magnification.

Agroecology

Seed germination studies were carried out both in situ and *ex situ*. For *in situ* method, seeds were sown at the natural forests where the seeds were collected (soil seed banking). For *ex situ* method, seeds were sowed in the home yard soil and nursery. In addition, following ISTA (2008) and Asomaning *et al*., (2011) various seed treatments were carried out in seed germinator (KEMI KSG-2) with and without light (30 ± 20C, 80% RH). The other methods adopt for germination studies are; soaking (normal, cold and hot water) of seeds up to 24 hrs, seeds pre-treated with different concentrations of GA3 (50, 100, 250, 500, 1000, 2000, 3000, 4000 and 5000 ppm). Vrikshayurveda methods for seed pre treatment with natural hormone sources like cow milk, coconut water, Aloe vera gel were also tested. Seed germination trials were also carried out with different soil samples (Forest condition- Forest soil–control & Nursery soil) and Nursery condition (Nursery soil–control & Forest soil). In the vegetative propagation method, the roots and stem including leaves were separated from the mother plant and planted in the humus forest soil with a space of 1 x 1 feet. Trials were also carried out with different soil samples (Forest condition- Forest soil–control & Nursery soil) and Nursery condition (Nursery soil–control & Forest soil).

Results and Discussion

Based on the herbarium and previous reports, more than 76 field trips were conducted to analyses the population structure and basic aspects of populations (Fig. 1). *T. zeylanicus* subsp. *travancoricus* has limited populations confined only in Agasthyamala Biosphere Reserve (120-1000m asl). The present populations are separated by diverse geographical barriers like mountains, rivers, human settlements, plantations and roads.

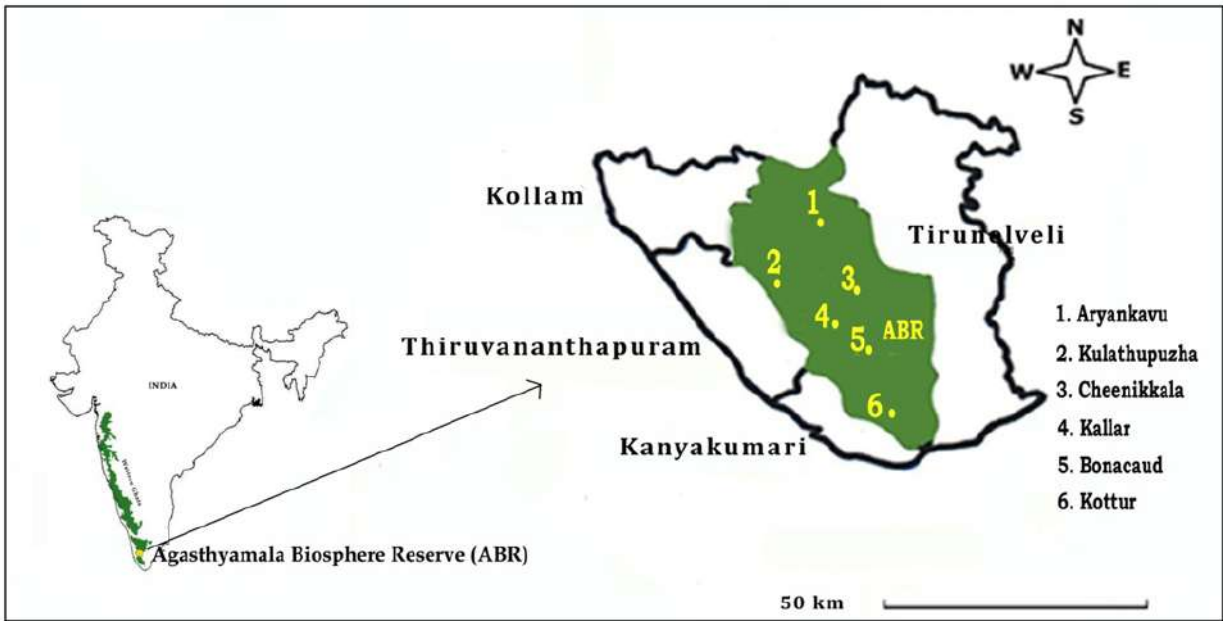

Fig. 1. Populations of *T. zeylanicus* subsp. *travancoricus* at southern Western Ghats

Phytogeographical distribution

The distributional status of *T. zeylanicus* subsp. *travancoricus* was documented with extensive field trips conducted to various forest areas of Agasthyamala Bioreserve (ABR). In ABR the plant is distributed in the Neyyar, Peppara and Shendurney Wildlife Sanctuaries and their adjoining areas of Achencoil, Thenmala, Thiruvananthapuram Divisions and Agasthyavanam Special Division. Also in these areas boundaries of the plant populations were marked using Garmin eTrex GPS and draw distributional maps prepared with the help of MaxEnt (version 3.4.1) and DIVA-GIS (version 7.5) software.

Spatial distribution

All located populations are in elevated hilly areas of 120-1000m asl. The aerial distance from Aryankavu population to Kulathupuzha population

is 13.61km, Kulathupuzhato Cheenikkala is 13.79 km, Cheenikkala to Kallar is 7.98 km, Kallar to Bonacaud is 5.12 km and Bonacaud to Kottur is 11.60 km (Fig. 2).

Niche characteristics of Arogyapacha

Analysis and documentation of atmospheric and soil factors in the selected Arogyapacha populations of Peppara, Shendurney and Neyyar Wildlife Sanctuaries were carried out during the year 2019-2022. The climatological factors of populations were documented in three major seasons such as summer (February to May), monsoon (June to October) and winter (November to January). The parameters recorded for the present study were atmospheric temperature (°C), relative humidity (RH, %) and sunlight intensity (Lux). Those parameters were directly measured by thermometer (Barigo), moisture meter (Barigo) and light meter (lutron LX – 1102) respectively. Soil temperature (°C) and moisture (%) were recorded using digital soil thermometer (Dimples model) and moisture meter (Lutron PMS - 713) respectively. The data collected during the field trips were calculated and tabulated as given below (Table 1, Fig. 3).

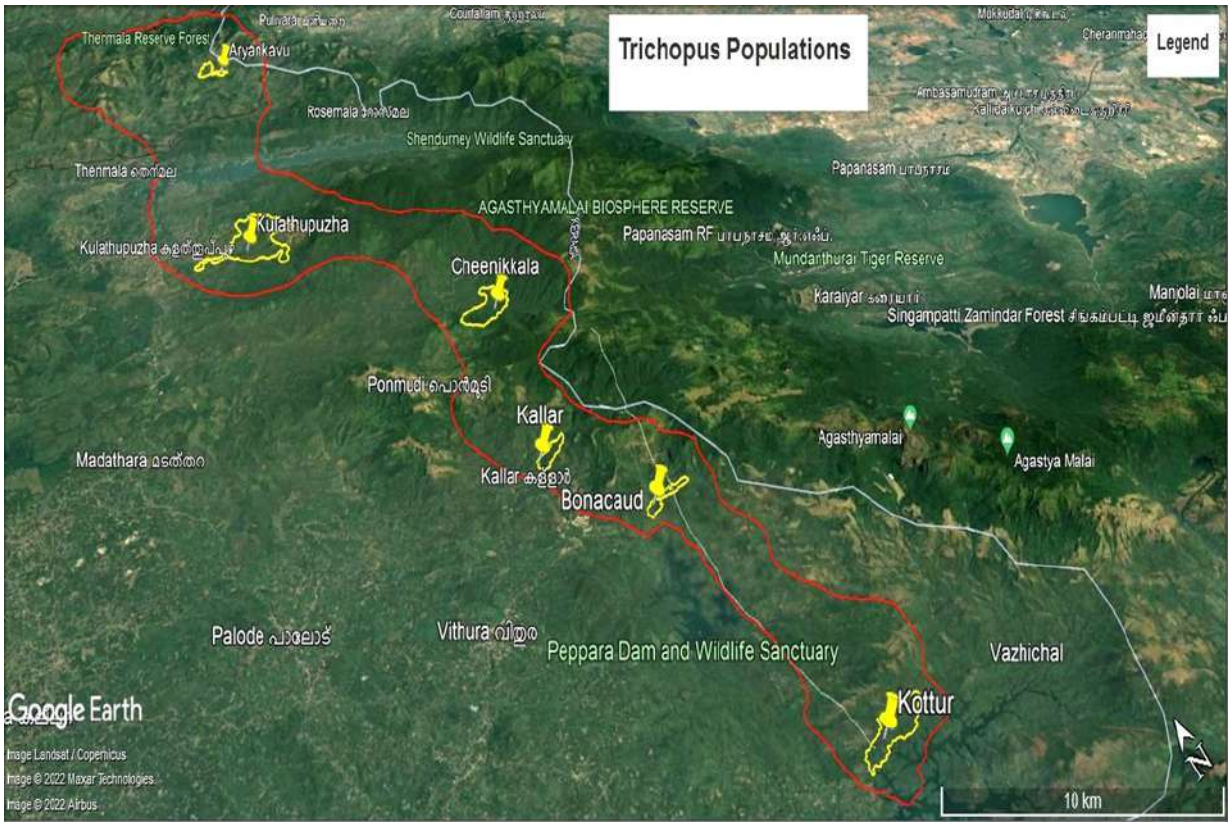

Fig. 2. Vegetation map showing the study area and study sites at ABR.
Red and yellow line indicated the area of occupancy and area of occurrence of *T. zeylanicus* subsp. *travancoricus* respectively (courtesy Google maps)

Percentage of mycorrhizal colonization in roots and rhizosphere soil

The soil samples of Arogyapacha with rich diversity of spores such as species of Acaulospora, Glomous and VAM fungi root colonization were recorded along with almost similar range of mycorrhizal colonization in roots different populations (Table 2, Fig. 4). Mycorrhizal association was studied with randomly selected plant root and soil samples (rhizosphere region) from all populations. The mycorrhizal colonization percentage in roots was calculated and mycorrhizal spores present in rhizosphere soil were isolated following the wet-sieving and decanting technique.

Agroecology

The growth cycle of *T. zeylanicus* subsp. *travancoricus* from sowing/planting to fruiting consist of five stages namely the seeding, branching,

flower-budding, flowering and fruiting phases. The propagation trials were carried out with mature seeds and vegetative parts of Arogyapacha with different conditions.

Seed germination studies

The seeds have strong seed coat, which takes more time to imbibe the water; which delays the seed germination. The major percentage of the fruits were foraged and damaged at natural

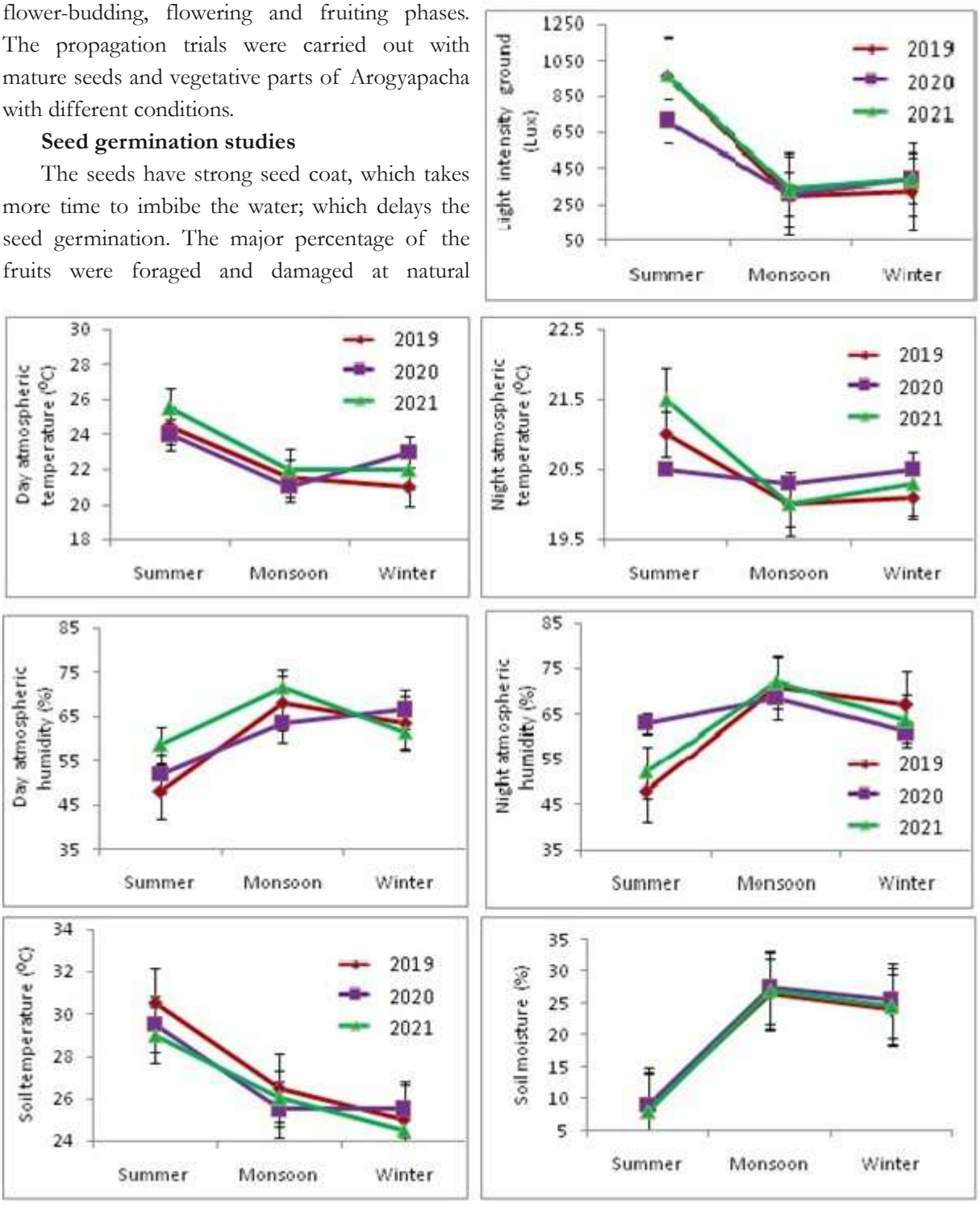

Fig. 3. Climatological and edaphological factors of the selected populations of Arogyapacha.

habitats. In the forest areas, seed germination percentage is 45- 55 %. These may be due to heavy rain and predation. Heavy rain washes out the dehisced seeds from the plant distributed area. Predation is high in the stage of fruit maturation. Anthropogenic activities (excessive collection of plants, leaves and fruits) also decrease the quantum of seed germination. Seed germination studies were carried out in natural as well as laboratory conditions. Natural seed germination occurs in 6-7 months after dehiscence (Fig. 5). In the laboratory conditions, seed germination studies were carried out with pre-treated seeds. The methods adopted for germination studies are; soaking (normal, cold

and hot water) of seeds for up to 24 hrs, seeds pre-treated with different concentrations of GA_3 (50, 100, 250, 500, 1000, 2000, 3000, 4000 and 5000 ppm). Non-treated seeds in the seed germinator took 5-6 months to germinate. Vrikshayurveda methods for seed pre treatment with natural hormone sources like cow milk, coconut water, aloe vera gel were also tested. The overalloutcome of these experiments revealed the proactive effect of 1000 ppm GA_3 pre-treatment. Seed germination trials were carried out with different soil samples (Forest condition- Forest soil–control& Nursery soil) and Nursery condition (Nursery soil–control & Forest soil). Forest soil was best which prompted further physicochemical and mycorrhizal soil studies.

Table 1. Nishe specific charactersof Arogyapacha

Altitude (m asl)	**120-1000**
Altitude (m asl)	120-1000
Atmospheric temperature (ºC)	20 - 24
Soil temperature (ºC)	19 - 23
Atmospheric humidity (%)	67 - 72
Soil moisture (%)	61 - 81
Light intensity (Lux)	850 - 1300
Soil pH	4.7 – 4.9

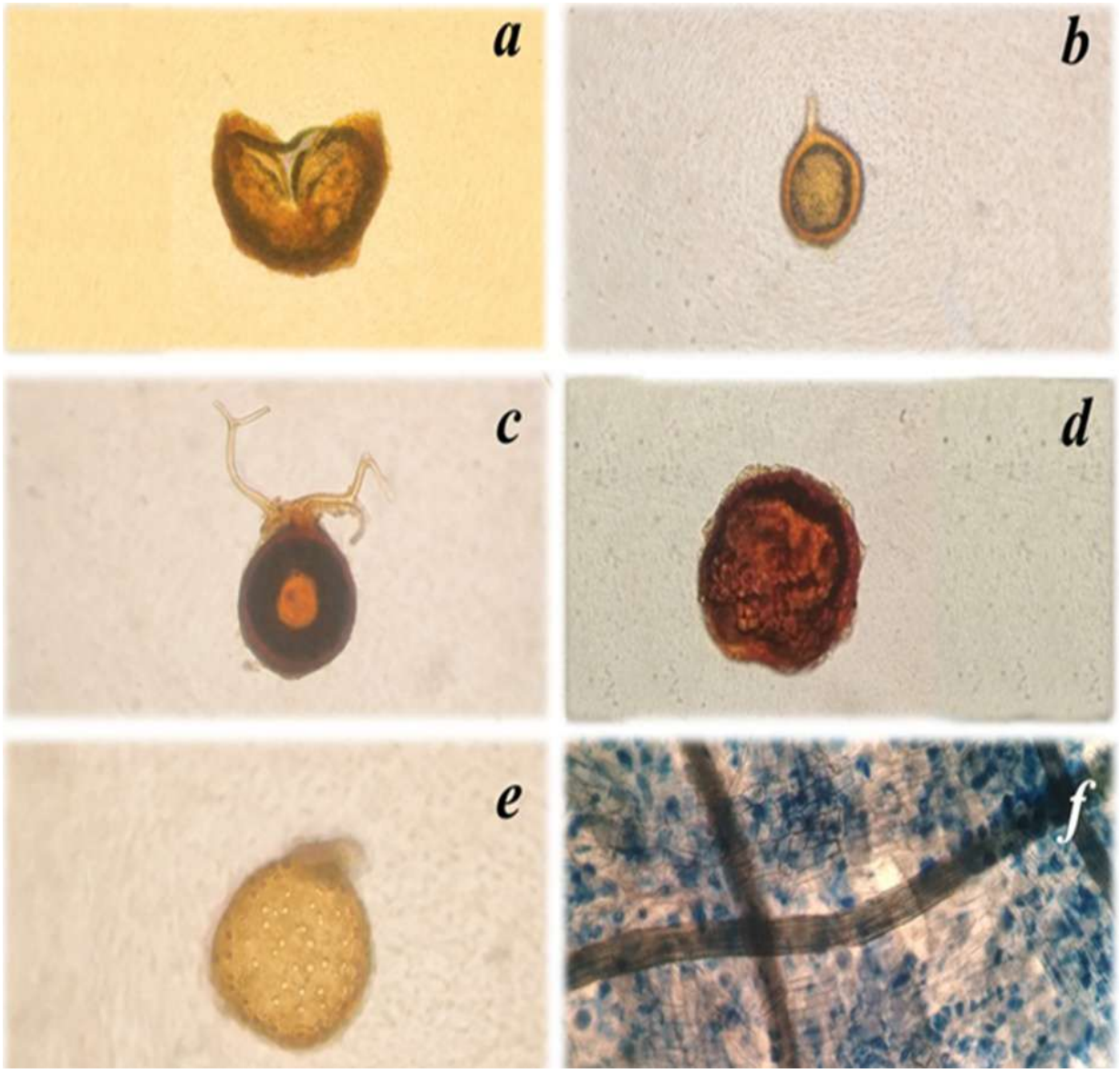

Fig. 4. Rhizosphere soil samples with rich diversity of spores: a; *Acaulospora* species, b-e; *Glomous* species, f; VAM fungi root colonization

Vegetative propagation

In this method, the roots and stem including leaves were separated from the mother plant were used for studies. Vegetative propagation trials were also carried out with different soil samples (Forest condition- Forest soil–control & Nursery soil) and Nursery condition (Nursery soil–control & Forest soil) (Figure 6). Mainly the propagules were selected from the reproductively mature plants. By this method 60-70% of the propagules showed good vigor.

