Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), a critically endangered endemic species of Kerala

Thesis submitted to the University of Calicut
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by

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CERTIFICATE

This is to certify that the thesis entitled, **"Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), a critically endangered endemic species of Kerala"** is an authentic record of research work carried out by **Mrs. Smitha P. S.** under my supervision in fulfilment of the requirement for the degree of Doctor of Philosophy, in Botany of the University of Calicut. The results embodied in this thesis have not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution. Also certified that the contents of the thesis have been checked using anti-plagiarism database, and no unacceptable similarity was found through the software check.

Thrissur
16 October 2023

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This is to certify that the adjudicators of the Ph.D. thesis of Ms. Smitha P.S, titled "Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), a critically endangered endemic species of Kerala", have not given any directions for corrections or suggestions for change in their reports. The content of the CD is the same as in the hard copy.

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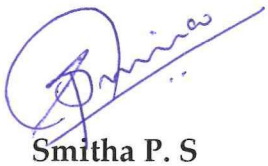
DECLARATION

I, **Smitha P. S.**, hereby declare that the thesis entitled **"Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), a critically endangered endemic species of Kerala"** submitted to the University of Calicut, for the award of the degree of Doctor of Philosophy in Botany is a bona fide record of the original research work carried out by me under the supervision and guidance of Dr Anto P. V, Assistant Professor, Department of Botany, St. Thomas' College (Autonomous), Thrissur and that it has not been submitted earlier either in part or full for the award of any degree/diploma to any candidate of any University.



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Smitha P.S

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ABBREVIATIONS

ft	Feet
° C	Degree Celsius
eV	Electron-volt
ng	Nano gram
µm	Micrometer
mm	Millimeter
µl	Microliter
µg	Microgram
ml	Milliliter
mg	Milligram
mM	Millimolar
M	Molar
kg	Kilogram
gm	Gram
v/w	volume per weight
w/v	Weight per volume
MW	Molecular Weight
bp	Base pair
cm	Centimeter
nm	Nanometer
A°	Angstrom
min	Minute
sec	Second
h	Hour
%	Percentage
∞	Infinite
rpm	Revolutions per minute
kV	Kilovolt
pH	Potential of Hydrogen
IC	Inhibitory concentration
λ	Lambda
α	Alpha
β	Beta
OD	Optical density
RT	Retention Time
W	Watt
FAA	Formalin Acetic acid-Alcohol
EDTA	Ethylenediaminetetraacetic Acid
GC-MS	Gas chromatography Mass Spectrometry

This dissertation is dedicated to my beloved parents, who instilled in me the virtues of perseverance and commitment and relentlessly encouraged me to strive for excellence

ABSTRACT

The objective of this research was to conduct a comprehensive investigation of *Syzygium travancoricum* Gamble (Myrtaceae), an endemic and endangered species of Kerala. In addition, another goal was to provide a taxonomic resolution to the ambiguities regarding the identity of this species due to its close resemblance to other species, *S. stocksii.* The study also aimed to evaluate the medicinal properties of this species. For this, various analytical techniques such as morphological, anatomical, histochemical, phytochemical, molecular and pharmacological methods were used. The study focused on morphologically closely related populations of *S. travancoricum* spread across five districts in Kerala. The morphological and anatomical studies revealed that populations collected from Pookode and Paliyeri Mookambika Kavu showed unique morphological and anatomical characteristics distinct from *S. travancoricum* and were closely related to *S. stocksii*. Histochemical analysis revealed the presence of tannins, lignin, starch and essential oils in various parts of *S. travancoricum*. Using the results of morphological and anatomical studies, the UPGMA phylogram of *S. travancoricum* showed that the plants found in Pookode and Palyeri Mookambika Kavu are closely related to *S. stocksii* and have diverged into a separate clade. Phytochemical analysis of *S. travancoricum* leaf and bark extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenols and terpenoids. Moreover, the extracts of *S. travancoricum* contained high levels of phenolic compounds (240.2 mg GAE/g)and flavonoids. Apart from this, the presence of vitamins, minerals, phenols, anthocyanins, carbohydrates, potassium and calcium was also found in their fruits. The essential oil GC-MS profiling identified that the leaves of *S. travancoricum* are rich in monoterpenoids and sesquiterpenoid compounds such as β-caryophyllene, caryophyllene epoxide, β-geraniol, α-farnesene, and α-selinene, monoterpenoids such as α-pinene, β-pinene, ocimene isomers,

myrcene, β-limonene, and α-terpineol. The Bayesian phylogenetic tree based on the concatenated dataset revealed paraphyletic relationships between two species, *S. travancoricum* and *S. stocksii.* Also, it supported the distinction of *S. travancoricum* as a distinct species from *S. stocksii*. The essential oil of the leaves of *S. travancoricum* effectively prevented the growth of different bacterial and fungal strains. Moreover, the study found that the leaves and bark of this plant are reservoirs of antioxidants, and their aromatic oil and extracts have the potential to work effectively against arthritis, inflammation and diabetes. As part of this study, about 200 trees were conserved through ex situ conservation strategies implemented by establishing green spaces in five grama panchayats of Thrissur, Palakkad and Pathanamthitta in Kerala.

Keywords: Anticancer, Anti-inflammatory, Antimicrobial, DNA barcoding, Ex situ conservation, GC-MS, UPGMA Phylogram, Species reinstatement

സംഗ്രഹം

മിര്ട്ടേസി സസ്യകുടുംബത്തിലെ അംഗവും, കേരളത്തിലെ തദ്ദേശീയവും വംശനാശഭീഷണി നേരിടുന്നതുമായ സൈസീജിയം ട്രാവന്കോറിക്കം എന്ന സസ്യത്തെക്കുറിച്ചുള്ള സമഗ്രമായ വിശകലനം എന്നതായിരുന്നു ഈ ഗവേഷണത്തിന്റെ ലക്ഷ്യം. ഇതോടൊപ്പം, രൂപസാദൃശ്യമുള്ള സൈസീജിയം സ്റ്റോക്സി എന്ന മറ്റൊരു സ്പീഷിസുമായി ഈ സസ്യത്തിനുള്ള വൈജാത്യങ്ങള് കണ്ടെത്തുകയും, ഔഷധഗുണങ്ങള് തിരിച്ചറിയുകയുമായിരുന്നു മറ്റൊരു ലക്ഷ്യം. ഇതിനായി മോര്ഫോളജിക്കല്, അനാട്ടമിക്കല്, ഹിസ്റ്റോകെമിക്കല്, ഫൈറ്റോകെമിക്കല്, മോളിക്യൂലര്, ഫാര്മക്കോളജിക്കല് എന്നീ വിവിധ വിശകലന സാങ്കേതികവിദ്യകള് ഉപയോഗിച്ചു. കേരളത്തിലെ അഞ്ച് ജില്ലകളിലായി വ്യാപിച്ചു കിടക്കുന്ന സൈസീജിയം ട്രാവന്കോറിക്കത്തെ കേന്ദ്രീകരിച്ചായിരുന്നു പഠനം.

സൈസീജിയം സ്റ്റോക്സിക്ക് സമാനമായ രൂപശാസ്ത്രപരമായ സ്വഭാവസവിശേഷതകള് ഉണ്ടെന്ന് രൂപശാസ്ത്ര പഠനവും, കോശഘടന സവിശേഷതകളില് വ്യതിയാനങ്ങള് ഉണ്ടെന്ന് കോശഘടന പഠനവും തെളിയിച്ചു. ഹിസ്റ്റോമെകമിക്കല് വിശകലനത്തില് സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ വിവിധ ഭാഗങ്ങളില് ടാനിന്, ലിഗ്നിന്, അന്നജം, സുഗന്ധ എണ്ണകള് (essential oil) എന്നിവയുടെ സാന്നിദ്ധ്യം കണ്ടെത്തി. മോര്ഫോളജിക്കല്, അനാട്ടമിക്കല് പഠന ഫലങ്ങള് ഉപയോഗിച്ച് നിര്മ്മിച്ച സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ യു.പി.ജി.എം.എ ഫൈലോഗ്രാം, പൂക്കോട്, പാലിയേരി മൂകാംബിക കാവ് എന്നിവടങ്ങളില് കാണപ്പെടുന്ന സസ്യങ്ങള്ക്ക് സൈസീജിയം സ്റ്റോക്സിയുമായി അടുത്ത ബന്ധമുള്ളവയായും, ഒരു പ്രത്യേക വംശമായി വ്യതിചലിച്ചതായും രേഖപ്പെടുത്തി.

സൈസീജിയം ട്രാവന്കോറിക്കം ഇലയുടെയും പുറംതൊലിയുടെയും ഫൈറ്റോകെമിക്കല് വിശകലനത്തില് ആല്ക്കലോയിഡുകള്, ഫ്ളേവനോയിഡുകള്, സാപ്പോണിനുകള്, ടാന്നിനുകള്, സ്റ്റീറോയിഡുകള്, ഫീനോളുകള്, ടെര്പിനോയിഡുകള് എന്നിവയുടെ സാന്നിദ്ധ്യം കണ്ടെത്തി. മാത്രമല്ല, സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ സത്തുകളില് ഫീനോളിക് സംയുക്തങ്ങളും (240.2 mg GAE/g), ഫ്ളേവനോയിഡുകളും ഉയര്ന്ന അളവില് അടങ്ങിയിട്ടുണ്ടെന്ന് പഠനത്തില് കണ്ടെത്തുകയും ചെയ്തു. ഇതുകൂടാതെ ഇവയുടെ ഫലങ്ങളില് വിറ്റാമിനുകള്, ധാതുക്കള്, ഫീനോളുകള്, ആന്തോസയാനിനുകള്, കാര്ബോഹൈഡ്രേറ്റുകള്, പൊട്ടാസ്യം, കാത്സ്യം എന്നിവയുടെ സാന്നിദ്ധ്യവും കണ്ടെത്തി. പ്രധാന രാസസംയുക്തങ്ങളായ β-കാരിയോഫില്ലിന്, കാരിയോഫില്ലിന് എപോക്സൈഡ്, β-ജെറാനിയോള്, α-ഫാര്നെസീന്, α-സെലിനൈന്, α-പൈനിന്, β-പൈനിന്, ഒസിമിന്, മൈര്സീന്, β-ലിമോണീന്, α-ടെര്പിനോള് തുടങ്ങിയ മോണോടെര്പെനോയിഡുകളും സെസ്കിടെര്പിനോയിഡ് സംയുക്തങ്ങളും സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ ഇലകളില് സമൃദ്ധമായി അടങ്ങിയിട്ടുണ്ടെന്ന്

സുഗന്ധതൈലം (essential oil) ജി.സി.എം.സ് പ്രൊഫൈലിങ്ങില് തിരിച്ചറിഞ്ഞു. കോണ്കാറ്റനേറ്റഡ് ഡാറ്റാസെറ്റിനെ അടിസ്ഥാനമാക്കിയുള്ള ബയേസ്യന് ഫൈലോജെനെറ്റിക് ട്രീ, സൈസീജിയം ട്രാവന്കോറിക്കം, സൈസീജിയം സ്റ്റോക്സി എന്നീ രണ്ട് സ്പീഷീസുകള് തമ്മിലുള്ള പാരഫൈലെറ്റിക് ബന്ധങ്ങള് വെളിപ്പെടുത്തുകയും സൈസീജിയം സ്റ്റോക്സിയില് നിന്ന് ഒരു പ്രത്യേക സ്പീഷിസായി സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ വ്യത്യാസത്തെ പിന്തുണയ്ക്കുകയും ചെയ്തു.