Fig. 5. Seed germination stages of Arogyapacha.

Fig. 6. Vegetative propagation stages of Arogyapacha

Conclusion

Cultivation practices of Arogyapacha by both seed and vegetative methods revealed that. 1. Collection of mature seeds (dehisced seeds) from natural habitat are difficult. 2. Dormancy of the seed delayed the germination (Dormancy can break through 1000 ppm GA_3 treatment). 3. Slow growth of seedling (took 1-2 years to attain normal plant size). Due to these limitations, vegetative propagation is found to be a technique for large scale production. In this method, the roots and stem including leaves were separated from the mother plant are used for multiplication. Site selection is very important in this case. The plant is growing in the humus soil with low light intensity under the shade with a distance of 1 x 1 feet. Repeated irrigation also helps to speedy growth. After some period, the plants become stunted in growth and become reproductively inactive. To overcome these issues, advised to simulate the soil conditions as forest soil by adding microbial supplements such as species of Acaulospora, Glomousetc., and other soil nutrients. The plants under cultivation may be in a wide fenced area to protect them from animal attacks.

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Chapter 22

IN VITRO PROPAGATION FOR CONSERVATION OF A RARE MEDICINAL PLANT *JUSTICIA GENDARUSSA* BURM. F BY NODAL EXPLANTS AND SHOOT REGENERATION FROM CALLUS

Deepamol P K, Amina S, Chithra Vijayan

Abstract *Justicia gendarussa* Burm.f. belonging to the Acanthaceae family, is well known for its multiple biological therapeutic usages in modern and traditional medicines since decades. A wide variety of biologically active constituents such as flavonoids, alkaloids, steroids, terpenoids, saponins, phenolic compounds and carbohydrates are present in this plant. Its over-exploitation for therapeutic uses and to meet the demand of the pharmaceutical industry, in raw materials supply, for the production of drugs has led to the considerable decline of the species in its natural habitats. An efficient system of shoot multiplication from nodal and inter-nodal explants and callus regeneration is reported in the present study. Significant multiple shoot formation was observed in medium fortified with BAP (3 mg/L) and Kn (2 mg/L). The produced micro shoots were rooted in half strength MS medium supplemented with IAA. The highest number roots were obtained with 0.04 IAA mg/L. The rooted plantlets acclimatized with 90% survival in natural habitats. The leaf and internode segments induced callus cultures efficiently on medium amended with various concentrations of phytohormones. Callus was induced from the leaf and internode explants on MS medium supplemented with different concentrations of NAA, IAA and 2,4-D. Optimum callus induction was obtained on MS medium supplemented with 2.0mg/L 2,4-D and 3.0mg/L NAA in leaf explants and with 0.5, 2 mg/L NAA, 1mg/L IAA in internode explants. Callus regeneration studies were also performed. The highest response was observed in MS medium containing 3mg/L BAP and 0.01mg/L NAA. The developed regeneration system can be exploited for genetic transformation studies, particularly when aimed at producing its high yielding cell lines for anti-diabetic phytochemicals. It also offers opportunities for exploring the expression of totipotency in the medicinal herb J. gendarussa.

Keywords: *Justicia gendarussa, Rare medicinal plant, conservation, explants, regeneration system.*

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Introduction

Justicia gendarussa Burm.f. is an evergreen scandent shrub belonging to the family Acanthaceae is commonly known as willow-leaved Justicia. Within the traditional medical system, different parts of *J. gendarussa* were mentioned to be useful in a variety of illnesses. The decoction of the leaves and tender shoots is diaphoretic and they are given in chronic rheumatism. Oil prepared from the leaves is useful in eczema, and the mixture of leaves is given internally for hemiplegia, cephalalgia and facial paralysis. Fresh leaves are used to treat oedema and earache. Root extract obtained from *J. gendarussa* is mostly prescribed for constipation, laxative action helps in normal bowel movement. The plant has been used by indigenous physicians and tribes to treat various ailments, including liver problems, tumors and inflammation (Ahmad and Hold-worth 2003).

Because of the wide variety of medicinal applications, the plant is typically harvested in the wild, a process to undermine natural biodiversity. The expanded global use of traditional medicines is leading to the fast depletion of this plant from its

habitat. Plant tissue culture offers an easy and reproducible method of micro propagation of medicinal plants on large scale by providing appropriate plant materials from elite germplasm lines.

The objectives of the present investigation is to establish *in vitro* cultures, by developing a rapid and high frequency plantlet regeneration protocol, from mature tissues of *J. gendarussa*, thus providing a continuous supply of a better source of elite plant, to be used as standard material for replantation of this plant in the forest and in the field of drug research as well as in the manufacturing of drugs.

Fig. 1. *Justicia gendarussa* Burm.f.

Methodology

J gendarussa were collected from the Botanical Garden,Sree Narayana College, Kollam. Segments of leaf, nodal and internodal segments were used as explants (Fig, 1). The explants were aseptically implanted on the culture medium after sterilization procedures. The cultures were then incubated at 25±2°C under 16/8 h photoperiod of 2000 lux light intensity with 55%-60% humidity. MS medium and half MS medium were used as basal media with various concentrations of plant growth regulators.

Results

To find out the effect of cytokinins and auxins on shoot multiplication from nodal explants 48 independent treatments were carried out. On each occasion, when an explant become brown, the surviving explants were transferred to new flask containing the culture medium. No significant results were obtained with combinations of cytokinins and different auxins in terms of multiple shoots. Only double shoots and single shoots were observed with respect to these treatments. As the cytokinin in combination with auxins, didn't gave the best results for shoot multiplication and elongation, futhermore treatments were carried out with cytokinins alone and by their combination. Plantlet regeneration had been successfully achieved using nodal explants cultured on MS medium fortified with BAP or BAP+Kn (Table 1 and 2). Multiple shoot induction was obtained when BAP (3mg/L) was used alone and in combination with Kinetin (BAP 3mg/L+Kn 2 mg/L) (Plate 1). Other hormonal combinations yield less response in terms of shoot multiplication. The best response was obtained on the 15th day onwards (Table 3).

The leaf explants showed brownish, compact, callus with slight proliferation in the medium containing 2,4-D 1mg/L (Plate 1) and profuse proliferation at 2,4-D 2 mg/L (Plate 1) after 2-3 weeks. A white minor callus was obtained on the MS medium fortified with NAA 2 mg/L and IAA 1 mg/L after 15 days. White callus with slight

Table 1. Effect of BAP and auxins on shoot multiplication from nodal explants.

Si.No	Plant growth regulators				Response	Shoot growth vigour
	BAP	NAA	IAA	2,4-D		
1.	2.5	0.04	*	*	Double shoot+green callus	++
2.	0.5	*	0.02	*	Single shoot + brown callus	+
3.	1.0	*	0.02	*	Double shoot+root	+
4.	2.0	*	0.02	*	Double shoot+brown callus	+++
5.	3.0	*	0.02	*	Double shoot + brown callus	+++
6.	1.0	*	*	1.5	Double shoot+ green callus	+++
7.	2.0	*	**	1.5	Double shoot+green callus	+++

Table 2. Effect of kinetin and auxins on shoot multiplication from nodal explants.

Si.No	Plant growth regulators				Response	Shoot growth vigour
	Kn	NAA	IAA	2,4-D		
1.	1.0	0.02	*	*	Double shoot+root	+
2.	1.5	0.02	*	*	Double shoot+ root	+
3.	2.0	0.02	*	*	Single shoot+brown callus +root	+++
4.	1.5	*	0.02	*	Single shoot+Brown callus+root	+++
5.	1.0	*	*	0.02	Double shoot+brown callus	+++

Table 3. Effect of BAP and kinetin on shoot multiplication

Si.No	Concentration Of BAP (mg/L)	Concentration of Kinetin (mg/L)	Response	Shoot growth vigour	No.of shoots
1.	0.5	*	Single shoot	+	1
2.	1.0	*	Double shoot	++	2
3.	3.0	*	Multiple shoot	+++	4
4.	*	1.5	Double shoot	++	2
5.	0.5	0.5	Double shoot	++	2
6.	2.0	2.0	Multiple shoot	+++	4
7.	3.0	2.0	Multiple shoot	+++	8

Table 4. Effect of growth regulators on callus induction in leaf explants

Si.No	PGRs used for callus induction	Concentration (mg/L)	Response	Degree of callusing
1.	NAA	2.0	White,friable callus	+
		3.0	White,friable callus with root	++
2.	2,4-D	1.0	Brown compact callus	++
		2.0	Brown compact callus	+++

Table 5. Effect of Auxins on callus induction in internode explants

Si.No	PGRs used for callus induction	Concentration (mg/L)	Response	Degree of callusing
1.	NAA	0.5	Green compact callus	+++
2.		1.0	Brown compact callus with root	++
3.		2.0	Green compact callus	+++
4.		3.0	Brown compact callus with root	++
2.	2,4-D	0.1	Green compact callus with root	+++
		2.0	Brown compact callus	+++
		3.0	Brown compact callus	+++

proliferation in the medium fortified with NAA 3 mg/L showed the formation of roots after 21 days (Plate 1). All other treatments do not give any results in terms of callusing (Table 4).

The effect of different auxins on callus induction in the internode of *J. gendarussa* was studied and the data was recorded after 1 week (Table 5). Green callus with higher growth proliferation was observed on medium fortified with IAA -1 mg/L (Plate 2), NAA-0.5 & 2 mg/L

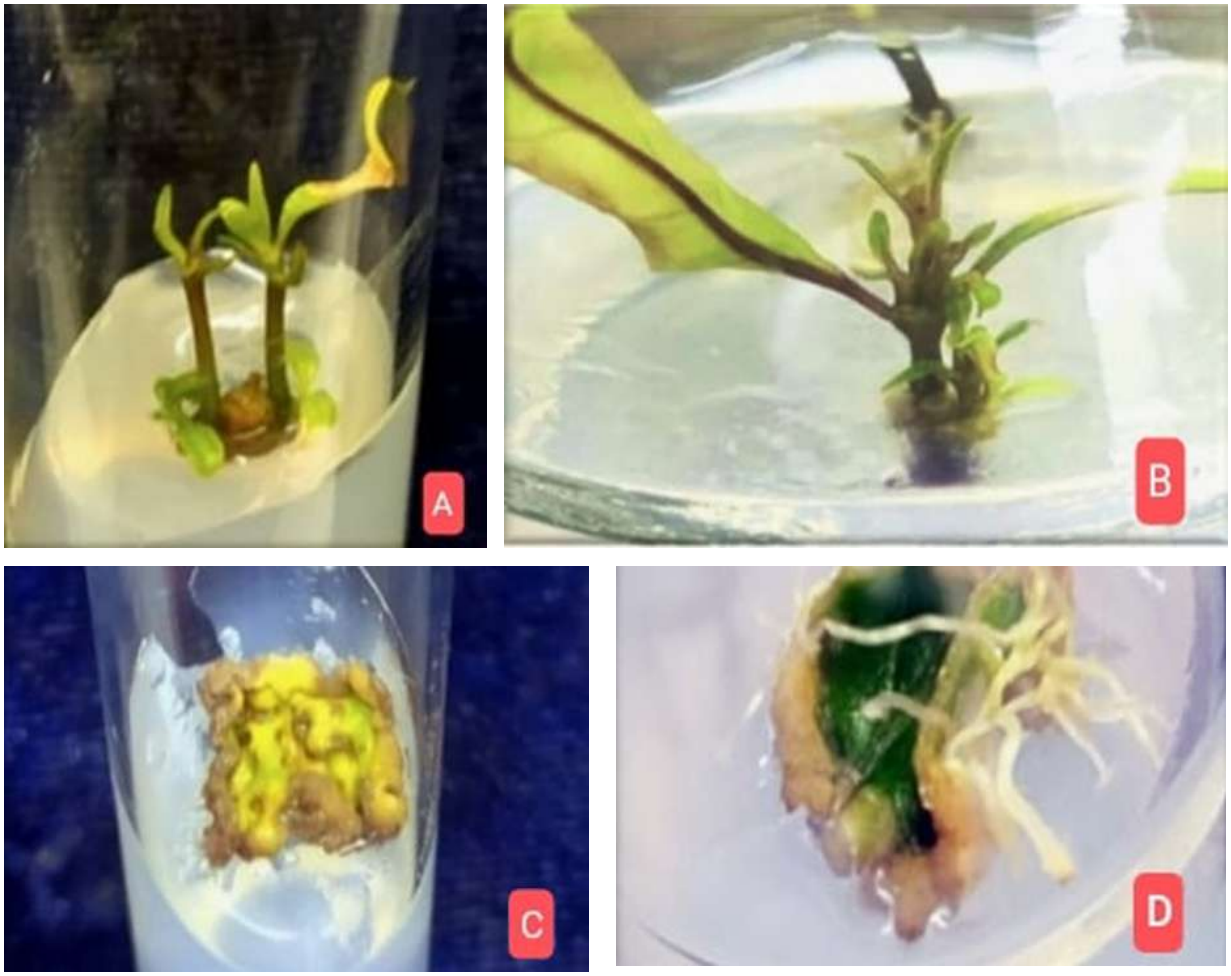

Plate 1. A and B: Shoot induction in BAP 3mg/L (4 shoots) and BAP 3mg/L+Kn 2mg/L (8 shoots), C: Callus formation at 2,4-D 1mg/L, D: Formation of roots in leaf explant in NAA 3mg/L.

Plate 2. Effect of different auxins on internodal explants; A: at IAA 1mg/L, B and C : NAA 0.5,2 mg/L and D: 2,4-D 0.1 mg/L.

Plate 3. A: Caulogenesis at BAP 3mg/L+NAA 0.01mg/L. B: Root formation from unrooted plantlets on half MS medium fortified with 0.04 mg/L. C: Effect of NAA (0.04mg/L) on root regeneration. D: Hardened in vitro plants successfully transplanted to the plastic cups.

Table 6. Effect of plant growth regulators on indirect organogenesis.

Si. No	PGRs used in shoot regeneration from callus		Response (No. of shoots)
	BAP (mg/L)	NAA(mg/L)	
1.	0.5	0.01	3
2.	2	0.01	5
3.	3	0.01	10

Table 7. Effect of plant growth hormones on root regeneration.

Si. No	Medium	PGRs used for root regeneration (mg/L)	Response (No. of roots)
1.	½ MS	0.02 IAA	5
2.	½ MS	0.03 IAA	11
3.	½ MS	0.04 IAA	16
4.	½ MS	0.04NAA	8

(Plate. 2), 2,4-D- 0.1 mg/L (Plate. 2). Brown compact callus was obtained in 2,4-D (2 mg/L) with higher proliferation rate. Root formation was also observed in certain combinations after 15-21 days.

To study the callus regeneration green chlorophyllous callus obtained from the internode was placed in a medium fortified with different concentrations of BAP & NAA. When the callus obtained from internodal explants were transferred to shoot inducing medium, it resulted in a significantly higher percentage of shoot proliferation in terms of total shoots per culture. The medium supplied with 3 mg/L BAP +0.01 mg/ L NAA obtained a higher number of multiple shoots ie, 10 shoots (Plate. 3). Other treatments showed less significant results in terms of callus regeneration (Table 6).

In the present study, adventitious rooting has been considered as a single-phase process in which auxins play a major role. Plant growth regulators IAA and NAA have been used in different concentrations (Table 7). An attempt was made to regenerate roots from unrooted plants obtained through direct organogenesis. Half MS medium fortified with IAA -0.04 mg/ L (16 roots) showed significant rooting (Plate 3). Roots formed were white in colour and longer. The highest concentration of NAA (0.04mg/L) showed maximum roots (8), (Plate 3) but elongation and growth of roots were poor.