സൈസീജിയം ട്രാവന്കോറിക്കത്തിന്റെ ഇലകളുടെ സുഗന്ധ തൈലം (essential oil) ബാക്ടീരിയകളുടേയും ഫംഗ്സുകളുടേയും വളര്ച്ചയെ ഫലപ്രദമായി തടയാന് കഴിവുള്ളവയാണ്. മാത്രമല്ല, ഈ സസ്യത്തിന്റെ ഇലയും, പുറംതൊലിയും ആന്റിഓകസിഡന്റുകളുടെ കലവറയാണെന്നും, ഇവയുടെ സുഗന്ധതൈലത്തിനും സത്തുകള്ക്കും അര്ബുദത്തിനും, വീക്കത്തിനും, പ്രമേഹത്തിനുമെതിരെ ഫലപ്രദമായി പ്രവര്ത്തിക്കാന് കഴിവുണ്ടെന്നും ഈ പഠനത്തില് കണ്ടെത്തി. കേരളത്തിലെ തൃശ്ശൂര്, പാലക്കാട്, പത്തനംതിട്ട എന്നിവിടങ്ങളിലെ അഞ്ച് ഗ്രാമപഞ്ചായത്തുകളില് ഹരിതയിടങ്ങള് സ്ഥാപിച്ച് നടപ്പിലാക്കിയ എക്സ്-സിറ്റു സംരക്ഷണ പദ്ധതിയിലൂടെ ഏകദേശം 200 മരങ്ങളുടെ സംരക്ഷണവും ഈ പഠനത്തിന്റെ ഭാഗമായി നടപ്പിലാക്കി.

Chapter 1

Introduction

CHAPTER 1 INTRODUCTION

Syzygium is the largest genus of flowering plants in Myrtaceae, represented by more than 1200 species worldwide. They are distributed in the tropical regions of Asia, Africa, Australia, and the southwestern Pacific (Parnell *et al*., 2007). However, Australia and Southeast Asia have the greatest diversity of *Syzygium* (Parnell *et al*., 2007; Govaerts *et al*., 2008). *Syzygium* was ranked 16th among the 57 largest genera of flowering plants by Frodin (2004), but according to Parnell *et al*. (2007), it could rank higher within the top 10. This is because many previously described species have yet to be transferred to *Syzygium,* and many new species have yet to be discovered. With a high rate of endemism, the *Syzygium* genus is found exclusively in forests, particularly evergreen and shola forests in the Western Ghats and north-eastern region. Even though *Syzygium* forms a very significant part of the endemic flora of India, it has not gained much attention from taxonomic, molecular, phytochemical, pharmacological, and conservation perspectives compared with other angiosperm families and genera.

1.1 Distribution of *Syzygium* species

Syzygium is predominantly of trees and is found in tropical regions with high diversity in Asia (Hussin *et al*., 1992). This paleotropical genus is primarily found in southeastern and southern Asia, southern China, Australia, Malesia, and New Caledonia. *Syzygium* is predominantly found in tropical and subtropical vegetation, including lowland to montane rainforests, swamps, savannahs, and limestone forests. *Syzygium* is an important food source for birds, insects, and small and large mammals (Parnell *et al*., 2007) because it is one of the most common tree genera in the forest ecosystem.

1.2 Distribution of *Syzygium* species in India

Myrtaceae of India was last revised by Duthie (1878-79) in Hooker's Flora of British India with 11 genera and 134 species. He classified the family Myrtaceae into three tribes, namely, Leptospermae (4 genera), Myrtaceae (5 genera) and Lecythideae (2 genera). Nair and Henry (1983) documented 16 genera and 107 species belonging to the family Myrtaceae Agonis (DC.) Sweet, *Angophora* Cavanilles, *Baeckea* L., *Callistemon* R.Br., *Eucalyptus* L.Herit., *Eugenia* L., *Feijoa* O. Berg., *Leptospermum* J.R. & G. Frost., *Melaleuca* L., *Meteoromyrtus* Gamble, *Myrtus* L., *Pimenta* Lindl., *Psidium* L., *Rhodomyrtus* (DC.) Rchb., *Syncarpia* Ten., *Syzygium* Gaertn. and *Tristania* R.Br. in the Flora of Tamil Nadu. However, the genus *Syzygium* Gaertn. reported a higher number of species.

Shareef and Kumar (2021) reported a total of 102 species of *Syzygium*, of which 44 are endemic to India. The Western Ghats and Northeastern parts of India are the two major centres of *Syzygium*, having 54 species with 28 endemics (Shareef & Kumar, 2021). The northeastern regions of India, including Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, stand the second highest in species diversity with 48 species and eight endemics, followed by Andaman-Nicobar Islands, which harbours 22 species with four endemics. *S. alternifolium*, *S. munro, and S. salicifolium* are four endemic species found in the northern and southern states of India. Moreover, *S. cumini* (L.) Skeels is the only known species occurring naturally and under cultivation in most parts of India. Similarly, other introduced species of *Syzygium,* such as *S. aqueum, S. aromaticum, S. jambos*, *S. samarangenese*, etc., are also widely cultivated in several states of India. About 75 species of *Syzygium* are currently enlisted as critically endangered and 104 species as endangered globally as per the recent IUCN Red List

category (IUCN, 2022). A few Indian endemic species, such as *S. palaghatense* and *S. travancoricum,* are enlisted as critically endangered species, whereas *S. caryophyllatum*, *S. andamanicum, S. bourdillonii, S. microphyllum, S. myhendre, S. parameswarani and S. stocksii* are enlisted as endangered species (Shareef & Kumar, 2021).

1.3 Medicinal significance of *Syzygium* species

Since immemorial, the family Myrtaceae, particularly the genus *Syzygium* Gaertn. and *Eugenia* L., have gained considerable interest due to their multiple benefits to mankind. The most economically significant member of this family is the clove (*S. aromaticum* (L.) Merr. & L. M. Perry). In addition to treating inflammatory diseases, clove oil possesses antioxidant properties and antimicrobial activities. Many species of *Syzygium* have edible fruits, including *S. aqueum* (Burm. f.) Alston, *S. cumini* (L.) Skeels, *S. jambos* (L.) Alston, and *S. malaccense* (L.) Merr. & L. M. Perry and are widely used in the vine industry. Moreover, the *Syzygium* species are rich repositories of phytochemicals and antioxidants, contributing to their pharmacological properties. Most of the species are rich in volatile oils with immense medicinal properties. Many fruits of the family have a rich history of use as edibles and traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world.

Syzygium travancoricum Gamble is an endemic, critically endangered and endemic tree species (IUCN, 2022) confined to the marshy swamps of the Southern Western Ghats. *S. travancoricum* is a medium-sized evergreen tree popularly known by the names, 'Poriyal', 'Kollinjaval', 'Vaathamkolli maram', 'Kulirmaavu' or 'Thenmaavu'. The bark of the tree is longitudinally fissured and greyish-brown in colour. Leaves are simple, petiolate and large.

Flowers are small, bisexual and white. Fruit is a berry with a deep purple colour containing a single seed. *S. travancoricum* is known for its medicinal value. Traditionally, this plant species has been used by local people to cure diabetes and arthritis. However, there are no reports on the phytochemical or pharmacological properties of this species to date. Bourdillon (1894) discovered *S. travancoricum* from the swampy lowlands (altitude less than 65 m) of Travancore in South Kerala. Later, Gamble (1919) described it in the Kew Bulletin and the Flora of the Presidency of Madras.

S. travancoricum has a unique habitat preference and is distributed in the evergreen to semi-evergreen forests and a few sacred groves in Thiruvananthapuram, Kollam, Pathanamthitta, Alappuzha and Thrissur districts (Sasidharan, 2004). They have also been reported from freshwater bodies or 'Myristica swamps' dominated by Myristicaceae members, especially *Myristica magnifica* and *Gymnacranthera farquhariana*. Krishnamoorthy (1960) first reported Myristica swamps from the Travancore region of South Western Ghats. As special abiotic conditions are prerequisites for developing Myristica swamp forests, these habitats have become highly restricted and fragmented (Chandran & Sivan, 1999).

Most of the existing populations of *S. travancoricum* have been reported from the marshy river sides and sacred groves in the southern Western Ghats (Sreekumar *et al*., 2020). The unique habitat preference of this species justifies the role of sacred groves in conserving biodiversity and ecological stability. 465 individuals of *S. travancoricum* are currently recorded from Kerala, with a maximum number of trees in Kalasamala Biodiversity Heritage Site, Thrissur, and Myristica swamps in Kulathupuzha. However, the plant populations are facing severe threats due to habitat modification, over-exploitation, pollution and invasion of exotic species (Sreekumar *et al*., 2020). Hence, urgent

interventions are required to restore the germplasm through efficient habitat management and ex situ conservation strategies.

1.4 Rationale of the present study

The genus *Syzygium* P. Browne ex Gaertn. consists of approximately 1200-1800 species worldwide (Parnell *et al*., 2007; Govaerts *et al*., 2008), of which 102 species are presently known to exist in India (Shareef & Kumar, 2021). The Western Ghats and the North Eastern region of India contain the greatest number of endemic species of any region (Shareef & Kumar, 2021). *Syzygium* is one of the most difficult genera to identify due to the scarcity of diagnostic morphological characteristics (Parnell *et al*., 2007; Craven, 2001; Schmid, 1972). In addition, the use of phenotypic classification to infer genetic relationships between genotypes in *Syzygium* remains controversial, and accurate species identification is essential for both resource utilization and genetic resource conservation.

Syzygium travancoricum Gamble is an endemic tree species of southern Western Ghats categorized as "Critically Endangered" according to the IUCN Red List category (IUCN, 2022). According to Ray (2011), the seedlings of this species are found in three sacred groves in Karnataka: Devaravattibana (Mattigar), Kadkod Choudammabana (Aralihonda), and Choudammabana (Dugdimane). It has also been reported from the freshwater Myristica swamps of Kaan forests in Uttara Kannada (Chandran *et al*., 2008, 2010), Kulathupuzha (Robi, 2009), and a relic Myristica swamp at Brahma Karmali, Valpoi Taluk in Goa (Prabhugaonkar *et al*., 2014). This species is also found in Pandiar, Nadugani, Tropical Gene Pool Garden, Kilnadugani, and Poonoor forest areas in Nadugani Village of Gudalur Taluk in Nilgiri District (Udhayavani *et al*., 2013) and Megamalai Wildlife Sanctuary (Karuppusamy & Ravichandran, 2016) in Tamil Nadu. The natural population of

S. travancoricum are facing a significant threat due to habitat destruction and poor regeneration potential (Sreekumar *et al*., 2020).

Recent misinterpretation and synonymization of this species with *S. stocksii* (Byng *et al*., 2015) have led to differing opinions among taxonomists regarding the taxonomic status of this species. Some taxonomists still consider it a distinct taxon (Sujanapal & Kunhikannan, 2017), while others consider it a synonym of *S. stocksii* (Shareef & Kumar, 2021). Hence, it is crucial to contribute good diagnostic characteristics for resolving the existing taxonomic bottlenecks among these species. Considering the critical need for a taxonomic resolution of these two species, the current study was conducted based on live and herbarium specimens of this difficult complex from the southern Western Ghats to resolve the conflicting viewpoints.

Moreover, there is a dearth of literature regarding the morpho-anatomical, histochemical, phytochemical and pharmacological perspectives of *Syzygium*, especially *S. travancoricum*. Due to their numerous medical applications in the Iranian, Ayurvedic, and Unani systems of medicine, only two species, *S. aromaticum* and *S. cumini*, have garnered the most attention despite widespread interest in and extensive scientific investigation of this genus (Ayyanar *et al*., 2013; Cock & Cheesman, 2018; Srivastava *et al*., 2014). Despite the initiatives taken by taxonomists throughout the past few decades, the correct assessment of species diversity still needs to be completed. While the conservation efforts are confined to protecting certain areas in situ or relocating a few endangered species to Botanic Gardens and Wildlife sanctuaries, no serious work is done to investigate the existing bottlenecks of endangered or rare species to help them grow and address their issues. Hence, it is imperative to perform bioprospecting, product development, and conservation of these species before they go extinct.

Hence, this study was chosen to characterize the endemic endangered species *S. travancoricum* by evaluating its morphological, anatomical, phytochemical, molecular and pharmacological potential. The morphological and anatomical studies will complement each other in species delineation. Morphological features or macroscopic characteristics of various plant organs, like bark, stem, leaf, inflorescence, flower, fruit and seed, are significant in identifying crude drugs. Furthermore, secondary metabolites such as flavonoids and phenols generally produced by plants for their defense mechanism have been implicated in the therapeutic properties of medicinal plants. The present study also aims to provide an in-depth analysis of the phytochemical elements and pharmacological effects of *S. travancoricum* to identify the potential use of this species in evidence-based medicine beyond their traditional medicinal applications. By conducting this evaluation, the gap between traditional knowledge and evidence-based research can be bridged, paving the way for future advancements in medicine. Apart from this, being an endemic endangered species, the study also aims to develop ex situ conservation strategies for germplasm conservation.

Objectives of the Study

- To evaluate the morphological characteristics of *S. travancoricum*
- To evaluate the anatomical and histochemical aspects of *S. travancoricum*
- To determine the phytochemical characteristics of *S. travancoricum*
- To understand the molecular phylogeny of *S. travancoricum*
- To determine the pharmacological potential of *S. travancoricum*
- To develop ex situ conservation strategies of *S. travancoricum*

Chapter 2

Review of Literature

CHAPTER 2 REVIEW OF LITERATURE

This review conducted a detailed literature search about all aspects of *Syzygium* using bibliographic databases such as PubMed, ScienceDirect, Wiley Online Library, SpringerLink, and Google Scholar. The search was carried out within the last ten years up to March 2023 for parameters including taxonomy, anatomy, phytochemistry, antimicrobial, antioxidant, anti-inflammatory, anticancer and anti-diabetic research. The literature search used the terms '*Syzygium*', '*Syzygium* taxonomy', '*Syzygium* essential oil', 'Bioactivities of *Syzygium*', and "phytoconstituents".

2.1. Role of morphological characters in the taxonomy of *Syzygium*

The genus *Syzygium* Gaertn. comprises trees with thick, granular bark and typically glabrous twigs. The union of the lateral nerves forms a clear or faint intramarginal vein; leaves are opposite, entire, pinni-nerved, and generally gland-dotted. Flowers are bisexual and arranged as panicles or axillary corymbose cymes; the calyx tube is hemispherical, globose, or turbinate above the ovary; calyx lobes 4 or 5, ovate to suborbicular, imbricate; petals 4 or 5, orbicular, pellucid-glandular; stamens numerous; anthers globose; filaments inflexed in bud; staminal disc broad or absent; ovary inferior, two-celled; few to many ovules in each cell; style 1, subulate; stigma simple; fruit is a berry, unilocular with few seeds.

In Hooker's Flora of British India, Duthie (1878-1879) classified the Indian specimens of this complex under Eugenia, with a total of 131 species divided into three sections (*Jambosa* with 31 species, *Syzygium* with 77 species, and *Eugenia* with 22 species). Wight (1842, 1846), Beddome (1872, 1874), and Duthie (1878-1879) described 21 of these 131 species from southern India. Later, researchers such as Gamble (1919) classified the south Indian

specimens of *Eugenia* into three distinct genera, including *Eugenia*, *Jambosa* DC., and *Syzygium*, along with ten new species. *Syzygium* is the largest genus of the 645 known tree species in the Western Ghats (Nayar *et al*., 2006), with 48 taxa, of which 27 are endemic to the region. According to Nayar *et al*. (2006), the Kerala portion of the Western Ghats is home to 39 taxa, including 19 endemic species. More than 1200 *Syzygium* species occur worldwide in tropical regions of Asia, Africa, Australia, and the southwestern Pacific (Parnell *et al*., 2007; Govaerts *et al*., 2008). The evergreen and shola forests in the Western Ghats and northeastern region contribute to high endemism in *Syzygium*.

Several new species were added to the peninsular Indian flora subsequently (Mohanan, 1996; Sheeba *et al*., 2003; Murugan & Manickam, 2004; Viswanathan & Manikandan, 2008; Shareef *et al*., 2010, 2012; Sujanapal *et al*., 2013). A new species, *S. dhaneshiana* was discovered and described from the southern Western Ghats of Kerala (Ratheesh *et al*., 2014). The bark of this species was greyish-white with a pale pinkish-brown blaze, yellowish-white flowers, goblet-shaped hypanthium and subglobose fruits, which were the characteristics of this species. Ravikumar (1999) reported two new species from the southern Western Ghats, *S. sriganesanii* K. Ravik. & V. Lakshm. and *S. zeylanicum var. megamalayanum*. *S. lanceolatum* is closely related to *S. sriganesanii* but also possessed distinct characteristics such as quadrangular branchlets, sessile leaves with an oblong-elliptic shape, and obconical fruits. Sujanapal *et al*. (2014) identified a new species of *Syzygium* Gaertn., named *S. sahyadricum* from the Western Ghats. This new species had peculiar characteristics, such as pale yellow-coloured tender leaves with horizontal secondary nerves. Based on the exhaustive research and analysis, the species *S. spathulatum* Thwaites. was renamed and confirmed to be *S. sahyadricum*. *S. parameswaranii* and *S. travancoricum* are the species only found in

Travancore. On the other hand, *S. bourdillonii* was a species that was only found in Kollam, and *S. palghatense* Gamble was a species that was only found in the Palakkad district (Shareef *et al*., 2013).

Shareef *et al*. (2014) described a new species of *Syzygium* Gaertn., *S. munnarensis* from Idukki, southern Western Ghats. Viswanathan and Manikandan (2008) described *S. agastyamalayanum*, as exclusive to the Kalakkad-Mundanthurai Tiger Reserve and considered a strict endemic. The study also assessed the conservation status of the species and determined it to be in a state of critical endangerment.

Andaman and Nicobar Islands are also an important centre of *Syzygium* with 17 species (Pandey & Divakar, 2008), with the addition of *S. hookeri* M.V. Ramana, Chorghae & Venu and *S. sanjappaiana* M.V. Ramana (Ramana *et al*., 2014). Many species are identified only by their type specimens, and some of these specimens have been relocated during floristic studies in some protected areas (Nayar *et al*., 2006). Shareef *et al*. (2013) discovered *S. chemunjianum* from the Agasthyamalai biosphere reserve. Specific characteristics possessed by this species include cream-coloured bark, elliptic leaves, a 2-tiered intramarginal nerve, and globose purple-coloured fruits. In addition, a new *Syzygium* Gaertn., *S. palodense*, was also discovered in the southern Western Ghats of Kerala. The species differed from *S. densiflorum* and *S. rubicundum* because they have slightly winged branchlets, sub-opposite or alternate leaves, thick and robust petioles, and dark purple obovoid fruits.

Despite its species diversity and abundance, *Syzygium* has received little attention compared to other angiosperm genera. Moreover, *Syzygium* is a significant component of India's endemic flora but has not received the attention it deserves from taxonomic, phytogeographic, and conservation perspectives (Sujanapal & Kunhikannan, 2017). Taxonomists used a

combination of inflorescence and flower characteristics, particularly the calyx tube and perianth, to determine the species of *Syzygium*. However, because of the overlapping of characters, one or a few character differences cannot be expected to differentiate the taxa in this genus. Moreover, given the current state of ignorance regarding *Syzygium* and *Eugenia*, unrestrained speculation by various trivial characters can only result in errors. Some of the recently described taxa reflected these ambiguities in their identities. Due to insufficient character sets, many researchers have reduced these taxa while revising a species or section (Sujanapal & Kunhikannan, 2017).

Byng *et al*. (2015) described the typification and nomenclatural notes on 44 taxa belonging to the family *Myrtaceae.* These taxa include members of the genera *Eugenia* L. and *Syzygium* Gaertn. documentation was done on nomenclatural notes and lectotypification of six species of *Eugenia* L. and twenty-nine species of *Syzygium* Gaertn. In that, *S. calcadense* (Bedd.) Chandra. was changed as *E. calcadensis* Bedd. and *M. wynadensis* (Bedd.) Gamble as *E. wynadensis* Bedd. It was determined that *S. megacarpum* was a basionym for *S. coarctatum*. Similarly, *S. microphyllum* was considered to be synonymous with *S. gambleanum*. Based on morphological characteristics such as the length of the petiole, shape of the leaves, and structure of the inflorescence, Byng *et al*. (2015) considered *S. travancoricum* Gamble as a synonym of *S. stocksii* (Duthie) Gamble. Similarly, *S. monatanum* was treated as a homonym of *S. tamilnadensis* Rathakr. & Chithra. based on its morphological peculiarities.

Later, Sujanapal *et al*. (2013) described a species from the Agasthyamala hills similar to *S. montanum* Gamble and named it *S. sasidharanii*. This tree had clusters of large yellow fruits and tender leaves that were reddish brown. Similarly, *S. zeylanicum* var. *lineare*, is distinguishable from *S. zeylanicum* (L.) DC. *var. megamalayanum* by having oblong-lanceolate leaves, a short petiole,

and a truncate calyx tube. Both *S. hookeri* and the *S. sanjappaiana* species were found in the Saddle Peak National Park of the Andaman and Nicobar Islands, a remote island off the coast of India (Venkat Ramana *et al*., 2014).