The plantlets were planted in poly cups containing sterilized mixture of sand and soil, irrigated and kept under fluorescent lights and covered with polythene bags (for maintaining humidity). 16/8 h photoperiod and 25±2°C was maintained, for a week, and then transferred to field conditions. The hardened plants when transferred to the field shown 90% survival (Plate 3).

Discussion

Production of medicinal and aromatic substances through the application of cell and tissue cultures is a novel approach to obtaining these substances in a large scale. The present work has been carried out in *J. gendarussa*, an important source of many pharmaceutically important secondary metabolites. Nowadays *J. gendarussa* is disappearing from our local areas and is listed as one of the plant species in India, vulnerable to extinction. Besides only very little research work have been done on this medicinal plant. For many species, the important morphological and physical process of shoot multiplication/ regeneration and elongation is often induced by the addition of plant hormones to the culture medium. The objective of this study was to find out the effects of different concentrations of various growth promoters in a culture medium on shoot regeneration and elongation. It is well known that auxins and cytokinin are effective for callus and organ formation in tissue culture of many plants (Yakauwa and Harada, 1982).

MS medium supplemented with BAP (3 mg/L) and Kn (2mg/L) displayed an efficient shoot multiplication. The highest number of shoots through direct organogenesis was obtained with cytokinins. Similar results was obtained in Sophora tonkinensis (Sonali *et al*, 2013), which cytokinins alone produced higher number of shoots. *In vitro* shoot regeneration of *Chlorophytum borivilianum* with combination of BAP and Kn also showed higher number of shoots (Ashraf *et al*, 2014). Direct regeneration techniques offer a viable tool for mass multiplication and germplasm conservation of rare and endangered medicinal plants for meeting

pharmaceutical needs. Direct regeneration of shoots without any intermediate callus phase can yield clonal plants for large scale propagation. More often the direct regeneration from explants yields adventitious shoots, each of which can develop into a new plant. There are several *in vitro* studies on the direct organogenesis of other medicinal plants using different sources of explants (Kawiak *et al.*, 2003). Plantlets obtained by direct organogenesis showed no phenotypic variation. The direct organogenesis method has the advantage of omitting the callus and significantly reducing the total number of stages in culture by the direct formation of new shoots from the explants (Mallick *et al.*, 2012). The present protocol deals with the direct organogenesis of *J. gendarussa* through adventitious shoot regeneration. A complete regeneration was achieved in 15 weeks of culture. Adventitious shoot regeneration from various explants is dependent on the interaction between plant growth regulator combinations and concentrations in the medium. As this protocol is rapid and reproducible; it can be applied for large scale regeneration, germplasm conservation and genetic transformation of *J. gendarussa*.

The efficient callus induction was observed on internode explants compared to leaf explants. White, green and brown callus was obtained with various auxins such as 2,4-D, IAA and NAA. The induction of callus in *J. gendarussa* was initially observed from the cut ends of the explants. This result is in agreement with the results of Bushrabi *et al.* (2008) where, they have reported the induction of callus from the cut ends of stem and leaf explants of *J. gendarussa*. The initiation of callus from the cut ends of the explants was reported in *Stephania cepharantha* Hayata (Suzuki et al., 1992), *Dalbergia sissoo* Roxb. (Singh and Chand, 2003), *Rauwolfia serpentina* L. Benth. (Salma *et al.*, 2008), *Scoparia dulcis* L. (Hassan *et al.*, 2008), *Mucuna pruriens* (L.) DC. (Vibha *et al.*, 2009).

The highest number of shoots from callus was obtained on MS medium fortified with BAP 3 mg/L+ NAA 0.01 mg/L. The medium supplied with 3 mg/L BAP +0.01 mg/L NAA obtained higher number of multiple shoots ie, 10 shoots. Other treatments showed less significant results in terms of callus regeneration. Thomas and Yoichiro (2010) reported a maximum of 12 shoots per callus clump from the leaf explants of *J. gendarussa* when cultured on MS medium supplemented with BAP + NAA at concentrations of 17.7+ 5.4µM. Agastian *et al.* (2006) reported the production of 2-3 shoots per callus clump in *J. gendarussa* when cultured on MS medium fortified with NAA+BAP at 0.5+0.1mg/L concentrations with a light intensity of 27µmol m-2 s-1.

Significant rooting showed in half strength MS medium with 0.04mg/L IAA. The induction of roots in the *in vitro* regenerated shoots was observed in previous studies of *Ocimum gratissimum* in MS medium supplemented with 0.5mg/l of IAA (Gopi *et al.*, 2006), *Psidium guajava* L. in MS medium supplemented with 2.5mg/l of IAA (Zamir *et al.*, 2007).

Conclusion

In vitro conservation of traditional medicinal plant germplasm is important to support chemical analysis and pharmacological and genetic transformation studies. With the resurgence of public interest in plant-based medicine and the rapid expansion of pharmaceutical industries, these *in vitro* techniques and innovative approaches will be useful. The callus cultures can be effectively exploited for secondary metabolite production which contains highly valuable alkaloids, which would avoid the destruction of plants in their natural habitats and thereby conservation of germplasm. Using *in vitro* propagation techniques it is now possible to produce a large number of pathogen-free uniform clones of elite, rare, and important native medicinal plants for reintroduction in their natural habitat and safe exchange of germplasm across international borders.

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Chapter 23

POPULATION STRUCTURE AND ECOLOGICAL NICHE MODELLING OF *GARCINIA IMBERTII* BOURD. A CRITICALLY ENDANGERED ENDEMIC TREE OF SOUTHERN WESTERN GHATS

Anto Mathew, Angala Mathew, Rameshkumar K B, Anilkumar C

Abstract *Garcinia imbertii* (formerly as: *Garcinia imberti*) Critically Endangered C2a(i) is an endemic, dioecious tree of the family Clusiaceae confined to the evergreen forest (600-1200 m asl) of Agasthyamala Biosphere Reserve of southern Western Ghats, India. Concerned with its Critically endangered status, this study was conducted to decipher the existing population structure as well as to understand the future existence of the species for augmenting species conservation. A total of six different distracted populations of *G. imbertii* were located in Thiruvananthapuram and Kollam districts of Kerala and Kalakkad Mundanthurai Tiger Reserve of Tirunelveli district of Tamil Nadu. The important value index of *G. imbertii* was high that indicates the dominance and ecological importance of the species. The biogeography of Chemunji was formed ideal for better growth and regeneration of the species with reverse J-shaped girth class distribution. Ecological niche modelling of field data with MaxEnt and DIVA-GIS indicated that many suitable *G. imbertii* habitats may disappear after years due to shift in climatic conditions. The area mapped on both current and future space of *G. imbertii* may decipher possible future spaces useful for the re-introduction and conservation.

Key words: *Agasthyamala Biosphere Reserve, Critically endangered, Ecological niche modelling, Garcinia imbertii*

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Introduction

Species conservation is one of the most important applications in ecology. According to Hamilton (1999), the conservation of tropical tree species is particularly significant as they give habitats and ecological niches for many species. The ecological and conservational aspects of most species of *Garcinia* genus are yet to be tackled. As an eco-component of evergreen forests, a study on the survival strategies including adaptive traits may throw light into their population aspects. An understanding of biology as well as specific interactive ecology may help to standardize species recovery and ecorestoration programmes.

India, as part of the tropical belt is one of the world's mega diversity centres covering approximately 2.4% of the total world flora (Singh and Dash, 2014). According to Arisdason and Lakshminarasimhan (2016), India possesses 47,513 species of flowering plants of which 28% are endemic. Conservation of endemic flora is of great concern because many of the world's plant species are on the verge of endangerment or extinction. The Western Ghats is an elevated chain of

mountain ranges (N 8o 21'-20o 41' and E 73o-77o) and cover a distance of 1600 km from the Tapti valley in Gujarat to Kanyakumari in Tamil Nadu. The protected areas of the Western Ghats include National Parks, Tiger Reserves, Reserve Forests and Wildlife Sanctuaries that harbour good number of endemic species. Of the 7,402 species of higher plants reported from the Western Ghats, 1,273 are endemic (Nayar *et al.*, 2014).

The Agasthyamala Biosphere Reserve (ABR) of southern tip of the Western Ghats is one of the most important species diversity centre and endemism in India (Henry *et al.*, 1984). The ABR was established in 2001 and comprises 3,500 Km2 out of which 1828 km² is in Kerala and 1672 km² in Tamil Nadu. The region supports high species diversity of endemic and endangered plants (Gupta *et al.*, 2014). According to the United Nations Educational and Cultural Organisation (UNESCO), the ABR is known to host approximately 2254 species of higher plants including 405 endemic taxa (UNESCO report, 2016). These areas have been reported as type localities for a number of plants, many of which are rare or endangered (Ramesh *et al.*, 1997). Chemunji peak area in ABR is the type locality for half a dozen endemic species (Varghese and Menon, 1999). The genus *Garcinia* is one of the important components of the flora of the ABR and most of them are enlisted as threatened with concern of extinction.

Garcinia imbertii Bourd. is a Critically Endangered C2a(i) (IUCN, 2020) and endemic tree belongs to the family Clusiaceae, confined only in the Agasthyamala Biosphere Reserve (ABR) of the south Western Ghats, India. The species is growing in the humid areas of tropical evergreen hilly and shola forests of Kerala and Tamil Nadu states. This species was first described by Bourdillon from (single century old) the collection made by Beddome (1879) from Tirunelveli hills and from Travancore hills (Bourdillon, 1899), later was relocated by Mohanan *et al.*, (1997) from Chemunji hills. Sasidharan (1999) reported the species occurrence in Shendurney Wildlife Sanctuary. Plant population structure in any forest ecosystem is mainly determined by frequency, intensity and magnitude of disturbances that occurred in forest (Armesto and Pickett 1985). Plant population status reflects the size and density, altitudinal range and regeneration capacity. The regeneration status of many tree species depends on size and age structure of their population (Saxena and Singh, 1984; Bhuyan *et al.*, 2003). The status of the regeneration of species was determined based on population size of seedlings and saplings species dominance within a specific niche is influenced by eco-physiological factors, interactions with other species and dispersal (Shaltout *et al.*, 2015). According to Jose (2001), the population study of a species including the niche characteristics, their structure and dynamism will help to access the trend of population behaviour and factors responsible for endangerment. Natality and mortality patterns also determine the population size that varies within species (Roff, 1992). Natural populations of *G. imbertii* have declined dramatically in recent times due to variations in eco-physiology and other stress. So information on population structure is essential for the in situ conservation of *G. imbertii* properly.

Ecological niche models (ENM) have become a proactive tool to predict and describe the ecological niche of a species. Population analysis and literature review of *G. imbertii* indicate the dwindling nature of the distracted populations. One such tool is the MaxEnt (Maximum Entropy) model that can assess the climate change influences on *G. imbertii* distribution. According to Dale *et al.*, (2001), climatic changes can alter the structure and function of an ecosystem by pathogen and insect outbreaks, introduced exotic species, drought, landslides and wind storms. Increasing temperature and altering patterns of rainfall contribute significantly on the spatial distribution of species and some habitats will be predicted to vanish totally (Anderegg *et al.*, 2015). Many researchers predicted that the future bio-climate will harmfully affect the distributions of many species (Remya *et al.*, 2015; Priti *et al.*, 2016; Pramanik *et al.*, 2018).

Materials and Methods

Six *G. imbertii* populations were identified for the present study based on literature survey and periodic field exploration trips to several forest

regions of the Western Ghats. Sankili hills, Cheenikkala hills, Ponmudi hills, Chemunji hills, Bonacaud and Athirumala hills and Poonkulam hills (Tamil Nadu) were the study sites. Frequent field trips were conducted during 2014-2022 to the above mentioned forest areas to decipher the existing population structure as well as the future existence of the species. For analysing the population structure and dynamism of *G. imbertii* and associates, continuous 3-5 days of field trips per week and a total of more than 130 trips to various forests were conducted and recorded the details.

Population structure analysis

A holistic study of all the plant species regarding their abundance (Abu), density (Den), frequency (%) (Fre), relative frequency (%) (Rfr), relative density (%) (Rde), relative dominance (%) (Rdo) and total basal area (TBA) were analysed following Misra (1968). Important value index (IVI) (Curtis, 1959) and Whiteford index (WFi) (Whiteford, 1949) were also calculated. Floristic wealth, structure and spatial distribution were studied. In addition, dominant associates were taken into consideration for studying the influence of associated species to *G. imbertii*. Population analysis was carried out by randomly established quadrats in all six populations. In each selected population, 25 quadrates were laid down with the size of each quadrat being 10 × 10 m followed by various workers in respect of phytosociological studies (Cottam and Curtis 1956; Saxena and Singh 1982; Lu *et al.*, 2004; Gupta and Yadav, 2005; Nautiyal 2008).

Ecological niche modelling

During the field surveys carried out in the Western Ghats, the latitude and longitude of *G. imbertii* occurrence were recorded using a global positioning system (GPS- Garmin eTrex 30). The species distribution was modelled using MaxEnt (version 3.4.1) and DIVA-GIS (version 7.5) software. The eco-physiological tolerances of *G. imbertii* was assessed using 19 bioclimatic variables of which 10 are having a higher percentage of contribution and permutation significance which was obtained from the WorldClim database for the period 1970–2000 (Fick and Hijmans, 2017). These 10 bioclimatic variables were again used to predict the possible climatic conditions of *G. imbertii* viz., Bio2, Bio5, Bio1, Bio4, Bio14, Bio19, Bio17, Bio11, Bio3 and Bio18. The study used bioclimatic layers for the period 2050s obtained from WorldClim (http://www.worldclim.org/CMIP5) and predict the future habitat aptness under varied climatic scenarios.

Results

G. imbertii has limited populations confined only in Agasthyamala Biosphere Reserve (600-1300 m asl) (Figure 1). Based on population mapping and

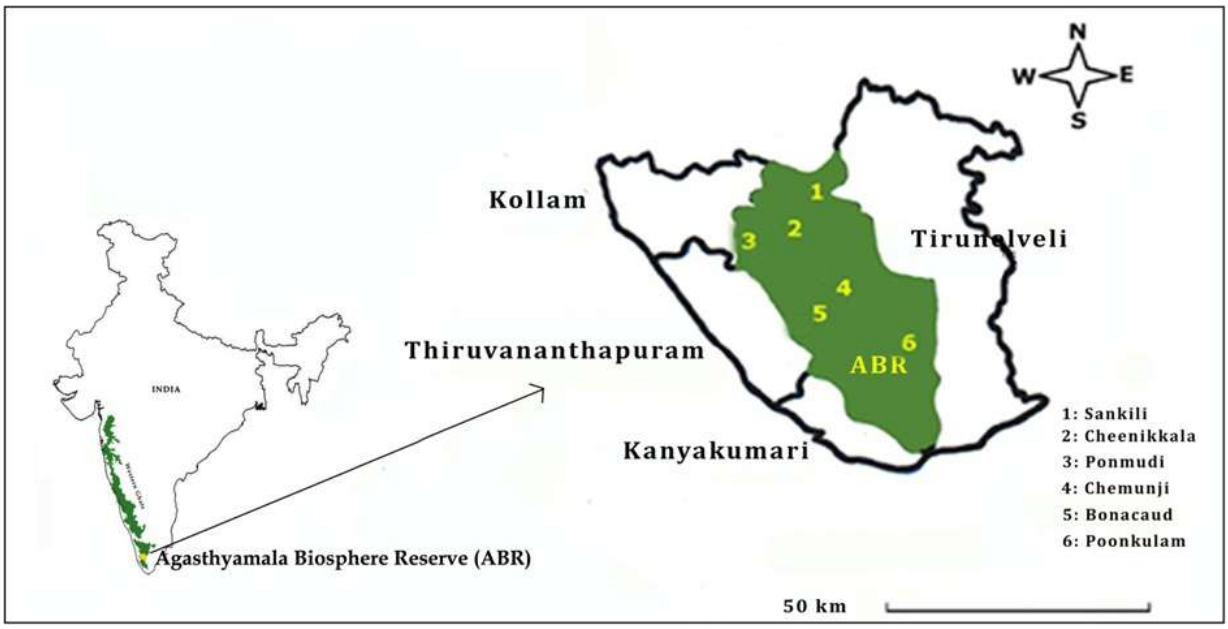

Fig. 1. Populations of *G. imbertii* at southern Western Ghats

Table 1. Population structure of *G. imbertii* at different populations

Population with elevation range (m asl)	No. of plants (100 x 100 m)	Den	Abu	Fre	Rde	Rdo	Rfr	IVI	Wfi
Sankili (920-1150)	45	1.80	2.50	72	27.1	55.8	7.76	90.7	0.03
Cheenikkala (910-1210)	49	1.96	2.58	76	37.9	46.0	12.9	96.9	0.03
Ponmudi (900-1003)	80	3.20	3.48	92	57.9	24.6	22.5	105.0	0.04
Chemunji (1010-1265)	160	6.40	6.40	100	54.4	57.8	11.7	124	0.06
Bonacaud (630-990)	68	2.72	3.40	80	43.0	37.1	6.41	86.5	0.04
Poonkulam (1050-1190)	37	1.48	2.06	72	35.2	47.4	10.7	93.3	0.03

Den- Density; **Abu**- Abundance; **Fre**- Frequency; **Rde**- Relative density; **Rdo**- Relative dominance; **Rfr**- Relative frequency; **IVI**- Important value index; **WFi**- Whiteford index

Table 2. Impact of altitude on seedling existence

Populations	Altitudinal level (m asl)	No. of seedlings per 0.01 Km^2 area
Sankili	Less than 950	0.12
	Between 950 and 1500	0.43
	Greater than 1500	0.16
Cheenikkala	Less than 950	0.41
	Between 950 and 1500	1.08
	Greater than 1500	0.44
Ponmudi	Less than 950	0.11
	Between 950 and 1500	15.21
	Greater than 1500	0.12
Chemunji	Less than 950	4.12
	Between 950 and 1500	18.1
	Greater than 1500	5.42
Bonacaud	Less than 950	1.11
	Between 950 and 1500	4.33
	Greater than 1500	1.11
Poonkulam	Less than 950	0.12
	Between 950 and 1500	2.28
	Greater than 1500	0.13

standard literature, the range of occurrence was estimated to be less than 80 km^2 and the area of occupancy was limited to less than 10 km^2. The present populations are separated by diverse geographical barriers like mountains, rivers, human settlements, plantations and roads.

Floristic wealth and population structure

The floristic diversity of different *G. imbertii* populations revealed that a total of 63 plant species belonging to 52 genera under 34 families. Four other *Garcinia* species were associated to *G. imbertii* populations such as *G. gummi-gutta*, *G. travancorica*, *G. rubro-echinata* and *G. gamblei*. In the present study, the most dominant family was the Lauraceae with six different species, and other dominant families such as Clusiaceae, Myrtaceae, Anacardiaceae, Rubiaceae

Table 3. Suitable climatic variables used in *G. imbertii* for MaxEnt modelling

Variables	Description	Source	Percent contribution	Permutation importance
Bio2	Mean Diurnal Range	WorldClim	30.7	86.6
Bio5	Maximum Temperature of Warmest Period	WorldClim	29.5	0.0
Bio1	Annual Mean Temperature	WorldClim	16.1	0.1
Bio4	Temperature Seasonality	WorldClim	4.9	0.0
Bio14	Precipitation of Driest Period	WorldClim	4.2	0.9
Bio19	Precipitation of Coldest Quarter	WorldClim	4.1	0.1
Bio17	Precipitation of Driest Quarter	WorldClim	3.8	0.0
Bio11	Mean Temperature of Coldest Quarter	WorldClim	3.1	0.0
Bio3	Isothermality	WorldClim	2.1	12.3
Bio18	Precipitation Of Warmest Quarter	WorldClim	1.5	0.0

Fig. 2. Jackknife of regularized training gain for *G. imbertii*

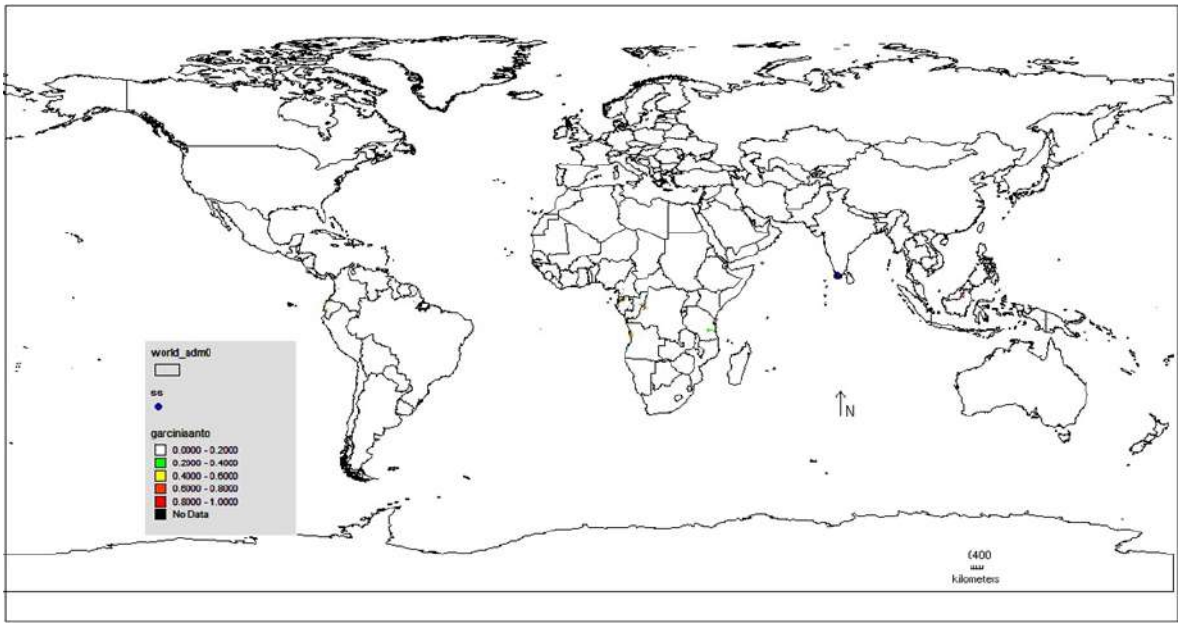

Fig. 3. Suitable climate spaces in the current world for potential distribution of *G. imbertii*

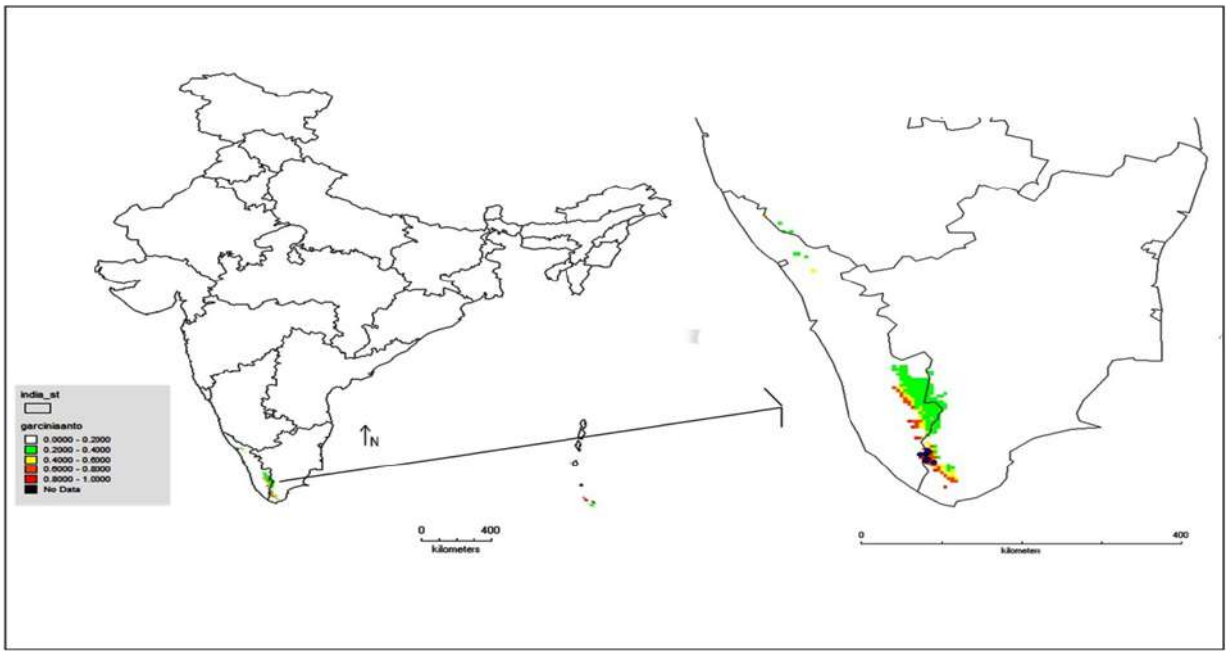

Fig. 4. Suitable climate spaces in current India for potential distribution of *G. imbertii* (Blue dots represented as *G. imbertii* populations)

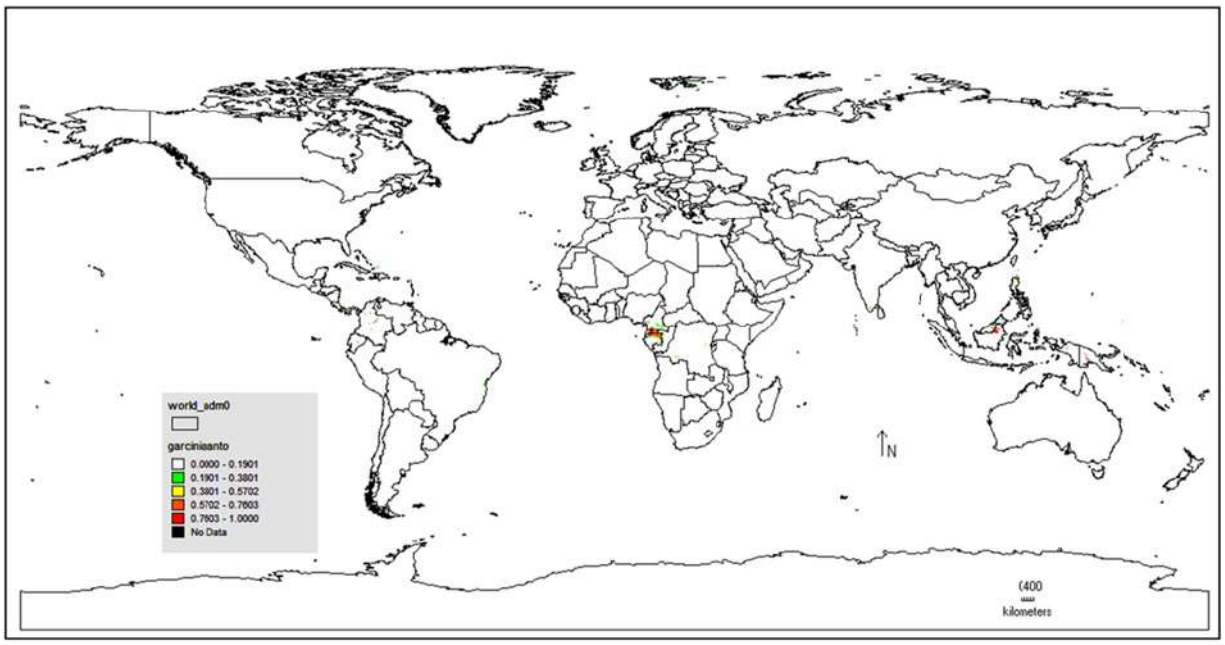

Fig. 5. Suitable climate spaces in the future world for potential distribution of *G. imbertii*

and Annonaceae contributed to the floristic wealth of this area. Species like *Artocarpus heterophyllus*, *Calamus rotang* and *Ochlandra travancorica* were present in all *G. imbertii* populations. Individuals of *Cinnamomum chemungia*num and *C. sulphuratum* present only in Chemunji and Bonacaud populations whereas, *C. malabatrum* existed in Sankili, Ponmudi, Chemunji and Poonkulam populations. Major dominant tree species were *Gluta travancorica*, *Semecarpus travancorica*, *Myristica beddomei*, *Palaquium ellipticum*, *Artocarpus heterophyllus* and *Cullenia exarillata*.

Different population parameters were given in Table 1. The maximum important value index (IVI) of *G. imbertii* was recorded with the more elevated population i.e., Chemunji (IVI; 124) than population at Bonacaud (IVI; 86.5). The result of population structure analysis clearly indicates elevation gradient plays a vital role in the dominance of *G. imbertii*.

Impact of altitude: *G. imbertii* is confined to

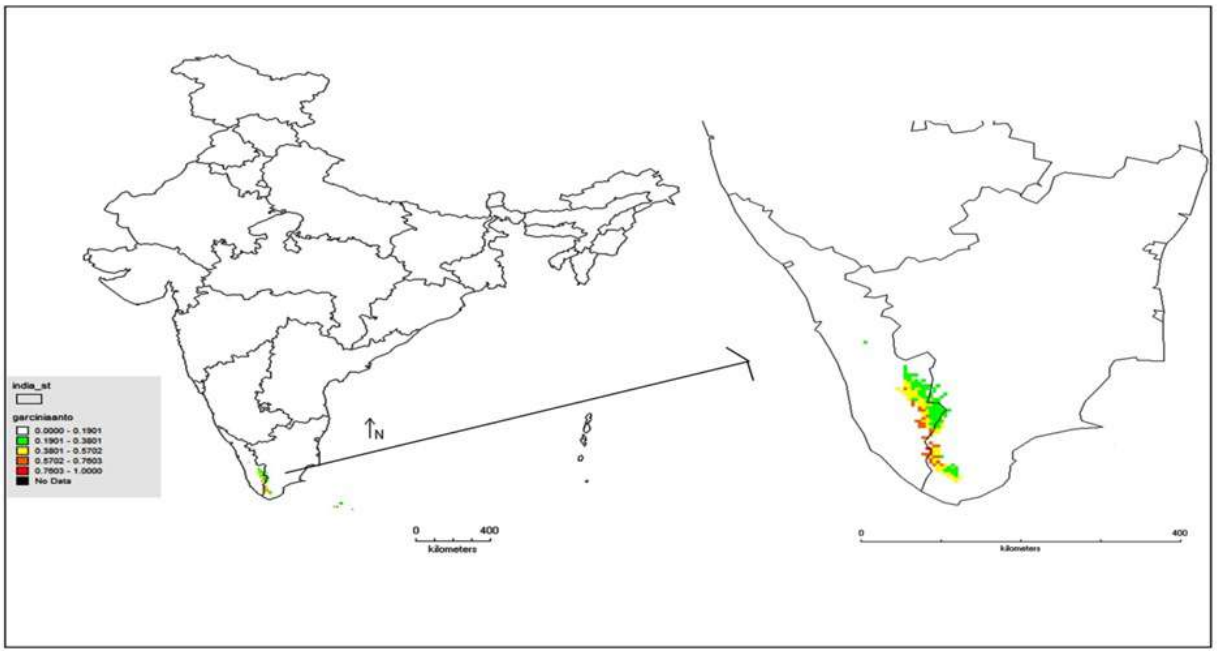

Fig. 6. Suitable climate spaces in the future India for potential distribution of *G. imbertii*

higher elevation range of 600-1300 m asl. The present population studies revealed that high frequency of *G. imbertii* seedlings at intermediate elevation range of 950 to 1500 m asl. The seedlings were not actively growing down or above the existing elevation range within the same population. Chemunji is the large population with 18.1 seedlings existed in 0.01 km^2 area of 950-1500 m asl, however only 4 to 5 seedlings were existed in down or above the existing elevation range (Table 2). In addition, decrease in the tree height and leaf length with an increase in altitude was also noted and observed.

Of the 19 bioclimatic variables used for modelling, the important variables influencing the spatial distribution of *G. imbertii* were mean diurnal range (30.7%), maximum temperature of warmest period (29.5%), annual mean temperature (16.1%) and temperature seasonality (4.9%) (Table 3). The cumulative contributions of 4 variables were 81.2%. The Jackknife test of variable importance exhibited a higher gain for mean diurnal range (bio2) (Fig. 2).

Current distribution of *G. imbertii*

Field observations revealed that currently the potential distribution of *G. imbertii* is being restricted to ABR of the southern Western Ghats. The present analysis also showed that the restricted distribution of this species to these regions and predicted the suitable habitats for this species which is extended towards the middle regions of the Western Ghats, India and certain other places of the world distribution (Fig. 3 & 4).

Anticipated future changes in *G. imbertii* **distribution**

The changes in the future potential distribution of *G. imbertii* was assessed by the difference among the current and future spatial distribution maps. The model predicted the areas under suitable habitats for *G. imbertii* would decrease after years due to varying climatic conditions (Fig. 5 & 6).

Discussion

The population floristic diversity analysis indicated that other allied *Garcinia* species such as *G. travancorica*, *G. rubro-echinata* and *G. gummi-gutta* were associated to *G. imbertii* and prevailing in Chemunji population. Shameer (2017) reported that Agasthyamala forests are rich in *Garcinia* diversity and endemism with 4 species i.e. *G. imbertii*, *G. travancorica*, *G. rubro-echinata* and *G. gamblei*. Shameer *et al.*, (2016) reported that *G. gummi-gutta* var. *gummi-gutta*, *G. talbotii* and *G. morella* were the allied *Garcinia* species in Shendurney Wildlife Sanctuary. Individuals of *Humboldtia decurrens* were mainly occurred in Sankili, Cheenikkala and Poonkulam forests where as *H. unijuga* var. *trijuga* individuals were occurred in Chemunji population.

The concept of importance value index is the expression of the ecological success and dominance of any species with a single number (Misra, 1968).

The IVI of each species in the population showed that *G. imbertii* has the highest value and become the dominant species in these populations. Maximum IVI of *G. imbertii* was recorded with the more elevated population such as Chemunji than population at Bonacaud. This observation clearly indicates that elevation gradient play vital role in the dominance of *G. imbertii* in their populations. Elevation associated temperature and humidity influenced the better growth and dominance (IVI) of associated species like *Cullenia exarillata*, *Myristica beddomei*, *Palaquium ellipticum*, *Hydnocarpus pentandrus*, *Mesua ferrea*, *Diospyros buxifolia*. Swamy *et al*., (2000) studied the population structure of Veerapuli and Kalamalai reserve forests of the Western Ghats and reported that *Hopea parviflora* (IVI of 103.8), *Terminalia paniculata* (IVI of 99.9), *Cullenia excelsa* (63.67), *Agrostistachys meeboldii* (63.65) and *Drypetes oblongifolia* (39.67) were the dominant species. Species richness in a forest is supported by climatic, edaphic and biotic aspects along with different elevation gradients (Ayyappan and Parthasarathy, 1999).

Chemunji is the most regenerating population and species diverse area compared to other populations. The Whiteford index of *G. imbertii* in Chemunji population was higher than the value 0.05, so the species is continuously dispersed in the population (Whiteford, 1949). The predominance of contagious species distribution in Chemunji area indicates abiotic and biotic interactions acting together as described by Mishra and Jeeva (2012). The Whiteford index of other populations indicated that *G. imbertii* and most of the associate species were randomly distributed in that area. Spatially random distribution of plant species may indication of either an ecological or endogenous biological process such as the grouping of seedlings around mother trees, response to climatic variables (varied soil moisture or nutrients) (Lindenmayer, 2009).