Shareef and Rasiya (2015) described the lectotype and status of *S. myhendrae* (Bedd. ex Brandis) Gamble from the southern Western Ghats. Shareef *et al*. (2013) discovered an endangered species, *S. ramavarma* (Bourd.) Chithra, of south Kerala. The young leaves of this species were purple and grey; the tree can sometimes produce adventitious roots from the main trunk, and the fruits can be consumed.

Ramasubbu (2018) discovered a new species of *Syzygium*, *S. bharathii* Ramas, from Tamil Nadu. Even though *S. benthamianum* (Wight ex Duthie) Gamble and *S. agastyamalayanum* are close relatives of this species, they differ in glabrous leaves with a wavy margin, a quadrangular peduncle, large flowers, a narrow pyriform hypanthium, and ovate-obovate seeds. Furthermore, Sreekala *et al*. (2019) described a new tree species, *S. ponmudianum*, from the Ponmudi hills of the Agasthyamalai Biosphere Reserve in Kerala. This species is similar to *S. benthamianum* and *S. myhendrae* but is distinguishable by its reddish-brown coloured cylindrical stem, large leaves with 22-29 pairs of secondary veins, deltoid and persistent calyx lobes, pyriform hypanthium, deep purple berries with ovate seeds. An evergreen tree species, *S. claviflorum* was discovered in the Agasthyamalai Biosphere Reserve in Kerala (Shareef *et al*., 2020). This species was previously reported from the Indo-Malaysian subkingdom and North Eastern Australia. Only Sikkim, Khasi Andaman, and Nicobar Island in India were reported to have the plant *S. claviflorum.* Still, its range has been extended to include southern parts of the Western Ghats. This species shared some characteristics in common with *S. lanceolatum* (Lam.) Wight & Arn., such as the shape of its crown and foliage.

2.2. Role of anatomical and histochemical characters in the taxonomy of *Syzygium*

According to the literature search, only very few studies have been carried out on the anatomical and histochemical aspects of *Syzygium*. One of the studies conducted by Kantachot *et al*. (2007) confirmed the importance of anatomical characters to identify taxa at the generic level among the species of *Syzygium*. The study investigated 11 genera and 28 taxa of members of Thai Myrtaceae. For the identification of species, characters like unifacial or bifacial leaves, midrib shape, petiole, margin, hypodermis, the shape of the vascular bundle at midrib and petiole, presence of bundle sheath extension, presence and absence of trichomes, types of stomata, epidermal cell wall, number of spongy layers, absence and presence and absence of scleroid at midrib and also the types of crystals were selected. The detailed analysis identified three types of stomata, anomocytic, paracytic and anisocytic, from all the species.

Similarly, the leaf anatomy of 44 species from the subfamily Myrtoideae was studied and identified by Cardoso *et al*. (2009). The percentage of palisade parenchyma, occurrence of an extension of vascular bundle sheath, absence or presence of adaxial hypodermis and position and shape of the vascular system in the midrib were some characteristic anatomical features recorded in all the genera. Hassan and El-Awadi (2013) described several applications of histochemistry in plant research, which include the detection and localisation of cellular components of active constituents such as protein, lipids, and carbohydrates, as well as a range of ionic elements occurring in cell solutions. Sajwan *et al*. (2014) revealed the presence of oil glands and clusters of calcium oxalate crystals in the parenchyma cells of the hypanthium of *S. aromaticum*. They later verified a positive test on the glands containing oil globules with red colouration when stained with Sudan III.

2.3. Role of Phytochemical characters in the taxonomy of *Syzygium*

2.3.1. Role of essential oil profiling in the taxonomy of *Syzygium*

As the conventional system of classification based on the analysis of morphological characters does not provide adequate data for identifying the systematic status of a taxon, phytochemical characters are considered a major tool in delineating taxonomic disputes. Moreover, phytochemicals significantly contribute to identifying the species and its classification. The composition of phytoconstituents in each plant species will help to determine the genus and species. Also, their structural configuration can describe the variations that occur in the chemical constituents.

The essential oil composition of leaves of *S. aromaticum* (L.) Merr. & L.M.Perry was identified by Raina *et al*. (2001). The dried leaves yielded about 4.8% of oil, and the compounds were identified with GC and GC-MS. The chemical analysis revealed about 16 compounds, and eugenol (94.4%) was identified as the major compound and β-caryophyllene (2.9%) as the second major compound. Noudogbesii *et al*. (2008) isolated the essential oils from dried leaves of *S. guineense* DC. The main constituents identified were α-humulene (39.5%), β-caryophyllene (20.1%), citronellyl pentanoate (15.2%), cis-calamenen-10-ol (14%), α-cadinol (12.7%), epi-α-cadinol (9.8%), viridiflorol (7.5%), δ-cadinene (7.5%) and caryophyllene oxide (7%).

The variation in the chemical composition of essential oils isolated from the leaves of *S. jambos* (L.) Alston was studied by Rezende *et al*. (2013). About 62 compounds were identified, and the dominant components are (E)-caryophyllene, α-humulene, α-zingiberene, hydroxytoluene butylated, caryophyllene alcohol, caryolan-8-ol, caryophyllene oxide, thujopsan-2-α-ol and n-heneicosane. In most aromatic plants, the variations in the composition

of chemical constituents present in the volatile oils are used to identify the species. Razafimamonjison *et al*. (2014) compared the chemical composition of essential oil of the stem, leaf and bud of *S. aromaticum* (L.) Merr. & L.M. Perry from Madagascar. A total of 121 samples of essential oil were isolated from stems (44 samples), leaves (32 samples) and buds (45 samples) and from that 10 phytoconstituents were identified and quantified. Eugenol was identified as the dominant constituent which showed a higher percentage in stem (87.52–96.65%) followed by leaf (75.04–83.58%) and bud (72.08–82.36%). β-caryophyllene was identified as the second major compound of leaf and stem, while in bud, eugenol acetate was the second major compound.

Rameshkumar *et al*. (2015) studied the essential oil composition of six species of *Syzygium* from the Western Ghats. The essential oil from the leaves of *S. arnottianum* Walp., *S. caryophyllatum* (L.) Alston, *S. hemisphericum* (Wight) Alston, *S. laetum* (Buch. Ham.) Gandhi, *S. lanceolatum* (Lam.) Wight & Arn and *S. zeylanicum* (L.) DC. var. *zeylanicum* were isolated, in which total of 57 compounds was detected through GC-MS. Among these, sesquiterpenoids were the dominant compound and caryophyllene was reported in all *Syzygium* spp. Caryophyllene and caryophyllene oxide were reported in five species; however, they were absent in *S. laetum* (Buch. Ham.) Gandhi. Conversely, α-farnesene (21.5%) and α- amorphene (12.1%) were the major compounds identified in *S. laetum* (Buch. Ham.) Gandhi.

The essential oil composition of leaves of *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. was identified by Siddique *et al*. (2017). By the GC-MS analysis, 28 compounds were detected, which mainly consisted of aromatic compounds (84.5%) followed by monoterpenoids (4.01%) and monoterpene hydrocarbons (1.2%). However, sesquiterpenoids and sesquiterpene hydrocarbons were reported in very small amounts (0.21% and 0.23%). Based on the geographical

distribution, unique climatic conditions, and environmental conditions, the composition of essential oil has shown variation in its concentration (Verma *et al*., 2015; Siddique *et al*., 2017).

2.3.2. Role of Phytochemical compounds in the taxonomy of *Syzygium*

An abundance of tannins and flavonols characterizes the family Myrtaceae as significant chemical constituents, of which the myricetin derivatives are frequently reported (Amaral *et al*., 2001). Several chemical investigations on the genus *Syzygium* have revealed the presence of triterpenes (Djoukeng *et al*., 2005; Kuiate *et al*., 2007), tannins, flavonoids (Vaishnava *et al*., 1992; Kuo *et al*., 2004), chromone derivatives (Tanaka *et al*., 1993), phenylpropanoids (Miyazawa & Hisama, 2003; Tanaka *et al*., 1993), and phloroglucinols derivatives (Zou *et al*., 2006). A monolignan was identified in *S. grijsii*, which showed chemotaxonomic significance (Miyazawa & Hisama, 2003). Phenylpropanoid isolated from *S. aromaticum* is the precursor of phenylpropanoids 9, 10 and 11 isolated from *S. grijsii*, showing that the two species are closely related.

The previous chemical investigation has demonstrated the presence of triterpenes, hydrolysable tannins, anthocyanins, flavonoids, chromone derivatives, phenylpropanoids, and phloroglucinols derivatives in the genus *Syzygium* (Reynerston *et al*., 2008; Ayyanar & Subash-Babu, 2012). Yao *et al*. (2013) reported the occurrence of different tetralin-type lignans, 8, 40-oxyneolignane and monolignans in the species *S. grijsii*. They suggested that this group of compounds could be used as taxonomic characters.

Hu *et al*. (2020) isolated two new oleanane triterpenoids and one known analogue from the leaves of *S. samarangense* (Blume) Merr. & L.M. Perry collected from Xishuang Banna Prefecture, Yunnan Province, China, which

not only provided new evidence for the chemical diversity of *Syzygium* plants but also served as potential chemotaxonomic markers for this species. Zhang *et al*. (2020) isolated a new monocyclic diterpenoid with three known diterpenoids and five sesquiterpenoids from the twigs and leaves of *S. fluviatile*, which was considered the chemotaxonomic makers of *S. fluviatile*.

2.4 Role of Molecular characters in the taxonomy of *Syzygium*

As per the literature, very few studies have been conducted on the molecular taxonomy and phylogeny of *Syzygium* to date. Understanding the phylogeny of *Syzygium* is hindered by the large size of the genus, the global distribution of its species, and the absence of systematic revision and monographic studies on the *Syzygium–Eugenia* group in its major centres of diversity (Nair & Rana, 2017). Wilson *et al*. (2001) reported that the fleshy fruited taxa were not associated with the monophyletic group, and the *mat*K analysis revealed that *Syzygium* Gaertn. and *Acmena* DC. had diverged independently from Myrtoideae. Lucas *et al*. (2005) studied the phylogenetic pattern of fleshy-fruited genera of the family Myrtaceae by sequencing the *psbA-trnH* region of plastid DNA and the ITS region of nuclear DNA. The combined analysis of nuclear and plastid DNA revealed that subtribe Myrciinae was monophyletic, whereas Myrtinae and Eugeniinae were polyphyletic.