Impact of altitude:

Altitude is directly associated to a variety of ecological variables such as temperature, humidityand soil characteristics. (Cierjacks *et al*., 2007) and *G. imbertii* growing in evergreen forests of fragmented ecosystems is also subjected to the effects of specific elevation range 600-1300 m asl that significantly influence plant growth. The physiognomy, floristic composition and structure of tropical forest vegetation showed remarkable changes with increasing altitude (Siebert, 2005). The present population studies revealed high frequency occurrence of *G. imbertii* seedlings at intermediate elevation range of 950 to 1500 m asl and are not actively growing down or above the existing elevation range within the same population. Cierjacks *et al*., (2007) reported that the numbers of inflorescences and seedlings of Polylepis incana exhibited a marginally significant reduction with increasing altitude. The shorter flowering period at high elevated regions may be the main reason for decreased regeneration of woody plant species in Spain (Vera, 1995).

The present observations reveal that the candidate trees showed some characteristic features towards high altitudes that they showed stunted growth and decreased leaf length with in the populations. This is in support with the findings of Korner (2003); Willis and Hulme (2004) that stated a decrease in plant height is an adaptation to the increasing altitude. Bennington and McGraw (1995) observed that increasing altitude and related decrease in plant height results from slower growth rate that supports the plants to use resources more effectively in extreme climatic variables. The stem height and canopy diameter of *Polylepis* species was significantly less at higher elevation as the decrease of air temperatures and soil help the species growth (Cierjacks *et al*., 2007). Korner and Cochrane (1985) argued that increasing altitude with decrease in plant height may favour species as the stem shortening helps the plants to avoid the harmful effects of the strong winds. Yaqoob and Nawchoo (2017) reported that decrease in leaf length, width and area of Ferula jaeschkeana with increasing altitude. Reduced light intensity at lower altitudes maximizes leaf surface area for light capture and it determines the overall plant productivity (Valladares *et al*., 2000).

Anticipated future changes in *G. imbertii* distribution

Ecological niche of a species is a chief factor

that governs and restricts the spatial distribution (Grinnell, 1917). This is one of the pioneer studies to assess the impact of climate change on the distribution of *G. imbertii*. Based on the MaxEnt model, the current distribution of the species is restricted to southern tip of the Western Ghats of India. Suitable climatic regions are spread to the elevated hills of the Western Ghats that support the species growth. The present study predicted the possible distribution of *G. imbertii* in Indonesia and African regions. Many Authors have already reported the distribution of the genus *Garcinia* in Africa (Berg 1979; Jones 1980) and Indonesia regions (Richards, 1990c). The regional climate at ABR such as the altitudinal temperature specificity and precipitation heavily influenced the *G. imbertii* distribution. The mean diurnal range (30.7%), maximum temperature of the warmest period (29.5%) and annual mean temperature (16.1%) restricted the species distribution. Pramanik *et al.*, (2018) reported the suitable area of potential distribution of G. indica in the Western Ghats on the basis of four bioclimatic variables such as temperature seasonality, isothermality, annual precipitation and precipitation of the wettest period. The results of the Jackknife test of variable importance also displayed a higher contribution of mean diurnal range. The model predicts that there is a significant reduction in the future spatial distribution pattern of *G. imbertii*. There is a possibility for the future climate to negatively influence the species regarding their distribution and many present habitats may disappear in the coming years due to varied climatic conditions. Climate variation is a factor that causes huge stress to the environment (Sanjo and Nameer, 2019). Pramanik *et al.*, (2018) reported that the future bio-climate may harmfully affect the distributions of *Garcinia* indica. Many workers predicted the decline of suitable space for several species like Myristicaceae in the Western Ghats (Priti *et al.*, 2016), Myristica dactyloides in Eastern Ghats (Remya *et al.*, 2015). Present study indicated that among the 19 bioclimatic variables, shifting mean diurnal range (Bio 2) is the major contributary stress factor for the existence of *G. imbertii*.

Conclusion

The findings in the present study could lead to a better understanding of the candidate species and may shed some light for in situ conservation of *G. imbertii*. The species is growing in the ecologically fragile areas endemic to ABR of the Western Ghats. The IUCN (1998) listed *G. imbertii* under 'Threatened Categories', and considered to be facing a very high risk of extinction in the wild (RED List-IUCN, 2019). The findings of the present study revealed that, area of occupancy of *G. imbertii* was limited to less than 10 Km^2 and that too in small fragmented and continually declined populations with extreme fluctuations in the number of mature individuals (IUCN criteria of Critically Endangered Category).

The current population status of *G. imbertii* cues to conserve the available populations by enforcing laws. Unsustainable ecotourism causes biodiversity loss through habitat loss and fragmentation, tourism urbanization, and varied climate changes. So 'Responsible Tourism' is recommended in Ponmudi as the *G. imbertii* population is found to be in a shola forest which is frequented by tourists. *Ex situ* restoration of *G. imbertii* was not successful due to niche specific requirements which urge to give more priority for conserving the species *in situ*. Restoration of more genetically diverse seedlings of *G. imbertii* at Ponmudi population will be better for population reinforcement because the individuals in this area are already with less density and genetic diversity. Field observations revealed that currently the potential distribution of *G. imbertii* is being restricted to ABR of the southern Western Ghats. The present analysis also showed that the restricted distribution of this species to these regions and predicted the suitable habitats for this species which is extended towards the middle regions of the Western Ghats, India and certain other places of the world distribution. The changes in the future potential distribution of *G. imbertii* was assessed by the difference among the current and future spatial distribution maps. The MaxEnt model predicted

that the future climate may severely influence the *G. imbertii* distribution and many suitable habitats may disappear after years due to varied climatic conditions. The area mapped for both current and future suitable climate space of *G. imbertii* may decipher possible future spaces useful for the re-introduction and conservation of the species.

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Chapter 24

LOSS OF GENETIC DIVERSITY IN CULTIVAR BANANAS INCREASES DROUGHT SUSCEPTIBILITY

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Abstract India leads the world in banana production with an annual output of about 14.2 million tonnes. Bananas are more drought sensitive because of repeated selection by agriculturalist leads to genetic uniformity due to the loss of most of its wild genes. They are quite sensitive to drought; however, genotypes with "B" genome are more tolerant to abiotic stresses than those solely based on "A" genome. Here in the present study two high yielding cultivar varieties which possess "A" genome (Red banana, AAA) and "B" genome (Nendran, AAB) were chosen. Water stress was imposed at different levels such as 0 %, 10 %, 20 % and 30 % using PEG (Poly ethylene glycol). Leaves and roots samples were collected after 24 hrs. of stress induction, for the biochemical and physiological analysis. Morphological changes include loss of leaf turgidity and leaf tip drying was found more in Red banana than in Nendran. Red banana showed higher MDA content as well as proline content suggesting more susceptibility towards drought while H_2O_2 content was found higher in Nendran. Chlorophyll content showed more or less similar effects. The present study also justifies that the genotype with "B" genome (Nendran) found to be more tolerant compared to the other one (Red banana) with A genome. This study has a significant potential to improve drought tolerance in cultivar banana through molecular breeding and crop improvement programs.

Key Words: *Drought sensitive, Red banana, Nendran, PEG, MDA, Proline, Cultivar banana.*

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Introduction

Musaceae family include 3 genus, *Ensete*, *Musella*, and *Musa* which is divided into two sections with 22 (*Eumusa*, Rhodochlamys) and 20 chromosomes (*Australimusa*, Callimusa) (Hakkinen 2013). Perrier *et al.* (2011) reported that *Eumusa* comprises of most of the edible bananas. Most of the cultivated varieties possess a genomic constitution as AAA, AAB, ABB (triploids). According to Hu *et al.* (2016) and Miao *et al.* (2017) molecular studies point out that the "A" genome provides more genes that are important for banana production and quality and hence can be considered as candidates in breeding programs. The presence of "B" genome is said to impart drought tolerance to these cultivars (Ravi *et al.*, 2013; Kissel *et al.*, 2015) because they were domesticated under more severe climatic conditions, such as wide temperature variation and soil water inadequacy, supporting better environmental stresses (Davey *et al.*, 2009). Thus commercially exploited varieties (with AAA genome) shows fruits with high yield, quality, long fingers and durability. They are more drought sensitive because of repeated selection by agriculturalist leads to genetic uniformity due to the loss of most of its wild genes.

Bananas and plantains belong to the family Musaceae and are cultivated throughout the humid tropics and subtropics. Because of the nutritional value they are considered the fourth most important crop worldwide after rice, wheat and corn. They are perennial showing relatively faster growth rate compared to other fruit crops, and produce fruit all the year round (Van *et al.*, 2011). Drought has emerged as one of the major constrains in banana production. Its effects are pronounced substantially in the tropics and subtropics of the world due to

climate change. Most of the drought stress studies were conducted either by withholding water or by the addition of organic compounds with osmotic action (Mannitol, polyethylene glycol and methyl viologen) on plants maintained in the green house or in the field or in the in-vitro conditions (Li *et al.*, 2013; Hu *et al.*, 2016). In order to find out whether the drought tolerant mechanism has significant relation with the "B" genome, objectives of the present study has been defined as Selection of two high yielding cultivar verities, representing "A" and "B" genome. Establishment of drought conditions using PEG 6000. Estimation of lipid peroxidation (MDA content), hydrogen peroxide (H_2O_2), proline content and total chlorophyll content of both cultivars.

Materials and Methods

Plant materials

Two high yielding cultivars were selected, Red banana and Nendren belongs to AAA and AAB genome respectively. Red banana (AAA triploid cultivar of the wild – Musa acuminate), possess a reddish skin in un-ripened condition (Fig. 1A) which turns reddish – purple on ripening (Fig. 1B) and are softer and sweeter than common Cavendish banana. Nendran have a distinct neck with thick green skin (Fig. 1C) turning yellow on ripening (Fig. 1D). Hardened, healthy, in-vitro plantlets developed by tissue culture techniques were collected from Biotechnology and Model floriculture Centre Kazhakootam, Thiruvananthapuram.

Fig. 1 (A) Red banana Unripe fruits. (B) Ripe fruits. (C) Nendran Unripe fruits. (D) Ripe fruits.

Establishment of drought conditions.

Drought was induced to evaluate its impact in the selected varieties of banana, Red banana and Nendran. Poly Ethylene Glycol (PEG), a high molecular weight compound that can reduce water potential of the medium, which in turn inhibits water and mineral absorption by the plants. Different levels of stress was imposed by preparing 10%, 20% and 30% PEG solution in distilled water. All the plantlets were well irrigated for 1 week, after that healthy plants with 3 to 4 leaf stage was selected for stress induction from both verities. Stress was imposed for a duration of 24 hrs. 10%, 20% and 30% were the three levels of stress imposed, while 0% was considered as control plants without PEG. Triplicates of the treatments were maintained for biochemical and physiological analysis.

Sample collection

The fresh leaves and roots of stressed as well as control (without PEG) plants were collected after 24 hrs. of stress induction from both the cultivars for further analyses.

Analytical methods

Estimation of lipid peroxidation (MDA content) (Heath and Packer, 1968)

The level of lipid peroxidation in the leaf and root were measured in terms of malondialdehyde (MDA) content. 250 mg of plant samples were homogenized with 5 ml of 0.1 % TCA (trichloroacetic acid) by using a mortar and pestle. The homogenate was centrifuged at 10000 rpm for 5 minutes. To the 1 mL of supernatant collected, 4 mL of 20 % TCA with 0.5 % TBA were added. The

mixture was heated at 95°C for 30 minutes and cooled it in an ice bath. Again the mixture was centrifuged at 10000 rpm for 10 minutes. Absorbance was read at 532 nm and 600 nm.

Estimation of proline content (Bates *et al.*, 1973)

The proline content of plant samples were estimated by the method of Bates *et al.* (1973). 0.5 g of fresh leaves and root samples stressed with 10 %, 20 % and 30 % PEG, along with control plants (0 % PEG) were collected after 24 hrs. of stress induction. They were macerated with 10 mL of aqueous sulphosalicylic acid (3 %) using mortar and pestle. The extracts were centrifuged at 4000 rpm for 10 minutes. The supernatant solution of 2 mL was taken in a test tube from each samples and to this 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were added. The solutions were kept in water bath for one hour at 100° C and were cooled under tap water. After cooling, the solutions were transferred in to a separating funnel and 4 mL of toluene were added. The funnel was uniformly shaken for 30 seconds. Two different layers were formed. The colorless bottom layer was discarded and the upper pink colour layer was collected. The optical density was recorded at 520 nm against blank as toluene. Acid ninhydrin: 2.5 g of ninhydrin was taken and mixed with 60 mL of glacial acetic acid and 40 mL of 6 M orthophosphoric acid. The solution was stirred well and slightly warmed in hot water bath until the content dissolved.

Estimation of hydrogen peroxide (H_2O_2) (Loreto and Velikova (2001).

Hydrogen peroxide in plant tissue (leaves and roots) was estimated according to Loreto and Velikova (2001). Samples (0.5 g each) collected after stress induction with 10%, 20%, 30% PEG were homogenized in 5mL of 0.1% trichloro acetic acid (TCA). The homogenates were centrifuged at 12000 rpm for 15 minutes at 4°C. The supernatants were collected and 0.5 mL (supernatant) was added to 0.5 mL of 10 mM potassium phosphate buffer (PH 7.0) and 1 mL of KI reagent (1M KI in distilled water). 0.1% TCA lacking leaf extract was considered as control. The reactions were carried out in the darkness for 1 hour. The absorbances were measured at 390 nm. The H_2O_2 content was calculated using a standard calibration curve prepared using different concentrations of H_2O_2.

Estimation of total chlorophyll content (Arnon, 1949)

To quantify the total chlorophyll content, chlorophyll a and chlorophyll b in the plant tissue was estimated using acetone. 10 g of fresh leaf samples collected from both stressed (10%, 20% and 30% PEG) as well as control plants were homogenized in 10 mL of 80% acetone using a mortar and pestle. The homogenates were filtered through the cheese cloth. The extracts were centrifuged at 5000 rpm for 5 minutes. The deep green supernatants were collected and the volume were measured. 1mL supernatant solution of each samples were taken and mixed with 4 mL of acetone. Then centrifuged at 2000 rpm for 5 minutes. The supernatants were collected and optical density were measured at 645 nm and 663 nm by using 80% acetone as blank.

Results

Morphological changes

In the present study, PEG was used to induce drought stress in plants. Plants treated with PEG 6000 at different concentration, 0%, 10%, 20% and 30% respectively to induce different levels of stress (Fig. 2). After 24 hrs. of treatment plants undergone morphological changes according to the concentration of PEG (stress imposed). Red banana plants stressed with 0% PEG did not showed much morphological variations (Fig. 3A). As the stress progressed plantlets exhibited different levels of stress responses. The plants stressed with 10% PEG showed flaccid lower leaves due to decreased leaf turgidity (Fig. 3B). As the stress progressed even the younger leaves appeared flaccid at 20% PEG (Fig. 3C), while 30% started wilting and also exhibited leaf tip drying (Fig. 3D). Nendran stressed with 0% PEG did not showed much morphological variations after 24 hrs of stress induction (Fig. 4A). The lower leaves showed flaccid condition at 10% PEG stress (Fig. 4B). Loss of leaf turgidity increased with 20% PEG stressed plants (Fig. 4C) while Nendran did not showed leaf tip drying even at 30% PEG stress (Fig. 4D).

Biochemical analysis

The concentration of MDA in the leaf samples was found to increase with increased level of stress induction in both the selected cultivars compared to the control samples. The higher amount of MDA content in red banana leaves were found in plants stressed with 30% PEG and lower was found with plants under 10% PEG stress. Similarly Nendran also showed increased MDA content in the leaf samples stressed with 30% PEG than the one stressed with 10% PEG (Fig. 5A). Root samples also showed increased MDA content with increased level of stress induction in both the selected cultivars compared to the control samples. 30% showed highest MDA with respect to 10% PEG in both red banana and Nendran root samples (Fig.

Fig. 2. Plants treated with PEG 6000 at different concentration 0%, 10%, 20% and 30% respectively to induce different levels of stress in both the cultivars (Nendran and Red banana).

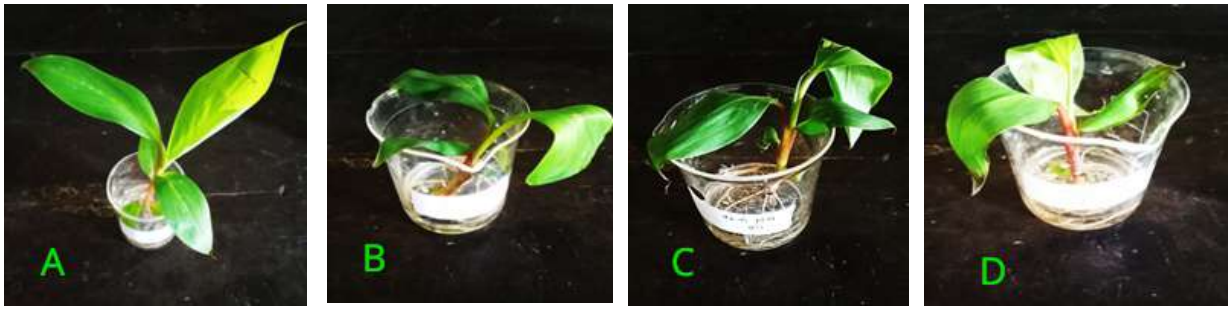

Fig.3. (A) Red banana plants after 24 hrs of stress induction 0 %, (B) 10 %, (C) 20 % and (D) 30 % respectively.

Fig. 4. (A) Nendran banana plants after 24 hrs of stress induction 0 %, (B) 10 %, (C) 20 % and (D) 30 % respectively.