The microsatellite loci of *Psidium guajava* L. were isolated and characterized by Risterucci *et al*. (2005). About 23 nuclear simple sequence repeat (SSR) loci were characterized from the species, and all the loci were polymorphic. Wilson *et al*. (2001) did the cladistics analysis of some groups in Myrtaceae using *mat*K sequence data. From the study, 11 clades were identified based on molecular data. Molecular phylogenetic data have been used as a valuable tool for resolving the taxonomic problems within Myrtaceae when morphological data are insufficient. The phylogenetic analysis of the

chloroplast genome can be used to identify higher relationships between species.

Biffin *et al*. (2006) studied the relationship among the group *Syzygium* Gaertn. by using parsimony and Bayesian analysis of cpDNA sequences. For the analysis, *mat*K and *ndh*F genes were used along with *rpl*16 intron. The study separated and validated the groups like *Acmena* DC., *Acmenosperma* Kausel, *Cleistocalyx* Blume, *Pilocalyx* and *Waterhousea* B. Hyland. The ovular, placental and seed morphological studies helped to identify evolutionary relationships among *Syzygium* Gaertn.

Biffin *et al*. (2010) analyzed the inter-tribe relationship based on *mat*K and ITS and observed that the data would support the modern classification of *Myrtaceae*. Based on the evolutionary relationship, an infrageneric classification was proposed for *Syzygium* by Craven and Biffin (2010). The evolutionary relationship was analyzed through plastid and nuclear DNA sequence data, and it was also supported by morphological evidence. The study resulted in six subgenera and seven sections of *Syzygium* Gaertn. The preliminary phylogenetic analysis of the Neotropical species of *Eugenia* L. was conducted by Mazine *et al*. (2014). The DNA sequence data from nuclear (*ITS, ETS*) and Plastid (*psb*A-*trn*H) genomes were used for the study, and a total of 70 samples were used for the analysis. Nine clades were recognized as morphologically identifiable groups within the genus *Eugenia* L. and were designated as two genera, *Eugenia* L. and *Myrcianthes* O. Berg. *Syzygium* Gaertn. was included within a heterogeneous group of species with unresolved connections.

Thornbill (2015) interpreted the distribution of the family Myrtaceae using molecular phylogeny. A total of 88 genera and 202 subfamilies of *Myrtaceae* of Gondwana origin were selected for the phylogenetic study, from which 22

sister groups were observed as geographically different and up to 6 groups resulted as the product of vicariance. Jo *et al*. (2016) sequenced the complete plastome of *P. guajava* L. The plastome contained 112 genes, including 78 protein-coding genes, 39 tRNA genes and four rRNA genes. The phylogenetic analysis of the study by the bootstrap method showed that *P. guajava* L. is a sister group of *Eugenia uniflora* L.

Vasconcelos *et al*. (2017) analyzed the phylogeny of the tribe Myrteae with 115 taxa from 46 genera by one nuclear (ITS) and seven chloroplast DNA regions. The chloroplast DNA includes *psb*A, *trn*H, *mat*K, *ndh*F, *trn*l-*trn*F, *trn*Q-*rps* 16, *rpl* 16 and *rpl* 32-*trn*L. The study analyzed the maximum likelihood and Bayesian inference for phylogeny construction. Three major clades were obtained from the tribe, including the Australian group, the Myrtus group and the neotropical lineage.

2.5. Pharmacological properties of *Syzygium*

The *Syzygium* genus is a rich source of phytochemical constituents. Various parts (leaves, seeds, fruits, barks, stem barks and flower buds) of *Syzygium* species are reported to have antioxidant, anticancer, toxicity, antimicrobial and antidiabetic activities due to abundant secondary metabolites such as terpenoids, chalcones, flavonoids, lignans, alkyl phloroglucinols, hydrolysable tannins and chromone derivatives (Srivastava *et al*., 2005; Ayyanar *et al*., 2013; Cock & Cheesman, 2018).

This review emphasizes the significance of *Syzygium* species from a pharmacological perspective, which supports their relevance in ethnomedicine. However, only two *Syzygium* species, *S. aromaticum* and *S. cumini* have drawn scientific attention because of their numerous medical uses in Iranian, Ayurvedic, and Unani systems of medicine. The lack of

sufficient ethnobotanical records highlights the pressing need for additional research on species that have received less attention. Such research is critical in expanding our understanding of these species and their potential benefits.

2.5.1 Antioxidant activity

The most researched *Syzygium* species for its antioxidant potential is *S. aromaticum* (clove) because of its significant economic importance as a source of essential oil.

Jayachandra and Devi (2012) examined the antioxidant activity of the methanolic extract of *S. cumini* bark extracts using DPPH assay, hydrogen peroxide scavenging assay, and FRAP assay. The IC_{50} value of the methanolic extract of *S. cumini* for DPPH and hydrogen peroxide scavenging activity were found to be 53.3% at a concentration of 600 mg/ml and 42.03% at 1.2 mg/ml, respectively. The FRAP value was found to be 810 μg $Fe2^{+}$/g. The extract showed significant activity in all antioxidant assays compared with the standard ascorbic acid.

Ismail *et al*. (2013) reported that a methanolic extract of *S. polyanthum* leaves showed mild antioxidant activity with an IC_{50} value of 90.85 μg/ml compared with the standard quercetin (24.09 μg/ml) using DPPH radical scavenging assay. Banadka *et al*. (2022) reported the antioxidant activity of *S. samarangense* fruit extract through in vitro models such as the ABTS method, DPPH assay and FRAP method. The IC_{50} value of the antioxidant activity of the fruit extract was found to be 140 μg/ml in the standard (L-ascorbic acid), The IC_{50} value for SSFE was found to be 175 μg/ml and 250 μg/ml for DPPH and ABTS scavenging activity, respectively.

Eshwarappa *et al*. (2014) examined the antioxidant activities of leaf gall extracts (aqueous and methanol) of *S. cumini* using DPPH, nitric oxide

scavenging, hydroxyl scavenging, and FRAP methods. The presence of phenolics, flavonoids, phytosterols, terpenoids, and reducing sugars was detected in both extracts, and the methanolic extract showed higher antioxidant potential than the standard ascorbic acid.

Shrikanta *et al*. (2015) detected a higher amount of resveratrol and polyphenol (gallic acid) and high antioxidant activity in *S. cumini* seeds. In another study, an aqueous leaf extract of *S. cumini* was shown to have a reduced DPPH radical, partial prevention of lipid peroxidation induced by $Fe2^{+}$/citrate, and effectiveness against mitochondrial swelling induced by $Ca2^{+}$, thereby indicating the potential of *S. cumini* leaf extract as a useful therapeutic for the treatment of diseases related to mitochondrial dysfunctions (Ecker *et al*., 2015). Ahmed *et al*. (2019) evaluated the phenolic contents and therapeutic activities of *S. cumini* leaves. The study reported a higher average amount of phenolic compounds (369.75 mg GAE/100g) and a higher antioxidant activity in the leaves (IC_{50} 133.07 μg/ml).

Kadri *et al*. (2019) reported that in addition to neutralising free radicals, *S. aromaticum* essential oil protects against other toxins. The antioxidant ability of the essential oil improved $CeCl_3$-induced neurotoxicity in the brains of rats, which alleviated cholinergic neural transmission and the state of memory in mice. According to Alfikri *et al*. (2020), clove produces the major essential oil component, Eugenol, during the flowering stage and is the most effective source of antioxidants when the trees are young. *S. aquem* displayed higher levels of phenolic compounds, anthocyanin content, and antioxidant activity (Itam *et al*., 2021). Uddin *et al.* (2022) confirmed the antioxidant potential of *S. cymosum*, *S. paniculatum*, and *S. caryophyllatum* and attributed polyphenols, tannins, and flavonoids as contributory compounds to their antioxidant potential.

2.5.2 Antimicrobial activity

Shafi *et al*. (2002) reported the antibacterial activity of the essential oils from the leaves of *S. cumini* and *S. travancoricum*, collected from Kerala, India, against *Bacillus sphaericus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *Salmonella typhimurium*. The essential oils showed considerable antibacterial activity, especially against *S. typhimurium*.

Javaid and Samad (2012) reported the antifungal activity of the methanolic extract of the leaves of *S. cumini* against two strains of *Alternaria alternata*, isolated from dying-back trees of *E. citriodora* and *E. globulus*. The methanolic extract concentrations (1%, 2%, and 5% w/v) significantly reduced the fungal biomass. There were reductions in the range of 82–88% due to different concentrations of the leaf extracts of *S. cumini*. The aqueous extract of the fruits of *S. cumini* was active against the growth of *F. oxysporum*, while the aqueous extract of the bark inhibited the growth of *Alternaria alternata* (Gupta & Bhadauria, 2015).

Kumar and Singh (2021) reported that the aqueous, methanolic, hexane, and ethyl acetate extracts of the leaves of *S. cumini* exhibited antimicrobial activity against dental caries-causing strains such as *S. viridans*, *S. mutans*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. Saroj *et al*. (2015) reported that the essential oil constituents of *S. cumini*, such as 7-hydroxycalamenene, 7-acetoxycalamenene, 1-epi-cubenol, α-terpineol, and (Z)-$\beta$and (E)-β-ocimene, are the potential antifungal agents against the phytopathogenic fungi, *Rhizoctonia solani* AG 4HG-III, and *Choanephora cucurbitarum*.

Singh *et al*. (2016) examined the antioxidant and antimicrobial activities of *S. cumini* fruit polyphenols against pathogenic strains such as *S. aureus*, methicillin-resistant *S. aureus*, *E. coli*, *K. pneumoniae*, and *C. albicans* in

comparison with polyphenol standards (gallic acid, quercetin, caffeic acid, sinapic acid, and delphinidin chloride). The *S. cumini* fruit polyphenol extract exhibited a broad-spectrum antimicrobial activity against reference pathogenic strains with a zone of inhibition and MIC in the range of 14.3–23.0 mm and 0.5–2.5 mg/ml, respectively. The largest zone of inhibition against *E. coli* (20 mm) was obtained with the methanolic extract.

2.5.3 Anti-inflammatory activity

Machado *et al*. (2013) detected the anti-inflammatory and apoptotic activity of the essential oil of *S. cumini* in vivo using mice. The anti-inflammatory action and chronic granulomatous inflammation in BALB/c mice intravenously infected with *M. bovis* BCG were evaluated by measuring and classifying the granulomas formed in the hepatic parenchyma. A reduction in the granulomatous area and a change in the pattern of the granulomas were found.

Siani *et al*. (2013) evaluated the anti-inflammatory activity of the essential oils from the leaves of *S. cumini*, as well as some of their terpene-enriched fractions (+V = more volatile; –V = less volatile) obtained by vacuum distillation. Eosinophil migration was inhibited by *S. cumini* (67%) and *S. cumini* (+V) (63%), and this efficacy was correlated with the presence of β-pinene and β-caryophyllene in oils. According to another study, in vitro research, the commercially available chemicals ursolic acid and myricitrin, as well as the ethanol leaf extract of *S. jambos*, significantly reduced the release of the inflammatory cytokines IL 8 and TNF-α by 74–99%, indicating anti-acne effects (Sharma *et al*. 2013). A study on the effects of *S. cumini* aqueous extract (SCC) on indomethacin-induced acute gastric ulceration revealed that SCC acted as an antioxidant, antiinflammation, and antiulcer agent against indomethacin (Chanudom & Tangpong, 2015).

Latief *et al*. (2015) investigated the anti-inflammatory and antioxidant effects of a flavonoid glucoside, trimeric myricetin rhamnoside (TMR), isolated from the leaves of *S. cumini*. The TMR was studied for anti-inflammatory activity in carrageenan-induced hind paw oedema and antioxidant activity in the lung by Cecal Ligation and Puncture (CLP)-induced sepsis in mice. This study concluded that the flavonoid has potential anti-inflammatory benefits and supports the pharmacological basis of using *S. cumini* as a traditional herbal medicine for treating inflammatory diseases.

The ethanol extract of the leaves also showed strong anti-inflammatory effects in carrageenan and histamine oedema rat models, according to in vivo studies (Hossain *et al*., 2016). Additionally, the soluble polysaccharide fraction demonstrated the ability to stimulate TNF-α, IL-1, and IL-10 secretion in a concentration-dependent manner (10–100 g/ml). At a 100 g/ml concentration, the aqueous extract reduced the inflammatory response brought on by LPS (Tamiello *et al*., 2018).

A more recent study on two isolated glycosylated flavonoids, the quercetin- 3-O-β-D-xylofuranosyl-(1 → 2)-α-L-rhamnopyranoside and myricetin-3-O-β-D-xylofuranosyl-(1 → 2)-α-L-rhamnopyranoside, isolated from the chloroform/ methanol fraction of *S. jambos* showed significant anti-inflammatory activity by reducing the levels of TNF-α, with IC_{50} values of 1.68 and 1.11 M, respectively in the RAW 264.7 cell line. Mahmoud *et al*. (2021) reported that the bark extract of *S. zeylanicum* reduced pro-inflammatory TNF-α and elevated anti-inflammatory IL-10 levels in the diabetic rat model and reduced pancreatic inflammation. Additionally, the levels of TNF-α, C-reactive protein, and fibrinogen were decreased by the flavonoids at 5 mg/kg in rats (Apaza Ticona *et al*., 2021). A research study evaluated the anti-inflammatory activity of *Syzygium* plant extracts by determining the levels of pro-

inflammatory mediators like IL-6, IL-1, and TNF-α and lipid peroxidases using alloxan-induced or LPS- or HFD-induced inflammation in diabetic rats or in vitro cell lines (Amir Rawa *et al*., 2022).

2.5.4 Anticancer activity and Cytotoxicity

The anticancer properties of many *Syzygium* species remain largely unexplored. However, a defining feature of the genus is that the fruit and the leaves possess high antioxidant activities. Thus, the high antioxidant contents of many *Syzygium* species may inhibit cancer formation and progression.

Prashar *et al*. (2006) reported the cytotoxicity of *S. aromaticum* oil and its major components on human skin cells. The essential oil extracted from clove is used as a topical application to relieve pain and promote healing in herbal medicine. It also finds use in the fragrance and flavouring industries. Clove oil has two major components, eugenol and β-caryophyllene, constituting 78% and 13% of the oil. The *in vitro* study demonstrated the cytotoxic properties of the oil and eugenol toward human fibroblasts and endothelial cells.

Another study examined the antiproliferative activity of *S. aromaticum* essential oil extracts against HeLa (cervical cancer), MCF-7 and MDA-MB-231 (breast cancer), DU-145 (prostate cancer), and Te-13 (oesophagal cancer) cell lines and reported that except DU-145 cells, the proliferation of all cell lines was inhibited significantly (Dwivedi *et al*., 2011). The same study also determined that the antiproliferative activity was generally due to apoptosis, with maximal cell death occurring within 24 hours at 300 µl extract /ml of cells. Furthermore, that study also examined the phytochemistry of the active extracts and reported that eugenol and β-caryophyllene were the major constituents, accounting for 79% and 13% of the crude *S. aromaticum* essential

oil, respectively. The natural essential oil and the extracts were not significantly toxic to normal human peripheral blood lymphocytes.

2.5.5 Antidiabetic activity

The hydroethanolic extract of *S. cumini* leaves also reduced hyperinsulinemia and insulin resistance by modifying β-cell insulin release In rat models of obesity induced by monosodium L-glutamate (MSG) (Sanches *et al*., 2016). Additionally, Sahana *et al*. (2010) showed that giving *S. cumini* seed powder to 30 newly diagnosed type 2 diabetic patients resulted in a decrease in insulin resistance. In rabbits with mild and severe type 2 diabetes induced by alloxan, it was discovered that the ethanolic seed extract of *S. cumini* stimulated insulin secretion made by pancreatic-β cells (Sharma *et al*., 2011). Serum glucose, insulin, and other diabetic markers were reduced in high-fat diet/streptozotocin (HFD/STZ)-induced diabetic rats treated with *S. cumini* aqueous seed extract at a dose of 400 mg/kg (Sharma *et al*., 2011). In another study, Shen *et al*. (2012) identified that TNF-treated FL83B cells showed insulin resistance to inflammation. As the cells were treated with the fruit extract of *S. samarangense*, the inhibition of c-Jun N-terminal kinase (JNK) prevented an inflammatory response. Phosphatidylinositol-3-kinase-Akt/PKB signalling was consequently activated to reduce insulin resistance brought on by TNF-α.

In a different study, vescalagin isolated from *S. samarangense* reduced insulin resistance in rats with hyperglycemia brought on by a high-fructose diet (Huang *et al*., 2016). Through activating the insulin signalling pathway, myricitrin, a compound isolated from the leaf extract of *S. malaccense*, exhibited insulin-like effects by promoting lipid accumulation, glucose uptake, and adiponectin secretion (Arumugham *et al*., 2016). The anti-diabetic potential of eight species of *Syzygium* was thoroughly reviewed by Zulcafli

et al. (2020). The review stated that the most researched mechanism of action in the anti-diabetic potential of *Syzygium* is the inhibition of enzymes involved in carbohydrate metabolisms, such as α-glucosidase, maltase, and α-amylase. On the other hand, *S. aqueum* leaf extract in STZ-induced diabetic rats decreased glucose levels, increased insulin secretion, and decreased collagen deposition in conjunction with its anti-inflammatory and antioxidant responses (Mahmoud *et al*., 2021). At a dose of 100 mg/kg, the aqueous extract of *S. paniculatum* fruits reduced the blockage of the insulin signalling pathway while improving the function of the insulin receptors (IR and IRS-1) in HFD-induced diabetic rats, which in turn reduced hepatic insulin resistance (Konda *et al*., 2021).

2.6. Conservation strategies

S. travancoricum is an IUCN red-listed critically endangered tree species confined to a specific habitat, especially evergreen forests, and a few scared groves in Thiruvanthapuram, Kollam, Pathanamthitta, Alapuzha and Thrissur districts (Sasidharan, 2004). This species is also reported from myristica swamps dominated by trees belonging to Myristicaceae, *Myristica magnifica* and *Gymnacranthera farquhariana*. Pascal (1988) stated that anthropogenic activities, specifically the conversion of swamps to paddy fields and construction, pose a grave threat to the existence of these habitats. Ramesh and Pascal (1997) considered Myristica swamps as unique areas at the ecosystem level. Several studies (Joyce *et al*., 2007; Nair *et al*., 2007) have also outlined many threats to these fragile ecosystems. Hence it becomes crucial to conserve the existing populations of this species from further endangerment by adopting appropriate conservation strategies.

Ex Situ conservation

Several species of *Syzygium*, including *S. cumini*, still occur in the rough and semiwild forests of the Western Ghats and northeastern India. Some particular examples of *Syzygium* species protected *in situ* include *S. laetum* in the Radhanagari Wildlife Sanctuary in Kolhapur, *S. lanceolatum (S. claviflorum)* (Shareef *et al*., 2010) and *S. sasidharanii* in the Agasthyamalai Biosphere Reserve of the southern Western Ghats (Sasidharan & Jomy, 1999); and *S. agastymalayanum* in the Kalakkad-Mundanthurai Tiger Reserve in Tamil Nadu. *S. travancoricum* is a critically endangered species found only in the swampy wetland habitats in Kerala. The populations are shrinking rapidly due to habitat destruction and intraspecific and interspecific competitions. Hence, ex situ conservation strategies are to be designed to conserve this species, which has immense socioeconomic importance and substantial ecological and livelihood support for local communities.

Chapter 3

Materials & Methods

CHAPTER 3 MATERIALS AND METHODS

3.1 Morphological characterisation of *Syzygium travancoricum*

For the morphological studies of the species, periodical field exploration trips were conducted to different forest areas for collecting specimens. The morphological characters of *Syzygium* populations were observed and compared with the type specimens and characters mentioned in the original protologue. Both visual and stereomicroscopic observations were used to evaluate the morphological characters. The characters were then validated by detailed studies on the morphology of vegetative and floral characters and also by consulting with different herbaria and monographs published by the taxonomists. About 21 morphological characters, including both vegetative and reproductive features, were examined and compared.

Systematic Position - (Bentham & Hooker's System of Classification)

Kingdom	: Plantae
Class	: Magnoliopsida
Order	: Myrtales
Family	: Myrtaceae
Genus	: *Syzygium*
Species	: *travancoricum* Gamble

3.1.1 Study Area

Kerala is located on the southwest coast of India along the Malabar Coast. It is wedged between the Arabian Sea and the Western Ghats within 10° 51′

1.8576'' N latitude and 76° 16′ 15.888'' E longitude, isolated from the Deccan Plateau by the mountainous belt of the Western Ghats. The average elevation of the Western Ghats in Kerala is 1,500 m (4920 ft) above sea level. The natural forest areas of JNTBGRI (Thiruvananthapuram), Idinjaar (Palode), Shendurney Wildlife Sanctuary (Kollam), Kalasamala sacred grove (Thrissur), Pookode and MSSRF (Wynad), and sacred groves at Konginichalkkavu and Paliyeri Mookambika Kavu, Kannur were selected for the study (Fig. 1).