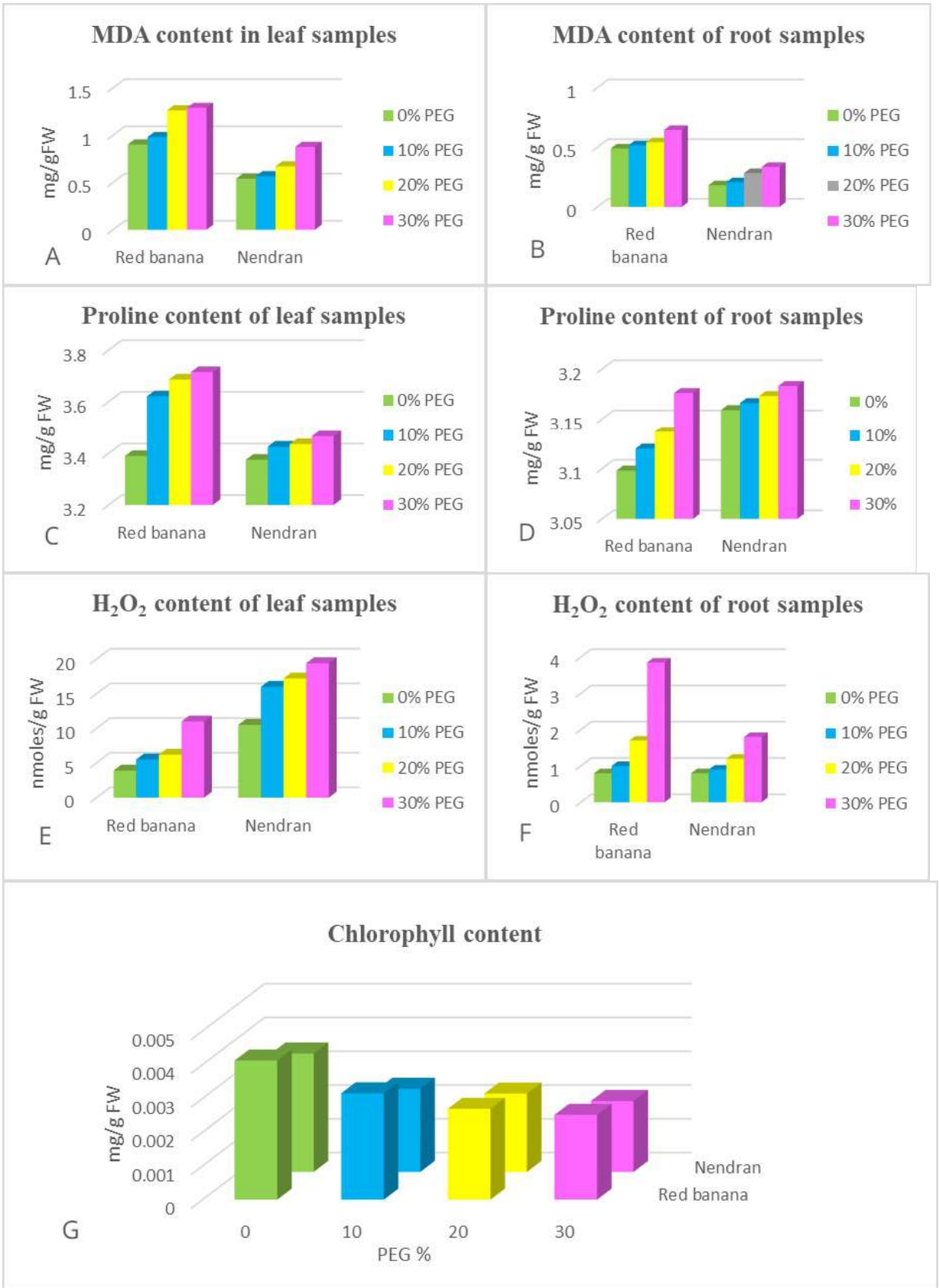

Fig. 5. MDA content in the leaf samples (A) and the root samples (B) of both cultivars (Red banana and Nendran) stressed with 0 %, 10 %, 20 % and 30 % PEG respectively. Proline content in the leaf samples (C) as well as root samples (D) of both cultivars. H_2O_2 content in the leaf samples (E) as well as root samples (F) of both cultivars. Chlorophyll content in the leaf samples (G) of both cultivars (Red banana and Nendran) stressed with 0 %, 10 %, 20 % and 30 % PEG respectively.

5B). With respect to both leaf and root samples MDA content was found to be higher in Red banana genotype than Nendran genotype.

Proline content in the leaf samples was found to increase with increased level of stress induction in both the selected cultivars compared to the control samples. Red banana plants stressed with 30 % PEG found higher amount of proline content in the leaf samples, while lower was found with 10 % PEG. Similarly result was found with Nendran increased proline content in the leaf samples stressed with 30 % PEG than the one stressed with 10 % PEG (Fig. 5C). Root samples of both the selected cultivars showed increased proline content with increased level of stress induction, compared to that of control samples. Highest proline content was found with 30 % stressed plants with respect to 10 % in both red banana and Nendran root samples (Fig. 5D). As far as leaf samples were concerned proline accumulation was found to be higher in Red banana than in Nendran. Root samples did not showed much variation.

Hydrogen peroxide accumulation was found to increase with increased stress induction in both the selected cultivars. Contrary to the above findings, higher H_2O_2 content was found with Nendran leaves stressed with 30 % PEG than Red banana leaves stressed with 30 % PEG. This difference was found with the leaf samples stressed with 10% PEG also (Fig. 5E). In the root samples H2O2 content was found higher in Red banana stressed with 30% PEG compared to the Nendran, 20% stressed plants also showed this variation. Both the cultivars stressed with 10% PEG did not showed much variation from that of control plants (Fig. 5F).

Physiological analysis

One of the major physiological changes that occurs during water deficit is chlorophyll degradation. In the present study chlorophyll content was found higher in the control plants (0% PEG) of both the cultivars. With the progression of stress induction the chlorophyll content found to decrease. Maximum chlorophyll degradation was found with both the cultivars stressed with 30% PEG. The reduction in chlorophyll content showed a more or less similar pattern in both the cultivars (Fig. 5G).

Discussion

Global climate change intensifies the adverse effects of various abiotic stresses (Fedoroff *et al.*, 2010). A substantial research has been done in order to find out drought tolerant banana with altered physiological, biochemical and molecular responses, which were mostly carried out in triploid ones. In the present study PEG - 6000 was used. PEG - a high molecular weight compound that could impart a reduction in water potential of the medium, thereby inhibiting water and mineral absorption by the plants (Davey *et al.*, 2009; Van Asten *et al.*, 2011; Bidabadi *et al.*, 2012).

In the present study the impact of water deficit was found to be different on both the cultivars. The leaves of both Red banana and Nendran stressed with 30% PEG showed higher MDA content than one stressed with 10 and 20% PEG, with respect to the control plants (0% PEG). Moreover the MDA content in the leaves of Red banana was found to be highest among the two varieties analysed. Similar pattern of membrane lipid peroxidation was found with the roots of both selected varieties. The roots of both Red banana and Nendran stressed with 30% PEG revealed higher MDA content than one stressed with 10 and 20% PEG, compared to the control plants. A significant increase in the MDA content in the roots of Red banana was observed with Nendran. The amount of MDA indicates the extent of membrane lipid peroxidation and serves as a signal to access drought- induced oxidative stress and the degree of plant sensitivity (Perez-Lopez *et al.*, 2009; Jain *et al.*, 2001). In this study the higher accumulation of MDA was found in Red banana genotypes with respect to the increased PEG treatment. The membrane lipid peroxidation found in both verities stressed with 10% PEG was negligible, showed the stress level induced was tolerable. According to Negi *et al.*, (2018) over expression of MusaSNAC1 in banana showed increased drought tolerance with lower MDA content. There were earlier reports on lower MDA content in many transgenic plants over expressing genes that positively regulate stress (Shekhawat *et al.*, 2013; Negi *et al.*, 2018).

In the present analysis the leaves of both Red banana and Nendran stressed with 30% PEG showed higher proline content than one stressed with 10 and 20% PEG, with respect to the control plants (0% PEG). The proline content in the leaves of Red banana was found to be highest among the two selected cultivars. The proline content was found to be increased with increased stress condition in both leaves and roots. But comparatively lower proline content was found with the root samples than in the leaves. Proline acts as an osmolyte and helps the plants to maintain tissue water potential under all kinds of stresses. Proline in many plant species might function as a source of solute for intercellular osmotic adjustment under water stress. The studies of Ismail (2004) in banana explained that the enhancement in free proline content could occur either due to 'de novo' synthesis of proline or breakdown of proline – rich protein or shift in metabolism. Transgenic plants over expressing drought responsive genes (SNAC1, WRKY,) showed increased proline accumulation with respect to control wild plants in rice, ramie, cotton and banana (Shekhawat *et al*., 2013; An *et al*., 2015). According to Arteaga *et al*., (2020) proline accumulation can be considered as stress response mechanism, but not as a measure of stress tolerance. With respect to that here in the present study Red banana can considered to be more stressed compared with that of Nandran. Since proline content was lower in roots than in leaves can be explained as leaves (source) were the main site for proline synthesis, and were transported from shoots to roots (sink) for osmotic regulation, growth, and other functions. (Sharma *et al*., 2011).

In the present study the leaves of both Red banana and Nendran stressed with 30 % PEG showed higher H_2O_2 content than one stressed with 10 and 20 % PEG, with respect to the control plants (0 % PEG). Besides the H2O2 content in the leaves of Nendran was found to be highest among the two varieties studied. In the case of roots higher accumulation of H_2O_2 found in Red banana variety with the increase in the PEG treatment. The generation and accumulation of reactive oxygen species is always an indication of stress in plants. The amount of H_2O_2 indicates the extent of membrane lipid peroxidation. On the other hand, H_2O_2 has also been found to act as a secondary messenger under stress conditions (Quan *et al*., 2008). However, the higher accumulation of H_2O_2 is involved in stomata closure induced by abscisic acid signaling (Zhang *et al*., 2001). The enhanced concentration of H_2O_2 may be due to the oxidative stress caused by water deficiency which causes the decline of CO_2 fixation. This leads to higher leakage of electrons to O2 to form O_2 ion which up on dismutation results in the formation of H_2O_2 and ultimately causes oxidative stress in plants (Hossain *et al*., 2015).

Besides affecting the major growth attributes stress induced significant changes in photosynthetic pigments. Chlorophyll is an important photosynthetic pigment which plays role in light absorption and energy transduction and is essential for photosynthesis. Chlorophyll content showed a significant decrease in present study which is in agreement with the earlier studies in thick pea and Phaseolus vulgaris (Ahmad *et al*., 2016; Taibi *et al*., 2016).The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species. The result showed almost similar pattern of chlorophyll reduction in both the varieties. The chlorophyll content decreased with the increased concentration of PEG. Yellowing of leaves and senescence normally occur before leaves abscise in plants under water deficit, which is associated with chlorophyll degradation and translocation of nutrients to newer leaves in crop plants (Boyer, 1976). Reduction in water use efficiency in this plant under water deficit level had a direct impact on photosynthetic pigment degradation, leading to reduce water oxidation in photosystem .

Sreedharan *et al*., (2013), cited banana as the second largest fruit crop in the world in terms of production. Drought stress has the utmost impact on crop yield worldwide and recommended as the most serious ecological problems challenging mankind. Modern molecular biological technologies were simplified elucidation of plant response mechanisms to water stress which in turn were

relevant to understand the genetic basis of drought tolerance of plants. A large number of studies focusing on drought stress under controlled conditions have provided important perceptions into plant stress responses.

Conclusion

Most of the edible bananas and are hybrids either from *M. accuminata* ("A" genome) or from crosses with *M. balbisiana* ("B" genome). Bananas are quite sensitive to drought; however, genotypes with "B" genome are more tolerant to abiotic stresses than those solely based on "A" genome. Here in the present study two high yielding varieties which possess "A" genome (Red banana, AAA) and "B" genome (Nendran, AAB) were chosen. Water stress was imposed using PEG 6000 in order to study their responses. Both the leaves as well as root samples were analyzed. MDA, proline, H_2O_2 and chlorophyll content analysed strongly suggested that the Red banana, found to be more susceptibility towards drought. Our study also conclude that the genotype with "B" genome (Nendran) found to be more tolerant compared to the other one (Red banana). This study has a significant potential to improve drought tolerance in cultivated banana through molecular breeding and crop improvement programs.

Acknowledgements

We thank the 'Fund for Improvement of S & T Infrastructure (FIST)' of the Department of Science & Technology, Government of India for the instruments we used throughout the study.

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Chapter 25

BIOMONITORING OF CURRENT STATUS OF HEAVY METAL LOAD IN THE WATER, SEDIMENT AND *CRASSOSTREA MADRASENSIS* OF ASHTAMUDI LAKE, KERALA

Parvathy N, Sulekha B T, Sheeba S

Abstract Ashtamudi (Ramsar Site 1204) wetland ecosystem is being exposed to various pollution threats from many pockets. Although metals are natural components of water, sediment and biotic systems, immense anthropogenic interferences have seriously hastened the rate of metal accumulation in the living systems. This will alarmingly affect the ecosystem's survival and sustenance and its components. Bioaccumulation of six heavy metals (Pb, Zn, Fe, Cu, Cr and Cd) were assessed seasonally in the water, sediment and an edible clam, *Crassostrea madrasensis* from three sampling stations (Neendakara, Dalavapuram, and Kureepuzha) of the Ashtamudi Lake. Metal Pollution Index (MPI) was used for the cumulative assessment of the heavy metal load in each sample of the respective sampling stations. The results obtained clearly confirmed that the study area was moderate to heavily contaminated with metals under study. The present findings of this study could be useful for the effective evaluation and implementation of suitable conservative measures as well as management strategies for the lake.

Key words: *Ashtamudi Wetland, Metal Pollution Index, C. madrasensis, bioaccumulation, biomagnifications*

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Introduction

Ashtamudi Lake, a Ramsar site (no.1204), has great importance for its valuable estuarine services. The lake is currently facing severe pollution threats from various sources. Among them, heavy metal pollution poses the greatest threat as it can easily locked on the estuarine bed and can take part in the food chain system (Loska and Wiechula, 2003). Besides the natural sources, the rapid growth of urbanization and industrialization scenario contributes to the major anthropgenical input of heavy metals into the aquatic system (Ali and Dzombak, 1996). Dumping of toxic effluents, land run off, sewage and trash waste discharges especially plastic components, etc. releases the metal constituents into the lake. The aquatic components viz. water, sediment and the dependent biotic communities will act as the major sink for many of these pollutant particles. Among aquatic biota, the benthic bivalves are more often chosen as a better biomonitoring system for its environmental contamination .

Since the estuarine quality has a deep influence on its dependent biotic communities, Ashtamudi Lake calls for immediate attention to its quality checking measurements and the strict implementation of its conservation strategies. Therefore, the present study focuses on biomonitoring of the current status of heavy metal load in the water, sediment and an edible bivalve *Crassostrea madrasensis* of the lake.

Materials and Methods

Study area

For the present investigation period during 2019, three sampling stations from the Ashtamudi estuarine system (Fig. 1) were selected for the seasonal collection of triplicated samples of water, sediment and *C. madrasensis*. Station I – Neendakara (8°56'09"N and 76°32'45"E) bar mouth, where the mechanized fishing boats are harboured and the oil spillage from the boats contributes the major source of pollution here. Station II- Dalavapuram, located only 2 Km away from the Bar mouth. Station III – Kureepuzha (8°55'10"N and 76°33'58"E), is placed near the previous waste dumping site of Kollam Corporation.

Sample preparation and heavy metal analysis

Water samples were collected, filtered (0.45 µm Whatman filter paper), and stored in a pre cleaned, acid washed polyethylene bottle. Acidify the sample with HNO_3 to a pH between 2-3. Store samples preferably at low temperatures to avoid evaporation. Samples preparation for the determination of heavy metals in the water samples were done by APDC- MIBK Extraction Procedure.

Sediment samples were collected using grab sampler and were stored at 4°C until extracted. The samples were firstly air dried and then, oven dried at 45°C for two days for evaporating the remaining moisture (Defew et al., 2005). Then 0.5 gm of samples were weighed, acid - digested (HNO_3 and HF acid, 1:3 ratio), for the analysis of six metals (Pb, Zn, Fe, Cu, Cr and Cd).

Live bivalves-*Crassostrea madrasensis* (Fig. 2) samples were collected in respective sterile polythene bags with the help of a local fisherman. The selected clams were pooled and the extracts (whole body except exoskeleton) were oven dried at 80-85°C, overnight. Approximately 0.5 gm of extracts were weighed and acid digested (nitric acid and perchloric acid, 8:1 ratio).

All the sample solutions were filtered, washed and diluted with metal free distilled water for the analysis of seven metals (Pb, Zn, Cu, Cr and Cd) in ICP-OES (Thermo Fischer iCAP 7200 DUO) . The samples were compared to the respective standard curve to determine the respective ppb levels of each heavy metals in the digested solutions.

Assessment of Metal Pollution Index

Metal Pollution Index, MPI calculation is a cumulative method for the evaluation of the comparative quality of the environmental

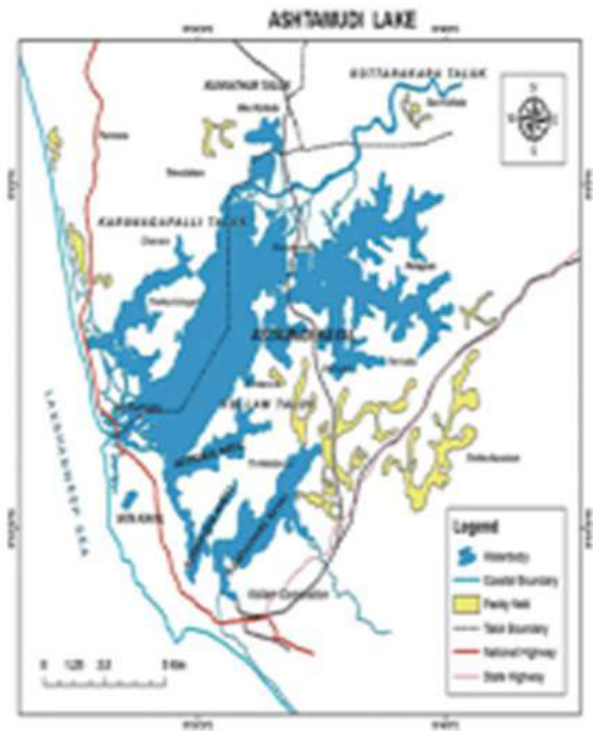

Fig. 1. Study area: Ashtamudi lake

Fig. 2. *Crassostrea madrasensis*

Table 1. Seasonal average values (ppb) of heavy metal load in the water samples from each sampling stations during the study period (2019).

Water							
	Pb	Zn	Fe	Cu	Cr	Cd	MPI
Neendakara							
Pre-Monsoon	0.1875 v	3.41983	52.7901^	7.72289	41.5388	BDL	4.705756
Monsoon	1.23 v	6.6875	100949^	134.4	29.94	BDL	38.66465^
Post-Monsoon	2.5 v	7.85167	1272.79^	12.86	7.90667	BDL	11.68094
Seasonal Average	1.30583	5.98633	34093.193	51.66	26.4618	BDL	38.75439
Dalavapuram							
Pre-Monsoon	BDL	2.72625 v	36.1878^	3.79245	7.70568	BDL	3.77242
Monsoon	BDL	5.77083 v	2398.58^	52.2083	9.54125	BDL	13.79465
Post-Monsoon	BDL	1.75333 v	692.628^	9.04875	6.70417	BDL	6.474167
Seasonal Average	BDL	3.41681	1042.4652	21.6831	7.9837	BDL	13.36724
Kureepuzha							
Pre-Monsoon	BDL	5.10417 v	49.0125^	13.457	8.24342	BDL	5.501969
Monsoon	BDL	4.42292 v	94.3646^	8.34375	22.9167	BDL	6.561036
Post-Monsoon	BDL	5.66208 v	2354.84^	13.8154	21.2879	BDL	12.55631
Seasonal Average	BDL	5.06306	832.741	11.8721	17.4827	BDL	9.779193

components in relation with the metal pollution load in the system (Lafabrie *et al*., 2008)

It is calculated as (Usero *et al*., 1997):

MPI = (Cf1 x Cf2 ……Cfn) 1/n

where Cfn is the concentration of metal n in the sample.

Results and Discussion

The seasonal analysis of the average values (ppb) of heavy metal load in the water, sediment and *C. madrasensis* samples from the respective sampling stations were summarized in table 1 to 3.

The results showed that among the three stations, the highest loaded metal was Fe in the water samples irrespective of all the three seasons under the study. In station I (Neendakara), the lowest accumulated metal was Pb in all three seasons. In station II (Dalavapuram) and station III (Kureepuzha), Zn was recorded as the least accumulated metal during pre-monsoon, monsoon and post-monsoon (Table 1). The highest MPI values were recorded during monsoon (38.664) in station I (Neendakara).

In the case of sediment samples, Fe was the most accumulated metal in all the sampling stations throughout the seasons. In the case of station I (Neendakara), Cd was the least accumulated metal during pre monsoon (61.3ppb) and Pb during monsoon (1486.25ppb) and post monsoon (1337.5ppb). On the other hand, the lowest accumulated metal was found to be Cd during pre monsoon and monsoon in station II (Dalavapuram) (71.25 and 25.00ppb respectively) and station III (Kureepuzha) (76.25 and 32.5ppb respectively). During post monsoon, Pb (338.3ppb) and Zn (5079.16ppb) were the lowest concentrated metal in the sediment samples of station II (Dalavapuram) and station III (Kureepuzha) respectively. Highest MPI value was recorded in station III (Kureepuzha) during monsoon season (table2).

In the case of tissue samples of *C. madrasensis* Fe and Cr were the highest and the lowest accumulated metal in station I (Neendakara) and Fe and Cd in station III (Kureepuzha) throughout the seasons. In the case of station II (Dalavapuram), Fe and Pb were the highest and lowest loaded metal in the tissue samples during pre monsoon and monsoon respectively. The highest value of MPI was observed in station III (Kureepuzha) during monsoon season. Bivalve samples were not obtained from station I (Neendakara) and station III (Kureepuzha) during monsoon and post monsoon season respectively (Table 3.).

Table 2. Seasonal average values (ppb) of heavy metal load in the sediment samples from each sampling stations during the study period (2019).

Sediment							
	Pb	Zn	Fe	Cu	Cr	Cd	MPI
Neendakara							
Pre-Monsoon	958.583	4227.71	7288090^	2784.67	6053.9	61.3^{v}	5.09×10^{21}
Monsoon	1486.25^{v}	7018.75	6664727^	3514.58	4824.58	BDL	196×10^{20}
Post-Monsoon	1337.5^{v}	3791.09	687217.1^	4819.25	2008.75	BDL	5.62×10^{18}
Seasonal Average	1260.78	5012.52	4880011	3706.17	4295.74	21.1	1.73×10^{21}
Dalavapuram							
Pre-Monsoon	984.295	32178.5	483118.9^	1825.42	3248.3	71.25^{v}	1.08×10^{21}
Monsoon	1635.42	5017.92	1330961^	4310.42	349518	25^{v}	6.86×10^{22}
Post-Monsoon	338.23^{v}	58650.3	567418.3^	1193.33	2223.96	BDL	4.98×10^{18}
Seasonal Average	985.98	31948.9	793833	2443.06	118330	32.4167	3.91×10^{22}
Kureepuzha							
Pre-Monsoon	41290.5	10343.2	991912.8^	5010.83	3697.41	76.25^{v}	9.97×10^{22}
Monsoon	2571.25	8543.33	10711238^	6539.59	18199.9	32.5^{v}	1.52×10^{23}
Post-Monsoon	12541.2	5079.167^{v}	5061127^	10501.9	11534.3	BDL	6.51×10^{21}
Seasonal Average	18801	7988.56	5588093	7350.76	11143.9	36.58	4.19×10^{23}

Table 3. Seasonal average values (ppb) of heavy metal load in *C. madrasensis* samples from each sampling stations during the study period (2019).

Crassostrea madrasensis							
	Pb	Zn	Fe	Cu	Cr	Cd	MPI
Neendakara							
Pre-Monsoon	112.5	4094.163	27510.17^	536.83	254.6667	27.5^{v}	601.9322
Monsoon	NA	NA	NA	NA	NA	NA	NA
Post-Monsoon	BDL	2047.082	13755.08^	268.415	127.333^{v}	BDL	99.34469
Seasonal Average	37.5	2047.082	13755.08	268.415	190.99	9.166667	262.9268
Dalavapuram							
Pre-Monsoon	58.75^{v}	1021.917	7507.583^	95	77.5	29.16667	121.8962
Monsoon	608.3333^{v}	2142.917	714945.8^	657.5	9767.917	BDL	1343.593
Post-Monsoon	BDL	BDL	2341.667^	BDL	BDL	BDL	3.642192
Seasonal Average	222.3611	1292.167	241598.4	250.8333	3281.806	9.722222	619.0292
Kureepuzha							
Pre-Monsoon	65	497.0833	4847^	86.66667	78.75	26.25^{v}	173.9581
Monsoon	1213.75	3260.417	874543.3^	17007.08	22988.75	BDL	3314.968^
Post-Monsoon	NA	NA	NA	NA	NA	NA	NA
Seasonal Average	426.25	1252.5	293130.1	5697.917	7689.167	8.75	1972.57

NA: Samples were not available from the respective stations at the time of sample collection.
BDL: Below Detection Limit
^: Highest recorded level among all the metals.
^{v} : Lowest recorded level among all the metals

Heavy metal toxicity can cause severe physiological as well as metabolic imbalances like defective thyroid gland, kidney problems, irreversible neurological as well as behavioral effects, reproductive and cardiovascular dysfunctioning etc (Homady *et al.*, 2002; Tchounwou *et al.*, 2012) etc. Metal accumulation in the water and sediment was comparatively lesser than the bivalves. The factors which lead to the increased adsorption of Zn in the estuaries are, suspended particles (Ahumada *et al.*, 2007), using Zn as an anti-corrosive agent, domestic discharges (Kamaruzzaman *et al.*, 2011) etc. This may be the source behind the accumulation of Zn in *C. madrasensis*. Although Cu, Cr and Zn have significant biological functioning related with protein complexes such as hemoglobin, metallothioneins etc, and the accumulation of the same beyond the permissible limits creates a toxic effect and physiological malfunctioning (Vallee and Walker, 1970; Da Silva, 1978; Pellerin and Amiard, 2009). cadmium and Pb are closely related to the sediment because of the nature of adsorption, hydrolysis and co-precipitation.

According to Olade, 1987 the lowest cadmium levels in the sediments may be due to tropical weathering effect . Environmental heavy metal accumulation in fishery resources were mainly contributed by several anthropogenic factors (Klein and Goldberg, 1970; Alina, 2012; Zarei, 2014; Da Silveira *et al.*, 2018; Esposito *et al.*, 2018). The factors contributing to the metal accumulation in the bivalves are feeding habit, filtering rate, growth rate, size and age of the organisms (Lau *et al.*, 1998; Jordaens *et al.*, 2006) etc. The analysis of the Metal Pollution Index (MPI) showed that the heavy metal accumulation in samples was in the decreasing order as follows: bivalves > sediment > water.

Conclusion and Recommendation

The present study reveals valuable information about the current heavy metal load in the sediment samples of the selected study stations of the Ashtamudi Lake. This reflects the water and sediment quality of the stations. The values of MPI indicate cumulative heavy metal load in the water, sediment and bivalve samples. This reveals the chances of transferring toxic metals from the water and sediments to its dependent biotic communities. This will become a part of the food chains and accumulate in the higher trophic levels through biomagnification. Therefore, immediate scientific implementation of the conservative strategies is recommended for maintaining the quality of the Lake as well as for the health of the aquatic biota.

Acknowledgement

This work was financially supported by the Council of Scientific and Industrial Research (CSIR)-HRDG, New Delhi, as grant for CSIR JRF fellowship for the PhD research work (of the author1), entitled, "Bioaccumulation of heavy metals in edible bivalves of Ashtamudi Lake, Kerala, India". The author1is also grateful to the respective Institution, Sree Narayana College, Kollam, Kerala, from which the grant for the facility to research is allotted.

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Chapter 26

STUDIES ON THE BIOLOGICAL ACTIVITIES OF A COMMERCIAL SAMPLE OF HAND SANITIZER

Thara A T, Latha Sadanandan

Abstract Bioefficacy of a marketed sample of alcohol based hand sanitizer (ABHS) Smartway was studied. The objectives were to find out their pH, to study it's antibacterial activity, studies on it's cytological effect, effect on plasmolysis and antioxidant activity. ABHS Smartway had 70% Isopropanol with gel base (as per specifications given) with pH 7.7 It showed antibacterial activity with a zone of inhibition of 15± 0.1mm against *S. aureus* and 14± 0.2mm against *P. aeruginosa*. Mitotic studies in root tip cells of *Allium cepa* showed that both 100% isopropanol (Control) and ABHS Smartway showed less number of mitotic cells. Onion root tip cells treated in distilled water showed maximum mitotic index and least mitotic abnormalities thus proving the deleterious effect of isopropanol at higher concentrations. Mitotic aberration in onion root tip cells increased with concentration of isopropanol with 45% aberration in ABHS Smartway and 70% abnormalities in Isopropanol. The abnormalities observed were nuclear lesion, Prophase erosion, strap shaped nuclei, chromosome clumping, diagonal orientation of chromosome, star metaphase and chromosome bridges. Studies on plasmolytic activity showed that both isopropanol and ABHS Smartway containing 70% isopropanol did not cause plasmolysis of lower epidermal cells in Rhoeo discolor leaves. This revealed that the pH of 7.7 (in Smartway) to 8 (in Isopropanol) did not cause any permeability problems. Antioxidant activity of ABHS Smartway in terms of DPPH scavenging percentage was 10.2% that is near to the maximum antioxidant activity (11.02%) reported in hand sanitizers. Though the selected ABHS Smartway showed positive results in terms of pH, antibacterial activity, antiplasmolytic activity and antioxidant activity, mitotic aberrations observed in the study recommends studies on the action of hand sanitizers on human skin regeneration before their constant application in the context of Covid 19 pandemic.

Key words: *Sanitizer, cytotoxic, antimicrobial, antioxidant, plasmolytic*

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Introduction

The success of the hand sanitization solely depends on the use of effective disinfecting agents formulated in various types and forms such as antimicrobial soaps, alcohol based or water based hand sanitizer, with the former being widely used. Most of the effective hand sanitizer products are alcohol based formulations containing 62% to 95 % of alcohol. The spread of the COVID-19 pandemic during 2020 – 2022 has played a significant role in the extensive use of hand sanitizers (Jane *et al*, 2020). The rigorous use of such alcohol based hand sanitizers (ABHS) prompted the authors to study their effect on biological systems.

Materials and Methods

Samples of ABHS Smartway were purchased and their biological activities evaluated. The objectives were to find out their pH, to study it's antibacterial activity on Staphylococcus aureus and *Pseudomonas aeruginosa*, studies on it's effect on mitosis in root tip cells of Allium cepa L., effect on plasmolysis of leaf epidermal cells of *Rhoeo discolor* (Lher.) Hance and antioxidant activity using DPPH method.

Determination of pH

The pH of the hand sanitizers were measured using Systronic digital pH meter to find out their compatibility to human skin.

Antibacterial assay

Test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* were collected from Institute of Microbial Technology, Microbial Type Culture Collection Centre (IMTECH), Chandigarh. Pure culture of the bacterial strains were maintained in 5 ml of Trypton Soya Broth (TSB). They were incubated at 37ºC for 2-8 hours till light to moderate turbidity developed and maintained at 2-8ºC. Mueller Hinton Agar (MHA) medium was used for bacterial culture. MHA was prepared and sterilized at 121ºC for 15 minutes. After sterilization, required volume of the medium (20 ml) was poured in the sterile petri dishes, allowed to solidify and stored at 2-8ºC. Agar well diffusion method was applied for determining anti bacterial activity. Three replicates were maintained.

Cytological studies

The roots *Allium cepa* L. were treated by suspending the sprouted bulbs in 100% concentrations of the hand sanitizer separately for 15min, 30min and 1 hour. Onion root tips suspended in distilled water and isopropanol served as negative control and positive control respectively. Root tip from each treatment were fixed between 11-12pm in Carnoy's fluid. To make the micropreparations the root tip from each treatments were transferred to vials containing 1 N Hydrochloric acid and kept at 600 C for 3-5 minutes in hot air oven. The hydrolysed root tips were then transferred to clean slide and squashed with Acetocarmine by using Acetocarmine squash technique (Sharma and Sharma, 1980). Prepared slides were then examined through OLYMPUS CH 20i Binocular stereomicroscope and photographs were taken. Mitotic index and chromosomal aberrations in each treatment were studied and tabulated.

Mitotic Index (MI) = (Total no.of DividingCells)/(Total No of Cells)×100

Percentage of abnormalities = (Total no.of abnormal Cells)/(Total No of Cells)×100

Plasmolytic activity

Different concentrations of hand sanitizer (25% and 50%) were prepared by diluting in distilled water. Lower epidermis of leaves of *R. discolor* was peeled off and placed in different watch glasses containing different concentrations (25%, 50% and 100%) of the hand sanitizers. Three replicates were maintained for each treatment. The treatments were incubated at room temperature for 15 min, 30 min and 1hour. Isopropanol was used as control. They were mounted on glass slides covered with coverslip and observed for plasmolysis under compound microscope. The results were tabulated and photographs taken.

Antioxidant activity

The free radical scavenging activity of the hand sanitizer was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Shimada *et al*., 1992). 0.1mM solution of DPPH in ethanol was prepared. 0.1ml of this solution was added to 3.0ml of hand sanitizer at 100% concentration. Thirty minutes later, the absorbance was measured at 517nm. Lower the absorbance of the reaction mixture, higher the free radical scavenging activity. A system with isopropanol instead of hand sanitizer served as control. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

DPPH scavenging % =

(A (control) – A (test))/A (control) x 100

Results

Determination of pH

The average pH of the selected hand sanitizer was 7.7

Antibacterial assay

The antibacterial activity of alcohol based hand sanitizer (ABHS) was assessed by the presence or absence of inhibition zone and zone diameter using Agar well diffusion method. The ABHS Smartway showed good zone of inhibition to both the pathogenic bacteria viz *Staphylococccus aureus* with average diameter of 15± 0.1mm and *Pseudomonas aeruginosa* with average diameter of 14±0.2 mm (Plate 1).

Cytological studies

Comparison of the mitotic indices of the different treatments showed that mitosis in the root tip cells of onion was least affected by water

(negative control) while both isopropanol (positive control) and ABHS Smartway reduced them. Maximum inhibition was found in the 100% isopropanol treatment for 30 minutes (Table 1) when compared to ABHS Smartway treatment that contain 70% isopropanol indicating the deleterious effect of isopropanol at high concentrations.

Comparison of the percentage of abnormal cells in onion root tip cells showed that the least number of abnormal cells were found in negative control ie water, while both isopropanol and ABHS Smartway increased the percentage of abnormal cells (Table 1). Maximum percentage of 70% abnormal cells were found in 100% concentration of isopropanol while it was limited to 45% in ABHS Smartway treatment even with increase in time duration to 1 minute. This also proves that 70% isopropanol in the ABHS is much safer than 100% isopropanol to living cells.

The major mitotic abnormalities observed were nuclear lesion, strap shaped nuclei, chromosome clumping, diagonal orientation of chromosome, star metaphase and chromosome bridges (Plate 2).The interphase stage showed nuclear lesions and strap shaped nuclei. Most of the cells in the interphase showed nuclear lesions. Double lesions were also observed. In prophase stage, prophase erosion was observed. Metaphase showed several abnormalities such as metaphase clumping, diagonal metaphase and star metaphase. Abnormality found in Anaphase was anaphase bridges. Telophase showed differential condensation of chromosomes. Minimum type of aberrations were observed in the onion root cells in water (negative control). Many types of abnormalities were common in root cells treated with Isopropanol and the ABHS Smartway (Table 2). This may be due to the adverse effects of Isopropanol at higher concentrations (70% and above).