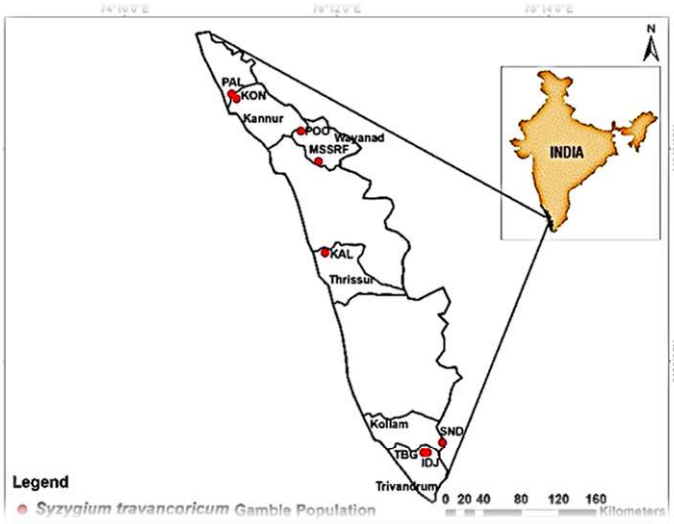


Fig. 1. Study Area

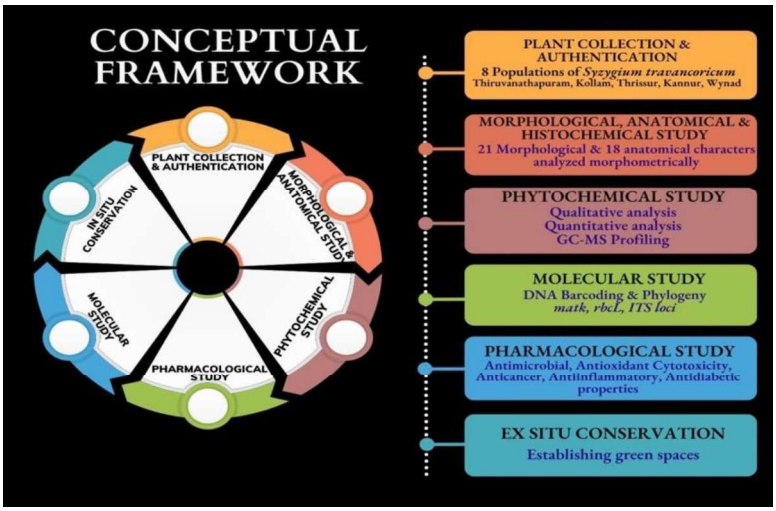


Fig. 2. Conceptual Framework of the Research Study

3.1.2 Sampling

The fresh plant specimens of *S. travancoricum* were collected from eight different populations distributed in the five districts of Kerala from March 2017 to June 2021 (Fig. 1). As part of the collection, about 15 field exploration trips were conducted.

During the field exploration, vegetative and reproductive stages were collected, and the specimens were carefully observed for the visual characteristic features. In case of the absence of flowering or fruiting during collection, the plant twigs with few leaves were collected to identify specimens. The collected flowering/fruiting twigs were tagged properly with respective accession numbers and carefully recorded. The field characters such as family, genus, species, local name, habitat, soil type, distribution, locality, altitude, taluk, district, habit, the height of the plant, brief description, and date of the collection were recorded in the field book for future reference.

3.1.3.1 Field observation and records

The specimens identified in the field were carefully observed and photographed with the help of a Leica EZ4W stereo digital microscope and Olympus Cx31 light microscope. For detailed analysis of reproductive structures, the specimens were carefully dissected and observed using a DM 100 dissection microscope. The morphological features such as habit, bark texture, blaze colour, young branchlets, stem, leaves, intramarginal nerves, peduncle, inflorescence, hypanthium, sepals, petals, stamen, anther, style, L.S of the ovary, number of ovules, fruits and seeds were analysed and documented.

3.1.3.2 Herbarium preparation and documentation

The herbarium specimens were prepared as per the standard guidelines recommended by Bridson and Forman (1992). The collected plant specimens were pressed with newspaper and placed inside the plant press with adequate pressure. The newspapers were periodically changed depending on the requirement, and the specimens were dried without damage. The dried specimens were mounted in hand-made herbarium sheets (42 x 26.5 cm) and labelled properly using standard herbarium labels (14.5 cm x 11 cm). The label is coded with details like the scientific name with citation, habit, habitat, notes, locality, altitude, district, state, date of collection, name of the collector and the person who identified. All the prepared herbarium sheets were submitted in well-preserved cabinets of the Post Graduate and Research Department of Botany, St Thomas' College (Autonomous), Thrissur, Kerala.

3.1.3.3 Identification and confirmation of specimens collected

The specimens collected from different locations were carefully worked out and compared with herbarium specimens of online herbaria accessed from Kew Herbarium online, MH (Botanical Survey of India, SRC, Coimbatore), CAL (Central National Herbarium, Howrah, Calcutta) and CALI (Department of Botany, University of Calicut, Malappuram). Also, the description of the specimens was compared with various standard literatures published through explorations by eminent taxonomists (Hooker, 1879; Gamble, 1919; Nair & Nayar, 1986; Bentham & Mueller, 1867; Sasidharan, 2004; Nayar *et al*., 2006). The plant names and citations were corrected by consulting authentic websites like POWO (http://www.powo.org), The Plant List (http://www.theplantlist.org), the Kew Herbarium Catalogue and the virtual herbarium network of BSI (https://ivh.bsi.gov.in). The conservation status of the species was also confirmed using the IUCN Red List category (IUCN, 2022).

3.1.3.4 Presentation of results

The morphological characters were presented systematically, starting with a morphological description of the species with citation and synonyms, vernacular names, distribution, flowering and fruiting period, and economic importance. About 21 morphological characters, including vegetative and reproductive features of eight *S. travancoricum* populations, were analysed. The list of morphological characters used in the study is presented in Appendix 1.

3.2 Anatomical and Histochemical Characterization

The study aimed to investigate the anatomical features of the bark, stem, petiole and lamina of *S. travancoricum* populations with special emphasis on the secretory structures and histochemical compounds.

3.2.1 Preparation of Specimens

As the preparation of specimens before the sectioning process is very important for obtaining successful results, in the present study, the specimens were prepared in two different ways: as fresh and fixed. First, fixed samples were used for the anatomical study, and for the histochemical study, fresh samples without fixation were used. The fixatives used in this experiment were FAA (Formalin-Acetic acid-Alcohol). The FAA mixture was prepared according to the procedure mentioned in the literature (Metcalfe & Chalk, 1979), with 90 ml of 50% alcohol, 5 ml of 99.9% acetic acid and 5 ml of 40% formalin. During sampling, the samples of stem, bark, petiole and lamina from randomly selected 3 to 5 trees were chosen from each population. They were placed in a resealable plastic bag filled with FAA for structural preservation. On the other hand, another batch of samples was thoroughly wrapped with moistened paper to prevent desiccation and kept in plastic bags for the histochemical studies.

3.2.2 Anatomical Investigation

About five fresh plant samples each of stem, bark, petiole and lamina of *S. travancoricum* distributed in five districts were collected during the period April 2017-June 2018. Standard anatomical procedures were used to prepare semipermanent slides and examine the anatomical characteristics (Cutler *et al*., 2008). Thin tissue sections of lamina, stem and petiole were obtained by hand, while hard structures such as bark were sectioned using a sliding microtome Automatic MT3. The sections were stained with diluted aqueous safranin, rinsed in water, and mounted in glycerine at a concentration of 40%. The prepared semipermanent histological slides were observed using a trinocular compound microscope (Leica DM 3000) with a 10X eyepiece attached to a Leica DFC 295 digital camera connected to a computer and Leica Application Suite software for the observation and capture of microscopic images at magnifications 4X, 10X, 20X, 40X with numerical apertures of 0.10, 0.25, 0.40, and 0.65 respectively. Images were examined and anatomical characteristics were compared. About 19 anatomical characters of eight *S. travancoricum* populations were analysed. The list of anatomical characters used in the study is presented in Appendix 2. All laboratory procedures were conducted in the DST-FIST Laboratory, Vimala College (Autonomous), Thrissur, Kerala.

3.2.2.1 Bark Anatomy

The chosen part of the bark was cross-sectioned using a sliding plant microtome, Automatic MT3. The sections were stained with diluted aqueous safranin, followed by rinsing in water and mounting in 40% glycerine. The semipermanent histological slides were analyzed using a trinocular compound microscope (Leica DM 3000) attached to Leica DFC 295 digital camera connected to the computer and Leica Application Suite software was used for

the observation and capture of microscopic images at magnification 4X, 10X, 20X and 40X. Images were observed, and the anatomical traits were compared.

3.2.2.2 Stem Anatomy

Thin sections of the stem were taken using a razor blade. The sections were stained with diluted aqueous safranin, followed by rinsing in water and mounting in 40% glycerine. The semipermanent histological slides were analyzed using a trinocular compound microscope (Leica DM 3000) attached to Leica DFC 295 digital camera connected to the computer, and Leica Application Suite software was used for the observation and capture of microscopic images at magnification 4X, 10X, 20X and 40X. Images were observed, and the anatomical traits were compared.

3.2.2.3 Petiole Anatomy

Thin sections of the petiole were taken using a razor blade. The sections were stained with diluted aqueous safranin, followed by rinsing in water and mounting in 40% glycerin. The semipermanent histological slides were analyzed using a trinocular compound microscope (Leica DM 3000) attached to Leica DFC 295 digital camera connected to the computer, and Leica Application Suite software was used for the observation and capture of microscopic images at magnification 4X, 10X, 20X and 40X. Images were observed, and the anatomical traits were compared.

3.2.2.4 Lamina Anatomy

Thin sections of the lamina were taken using a razor blade. The sections were stained with diluted aqueous safranin, followed by rinsing in water and mounting in 40% glycerin. The semipermanent histological slides were analyzed using a trinocular compound microscope (Leica DM 3000) attached

to Leica DFC 295 digital camera connected to the computer, and Leica Application Suite software was used for the observation and capture of microscopic images at magnification 4X, 10X, 20X and 40X. Images were observed and the anatomical traits were compared.

3.2.2.5 Comparative morphological and anatomical characterization

A comparative evaluation of morphological (21 characters) and anatomical (18 characters) characteristics of eight populations of *S. travancoricum* was carried out.

3.2.3 Histochemical Investigation

3.2.3.1 Reagents used

i. Safranin stain: The stain was prepared by dissolving 1 g safranin in 100 ml distilled water.

ii. Ferric chloride: The solution was prepared by dissolving 5 g of $FeCl_3$ in 100 ml of water.

iii. Hydrochloric acid (HCl)

iv. Iodine solution: The solution was prepared by dissolving 2.6 g of Iodine and 3g of KI in 100 ml water.

v. Phloroglucinol S: The solution was prepared by dissolving 1ml of phloroglucinol in 100ml of ethanol.

vi. Sudan red: The dye was prepared by dissolving 0.5 g of Sudan red in 100 ml glacial acetic acid AR.

3.2.3.2 Procedure

The fresh samples of stem, petiole and lamina were cross-sectioned using a razor blade and the bark was sectioned using a sliding plant microtome, Automatic MT3. Later the sections were treated with histochemical reagents

such as; Ferric chloride reagent for the detection of tannin compounds, Phloroglucinol S for the detection of lignin, Iodine solution for the detection of starch and aleurone grains, and Sudan Red for the detection of oil compounds and total lipids.