Plasmolytic activity

The tissues treated tin both treatments and control (Isopropanol) did not show plasmolysis in different time durations. (Plate 2). This indicated that the ABHS Smartway does not cause

Table 1. Comparison of average Mitotic Indices and percentage of abnormal cells of onion root tip in various treatments and duration

Treatment duration (Minutes)	Positive Control (Isopropanol)		Negative Control (distilled water)		ABHS Smartway	
	Mitotic Index	% of abnormal cells	Mitotic Index	% of abnormal cells	Mitotic Index	% of abnormal cells
15	12.7	51.96	12.5	11.6	6.36	64.54
30	4.13	55.17	11.6	10.5	7.58	44.80
60	6.41	70.51	10.9	9.2	5.20	45.87

Table 2: Various types of mitotic aberrations found in different controls and treatments

Si.No	Positive Control (Isopropanol)	Negative Control (Distilled water)	ABHS (Smartway)
1	Single nuclear lesion	Single nuclear lesion	Single nuclear lesion
2	Double nuclear lesion	Double nuclear lesion	Double nuclear lesion
3	Prophase erosion	Prophase erosion	Prophase erosion
4	Anaphase with fragments		Anaphase with fragments
5	Diagonal metaphase		Diagonal metaphase
6	Ring chromosome at Metaphase		Ring chromosome at Metaphase
7	Metaphase clumping		Metaphase clumping
8	Star metaphase		Star metaphase
9	Strap shaped nucleus		Strap shaped nucleus

plasmolysis of plant cells even when applied for one hour.

Antioxidant activity

The average absorbance of ABHS Smartway and the control Isopropanol were 0.219 and 0.243 at 517nm. The result indicated that the ABHS Smartway has the ability to donate hydrogen as free radical that is scavenged by DPPH. Table 3 shows the absorbance exhibited by the ABHS Smartway and Control Isopropanol at their 100% concentrations. From the average of three trials, the average DPPH scavenging percentage of ABHS Smartway was found to be 10.2 %.

Discussion

Human skin pH ranges from 4.5 to 6.5 (Heather, 2012). The skin pH and the buffering capacity of the skin surface are made up of the contributions from all the components of the

Table 3. Absorbance of the Control and ABHS Smartway in DPPH Assay

Si. No	Control (Isopropanol)	Treatment (Smartway)	DPPH scavenging percentage
1	0.198	0.185	6.56
2	0.283	0.253	10.6
3	0.250	0.220	12.0
Average	0.244	0.219	10.2

Plate 1. Treatment of ABHS Smartway in bacterial culture plates containing P. aeruginosa (left) and S. aureus (right)

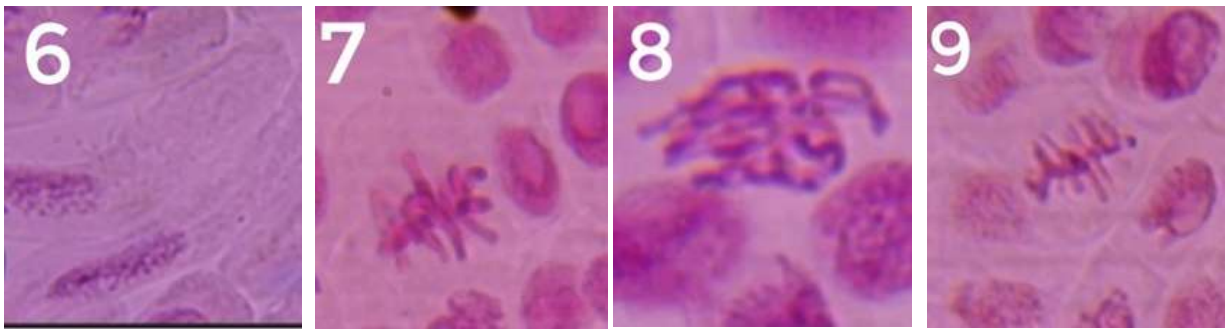

Plate 2. Mitotic aberrations observed in various treatments
6. Strap shaped nucleus 7. Star Metaphase
8. Metaphase clumping 9. Diagonal Metaphase

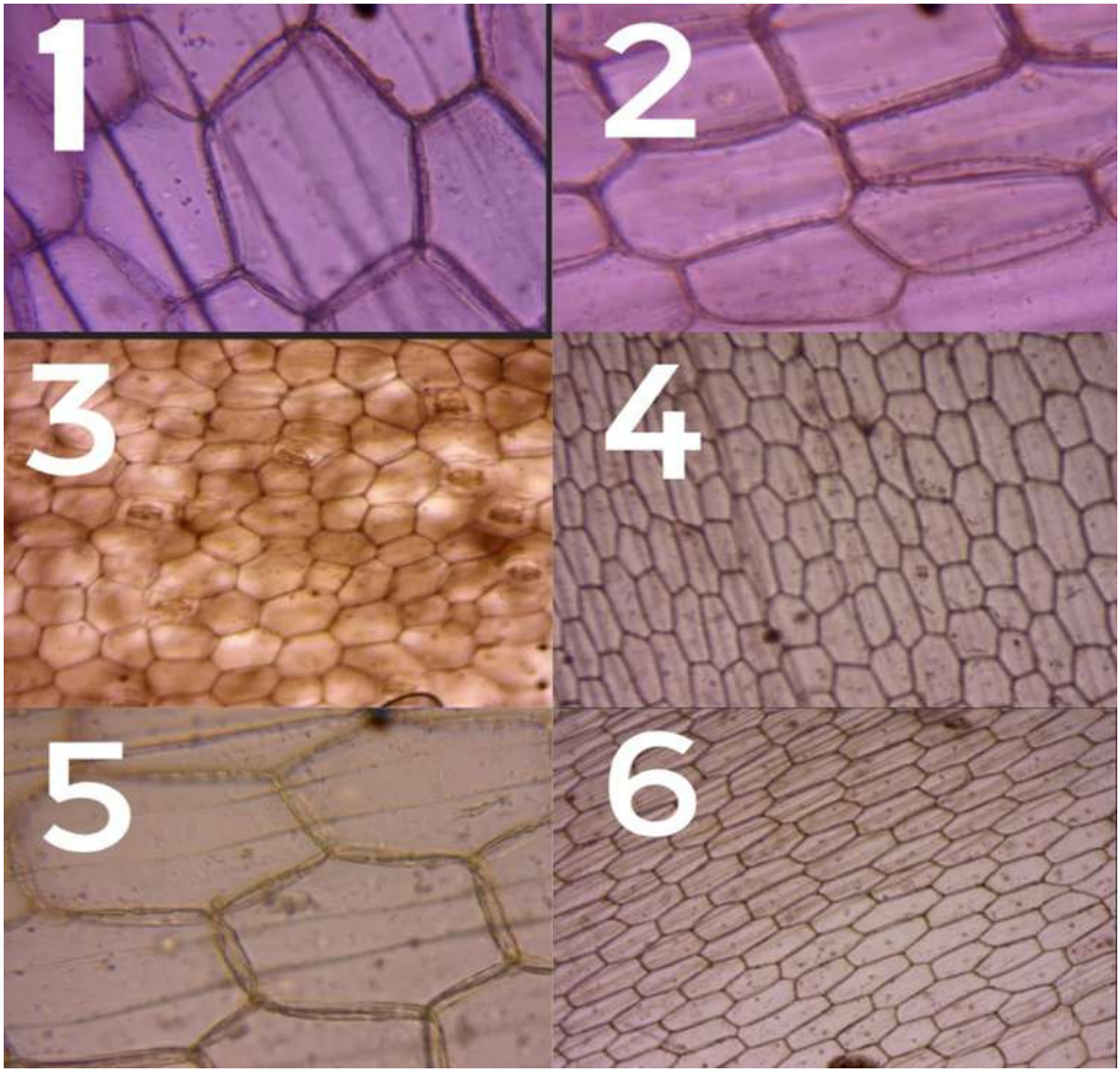

Plate 3. Unplasmolysed lower epidermal tissue of *R. discolor* in various treatments
1) Isopropanol after 15minutes. 2) Isopropanol after 30 minutes 3) Isopropanol after 1 hour 4) ABHS Smartway after 15 minutes 5) ABHS Smartway after 30 minutes 6) ABHS Smartway after 1 hour

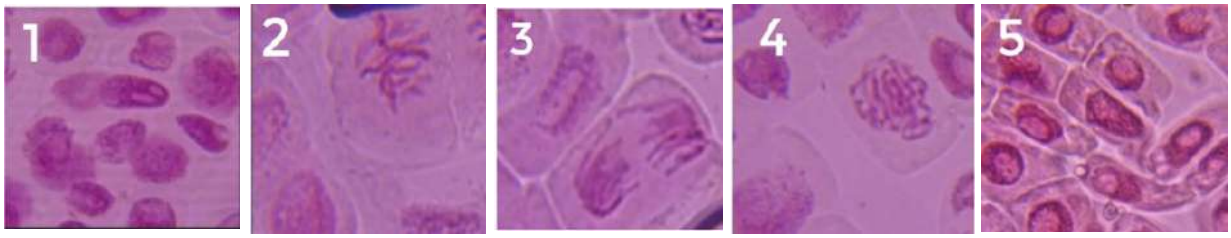

Plate 4. Mitotic aberrations observed in various treatments
1.Double nuclear lesions 2. Ring chromosome at Metaphase 3.Anaphase with fragments 4. Prophase erosion 5. Single nuclear lesions

stratum corneum as well as the secretions from sebaceous and sweat glands (Dikstein and Zlotogorski, 1994).The use of synthetic detergents formulated at the same pH as skin and even tap water also leads to a rise of the skin surface pH, however to a lesser extent and for a shorter period of time (Korting and Braun-Falco,1995). A study conducted by Oke and coworkers revealed that Dettol hand sanitizer was effective only against P. aeruginosa whereas it was not effective against S. aureus and *E. coli* (Oke *et al.*, 2013). A study conducted by Mondal and Kolhapure showed that

PureHands, the herbal hand sanitizer, was effective against *E. coli*, *Proteus mirabilis*, *Shigella sonnei*, *S. aureus*, and *S. epidermidis* (Mondal and Kolhapure 2004). Isopropanol denatures proteins in the plasma membrane of microbes, dissolving membrane lipids that neutralizes them (Jane *et al*., 2020). Earlier studies showed that hand sanitizer has the highest antioxidant activity of 11.02% while *Ocimum sanctum* showed 10.76%, *Azadirachta indica* showed 6.18% and followed by *Citrus limon* showed 1.08% DPPH scavenging activity. These phenomena can be attributed towards the presence of phenolic constituents in formulation and herbal extract which in turn indicates the stability of the formulation. (Acharya *et al*, 2018).

Conclusions

The above work finds relevance in finding the pros and cons of a hand sanitizer. The antibacterial study shows that the ABHS Smartway has activity against *S.aureus* that acts as a commensal of the human microbiota that can become an opportunistic pathogen. It showed antibacterial effect on *P. aeruginosa*, a known multidrug - resistant pathogen. This action of ABHS Smartway against both a Gram positive and a Gram negative bacteria is worth addressing. Studies on plamolysis also showed a positive impact on the hand sanitizer Smartway as the water potential of the plasma membrane of *R.discolor* was unaffected, thus maintaining the plasma membrane intact. So far no studies were carried out on the plasmolytic activity of hand sanitizers. It also showed a good antioxidant activity of 10.2% that is nearly the maximum antioxidant activity (11.02%) reported in hand sanitizers. Though it has these positive aspects, the studies on mitosis of onion root tip cells showed negative effect with less mitotic index and many types of mitotic aberrations that is of great concern. When the skin flora distribution is disrupted, due to the long – term use of topical hand sanitizers, the non-pathogenic microflora may become virulent. To reduce the incidence of infection, the microbiota balance is restored and maintained through constant skin regeneration. The whole process takes about 28 days, starting from the mitotic division of basal epithelium to desquamation. When the dead keratinocytes in the uppermost epidermal layer, the stratum corneum are sloughed off, it takes away the microbes that colonized the skin surface. This continuous process significantly limits the invasion of bacteria while achieving a balanced growth among the microbial populations. Therefore, the action of hand sanitizers on mitosis required for skin regeneration needs to be addressed.

Therefore, the study recommends further studies on the action of hand sanitizers and other hand disinfectants on human skin before their constant application in the context of Covid 19 pandemic.

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Chapter 27

EX-SITU CONSERVATION OF SEVEN ENDEMIC AND ENDANGERED TREE SPECIES AT SREE NARAYANA COLLEGE, KOLLAM - A CASE STUDY

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Abstract Biodiversity is essential for human survival and economic well-being and for ecosystem function and stability. Successful strategies for people's participation in preserving biodiversity are lacking. India has a rich tradition of conservation, and with growing inputs from the Government, scientists and NGOs, should provide leadership in developing appropriate methodologies and strategies for biodiversity assessment and conservation. Various tree species in India have been used for medicine, food, timber, fuel, fibers, ornamental, cultural and spiritual purposes. Due to over exploitation for timber and other products, habitat loss, forest clearance, disease and climate change, many tree species are facing endangerment. A range of conservation approaches are required to conserve the rare trees of India, that is, *in situ* conservation, reintroduction, species recovery and *ex situ* conservation. Furthermore, urgent restoration and conservation efforts are needed to improve the conservation status of tree species on the ground. *Ex situ* conservation is an essential step for preventing the extinction of rare and endangered trees. Saplings of 7 endemic, endangered tree species viz *Buchanania barberi* Gamble., *Buchanania lanceolata* Wight., *Artocarpus gomezianus* Wall., ex Trecul subsp. *zeylanicus* Jarret., *Flacourtia montana* Graham., *Syzygium palodense* Shareef, E. S. S. Kumar & Shaju.., *Baccaurea courtallensis* (Wight) Muell. Arg., *Syzygium mundagam* (Bourd.) Chithra., collected from JNTBGRI, Palode on 10-03-2021 attained height of 0.3 m were planted in the pits sized 2.5 height x 2.5 Depth x 2.5 Width (in feet) at S.N. College in Kollam dist. in 2021. The planted area is protected with metallic fencing. The routine watering and manuring were done with the help of teachers and students. The survival rate is high due to better protection and less human disturbance. After 21 months plants had attained an average height of above one meter with a survival rate of 100%. It is the preliminary results for documented trial may do future conservation efforts not only for these species but also for other IUCN Red Listed species of the genus worldwide.

Key Words-Biodiversity, Ex situ conservation, Endemic

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Introduction

Biodiversity mainly refers to the variety and variability of life existing on the planet Earth. The term biodiversity usually refers to the process of measuring the variation at the genetic, species, and ecosystem level. Biodiversity plays a vital role in boosting the ecosystem. The factors responsible for the cause of changes in biodiversity are: pollution, invasive species, overexploitation, and change in climatic conditions. We all need to conserve biodiversity, as it leads to the conservation of essential ecological diversity to preserve the continuity of food chains. *Ex-situ* conservation is the strategy practiced for the preservation of a variety of living species globally. This method involves removing threatened plants from their native environment and relocating them to a designated location where they can be protected and given specialized care.

Materials and methods

The saplings of *Buchanania barberi* Gamble (Anacardiaceae), *Buchanania lanceolata* Wight, (Anacardiaceae), *Artocarpus gomezianus* Wall. ex Trecul subsp. *zeylanicus* Jarrett (Moraceae), *Flacourtia*

Table 1. Reintroduction of seedlings growth during 2021and 2022

Period of growth	Height of sapling	Survival (%)
6 months	0.3m	100
12 months	0.6m	100
21 months	Above 1m	100

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Artocarpus gomezianus Wall. ex Trecul subsp. *zeylanicus* Jarrett (Moraceae),
Baccaurea courtallensis (Wight) Muell. Arg. (Euphorbiaceae)
Buchanania barberi Gamble (Anacardiaceae)

montana Graham. (Flacourtiaceae), *Syzygium palodense* Shareef, E. S. S. Kumar & Shaju., (Myrtaceae), *Baccaurea courtallensis* (Wight) Muell. Arg. (Euphorbiaceae), *Syzygium mundagam* (Bourd.) Chithra (Myrtaceae) sized about 30 cm saplings were collected from JNTBGRI, Palode. Planted the above mentioned saplings planted in the pits sized 2.5 height x 2.5 Depth x 2.5 Width (in feet) at S. N. College, Kollam in March 2021.The planted area is protected with metallic fencing The routine watering, manuring and monitoring was done with the help of teachers and students

Result and Discussion

Planted saplings in the pits sized 2.5 height x 2.5 Depth x 2.5 Width (in feet) at S.N. College, Kollam in March 2021(Table 1). The planted area is protected with metallic fencing. The routine watering, manuring and monitoring was done with the help of teachers and students. These saplings require acclimatization to environmental

conditions for successful growth (Aguraiuja, 2011; Maschinski and Albrecht, 2017). After 1 year saplings attain 0.6m. After 1 year and 9 months (21 months) plants had attained an average height of above 1m with a survival rate of 100%.

This is considered a short-term success, but for long-term success, monitoring should be continued for many years (Silcock et al., 2019). It is the preliminary results for documented trial. May do future conservation efforts not only for these species but also for other IUCN Red Listed species of the genus worldwide. *Ex situ* conservation requires regular assessments on the materials, viability and prompt regeneration, which vary depending on the crop species and their reproductive systems. *Ex situ* conservationists will have to face and strive to resolve these issues in the upcoming ten to fifty years, much alone the entire new millennium. They will be important for the advancement of humanity. To ensure that the world's natural resources are accessible to everyone who needs them, we must work to preserve sections of the natural, or at least the semi-natural environment, not just for living and leisure, but also to ensure that they are preserved for future generations.

Acknowledgement

The authors are thankful to Director, JNTBGRI and Principal S N College, Kollam for providing the necessary facilities and support.

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BIODIVERSITY CHALLENGES AND THREATS; CURRENT SCENARIO

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