To determine the presence of starch, tissue sections were stained with iodine solution, and the appearance of deep blue colour indicated the deposition of starch grains. The presence of tannin was detected using ferric chloride solution where a bluish-black or greyish-black colour indicated the presence of tannins. For examining the lignified cells, the section was first moistened with phloroglucinol solution, allowed to dry and then treated with one drop of concentrated hydrochloric acid. A pink colour indicated the presence of lignin. The sections were stained with Sudan red to examine the presence of essential oil. The appearance of a deep orange-pink colour inside the cells confirmed the presence of essential oil and lipid compounds.

Each reagent was dropped onto tissue sections in a petri dish for 15 minutes. The tissue sections were rinsed twice with distilled water, transferred onto a glass slide using a camel brush and covered with a cover slip. A Trinocular microscope (Model Leica DM 3000) attached to Leica DFC 295 digital camera connected to the computer and Leica Application Suite software was used for the observation and transferring of microscopic images of the samples. Images were captured at magnifications 4X, 10X, 20X and 40X with numerical apertures of 0.10, 0.25, 0.40, and 0.65, respectively, and the histochemical characters were compared.

3.2.4 Phylogenetic analysis using morphological and anatomical characters

The morpho-anatomical characters (39 characters) were scored and compared between eight *Syzygium* populations and 25 other *Syzygium*

species. Among these, 21 characters were morphological, while 18 were anatomical characters. Visual and stereomicroscopic observations were used to analyze the morphological characters (Cheong & Ranghoo-Sanmukhiya, 2013). All morphological information was encoded using binary or multistate characters (Appendix 3) (Cheong & Ranghoo-Sanmukhiya, 2013). The data matrix containing characters with multiple states is summarized in Appendix 4. All characters were viewed as unordered and equal in weight. A phylogenetic tree was constructed employing UPGMA (Unweighted Pair Group Method with Arithmetic Mean) using PAUP* (Phylogenetic Analysis Using Parsimony) ver. 4.0b 10 (Swofford, 2002).

3.3 Phytochemical characterization

In the present study, phytochemical characterization was carried out in two phases: (i) qualitative and quantitative analysis of crude plant extracts and (ii) Leaf essential oil profiling using GC-MS.

3.3.1 Qualitative analysis

3.3.1.1 Preparation of extracts for phytochemical screening

The standard procedures of phytochemical screening (Kokate *et al*., 1996; Harborne, 1984) were employed to identify the major classes of compounds present in the leaf and bark extracts of *S. travancoricum.*

(i) Water extract: About 50 gm of powdered samples (leaf and bark) were suspended in 500 ml of distilled water. Extraction was done using a Soxhlet apparatus for one hour, then filtered using Whatman filter paper. Extracts were then evaporated at 45°C using a rotary evaporator (Yamato Rotary evaporator) to remove any solvent trace, transferred into sterile bottles, and refrigerated until further analysis.

(ii) Ethanolic extract: About 50 gm of the powdered samples (leaf and bark) were suspended in 500 ml of 95% ethanol. Extraction was done using a Soxhlet apparatus for one hour, filtered using Whatman No.1 filter paper. Extracts were then evaporated at 45°C using a rotary evaporator (Yamato Rotary evaporator) to remove any solvent trace and then transferred into sterile bottles and refrigerated until further analysis. About 1mg/ml of the extracts (water and ethanol) were dissolved in respective solvents and were used as the test solution for further phytochemical analysis.

3.3.1.2 Test for steroids

Liebermann Burchard test: About 1 ml of test solution was mixed with 1 ml of chloroform and a few drops of acetic anhydride. This was followed by the addition of a few drops of conc. H_2SO_4 and mixed carefully. The appearance of a deep green colour indicated the presence of steroids, and the appearance of pink colour indicated the presence of terpenoids.

3.3.1.3 Test for terpenoids

Salkowski test: About 1 ml of test solution was mixed with 1 ml of chloroform and 2 ml of conc. H_2SO_4 was carefully added and thoroughly shaken. The appearance of reddish-brown interphase indicated the presence of terpenoids.

3.3.1.4 Test for Phenols

Ferric chloride test: About 1 ml of test solution was mixed with a few drops of 1% $FeCl_3$ solution. The appearance of blue colour indicated the presence of phenols.

3.3.1.5 Test for tannins:

a. **Gelatin test:** About 1 ml of test solution was mixed with 1 ml of 1% gelatin solution followed by a few drops of 10% NaCl solution and mixed thoroughly. The appearance of a white precipitate indicated the presence of tannins.

b. **Braymer's test:** To 5 ml of test solution was added 3 drops of 10% $FeCl_3$ solution. The formation of a blue-green colour indicated the presence of tannins.

3.3.1.6 Test for flavonoids:

a. **Shinoda Test:** The extract was mixed with a few fragments of magnesium ribbon, followed by the dropwise addition of concentrated HCl. The appearance of pink scarlet colour indicated the presence of flavonoids.

b. **Lead acetate Test:** To the plant aqueous extract, 10% lead acetate solution was added. The appearance of a yellow precipitate indicated the presence of flavonoids.

3.3.1.7 Test for Coumarins:

NaOH Test: The test solution was mixed with 10% NaOH and a few drops of chloroform. The appearance of yellow colour indicated the presence of coumarins.

3.3.1.8 Test for Alkaloids:

a. **Mayer's test:** To 1 ml of the test solution, a few drops of Mayer's reagent were added. The formation of a yellowish or white precipitate indicated the presence of alkaloids.

b. **Wagner's test:** To 1ml of the test solution, a few drops of Wagner's reagent were added. The formation of a brown to reddish precipitate indicated the presence of alkaloids.

c. **Dragendorff's test**: To 1ml of the test solution, a few drops of Dragendorff's reagent were added. The appearance of an orange-brown precipitate indicated the presence of alkaloids.

3.3.1.9 Test for Saponins:

Froth test: To 2 ml of the test solution, 10 ml of distilled water was added. It was shaken well for a few minutes and was allowed to stand for some time. Persistent foam for 10 minutes indicated the presence of saponins.

3.3.1.10 Test for Carbohydrates:

a. **Molisch's test:** The test solution was treated with 2 to 3 drops of 1% alcoholic alpha naphthol and 2 ml conc. H_2SO_4 was added along the sides of the test tube. A violet ring was observed at the junction of two layers indicating the presence of carbohydrates.

Test for Reducing Sugars (Fehling's Test)

To 1 ml of test solution, 0.5 ml of Fehling's solution, A and B were added. The mixture was boiled in a water bath for 2 to 5 minutes. The appearance of a brick-red precipitate indicated the presence of reducing sugars.

3.3.1.11 Test for Proteins

a. Ninhydrin reagent: To 1 ml of test solution, 0.5 ml of Ninhydrin reagent was added and mixed thoroughly. The appearance of a purple colour showed the presence of proteins and free amino acids.

b. Biurette test: To 1 ml of test solution, 0.5 ml of Biurette reagent was added and mixed thoroughly. The purple colour showed the presence of proteins and free amino acids.

3.3.2 Quantitative analysis

3.3.2.1 Estimation of total phenolic compounds

The standard procedure proposed by Singleton *et al*. (1999) was employed to determine total phenolic compounds in various plant extracts. Firstly, the stock solutions of plant extracts were prepared by mixing 1 mg of plant extracts (water and ethanol) in 1 ml of methanol to get a 1 mg/1 ml concentration. Using these stock solutions, 50 µl extract concentration was prepared by pipetting 50 µl of the stock solution and mixing it with 950 µl distilled water. To this solution, 5 ml of Folin-Ciocalteu reagent was added, vortexed, and left for 5 minutes. Finally, 4 ml of 20% Na_2CO_3 solution was added and incubated for 30 minutes. The percentage of light transmittance was determined at 756 nm using a UV-vis spectrophotometer. The total phenolic compounds was calculated using a standard curve where gallic acid (GA) was used as the standard. About 10 mg of gallic acid was dissolved in 10 ml of distilled water in a volumetric flask and stirred well. Serial dilutions of gallic acid stock solution at 20, 30, 50, and 100 µl were prepared to determine the total phenolic compounds. For this, 20 µl, 30 µl, 50 µl and 100 µl of stock solution of GA was pipetted out and mixed with 980 µl, 970 µl, and 950 µl of distilled water, respectively. The gallic acid standard curve was plotted on a graph, which was further used to determine the total phenolic compounds in various plant extracts. Each analysis was performed in triplicate, and the values were expressed in mean ± SD. Results were expressed as mg GAE (gallic acid equivalents)/g dry extract.

3.3.2.3 Estimation of total flavonoid content

The total flavonoid content in the plant extracts was determined using the method proposed by Chatattikun *et al.* (2013). This method is based on the formation of the flavonoid-aluminium complex, which has an absorptive maximum of 415nm. 1 ml of the plant extract was mixed with 4 ml of methanol and added with 1 ml of 10 % aluminium chloride hexahydrate (1g/10 ml distilled water), followed by 1 ml of 1M sodium acetate solution. The absorption at 415 nm was read after 45 minutes. The mixture was kept in the dark. About 3–4 ml of distilled water was taken as control. Quercetin was used as the standard reagent for the procedure. A calibration curve was prepared using different concentrations of Quercetin. A stock solution was prepared by weighing 10 mg of quercetin in 10 ml of distilled water. From this, serial dilutions were made to get 20, 40, 60, 80, and 100 µl concentrations of quercetin. The absorbance value was measured using a UV-vis spectrophotometer and was plotted on a graph. Each analysis was performed in triplicate, and the values were expressed in mean ± SD. The results were expressed as mg QE (quercetin equivalents)/g dry extract.

3.3.2.4 Proximate Analysis

3.3.2.4.1 Plant Materials

Approximately 200 g of fresh fruits of *S. travancoricum* was collected from the field. The fruits were washed in running tap water to remove dirt and dust particles and patted dry. The fruits were sorted into three categories: fully ripened, semi-ripened and raw, depending on their ripening stage. The fresh pulp including the peel were separated from the seeds and ground into a fine paste, and stored in airtight containers at 4°C until analyzed.

Standard protocols (Sadasivam & Manickyam, 1992) and instruments were used for colourimetric estimation. UV Spectrophotometer (Eli Co India Ltd, Model SL 160) was used for the estimation of total carbohydrates, protein, amino acids, ascorbic acid, dietary fibre, total flavonoids, phenol, pectin, the Atomic Adsorption Spectrophotometer (Eli Co India Ltd, SL 194) and Inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific ICAP Qc) were used for the detection of mineral components and heavy metals of the samples respectively.

3.3.2.4.2 Estimation of carbohydrates (Phenol & Sulphuric Acid method)

The standard solution (glucose) at 0.2, 0.4, 0.6, 0.8, and 1 ml (0.1mg/ml) were taken in test tubes. Distilled water was added to each test tube to bring the final volume to 1 ml. In each tube, 1 ml of 5% phenol and 5 ml of 96% sulphuric acid were sequentially added and thoroughly mixed to ensure complete mixing with the standard. After a duration of 10 minutes, all the tubes were transferred to a water bath set at a temperature range of 25–30°C and left for a period of 15 minutes. A blank sample was prepared by adding 1 ml of distilled water. The optical density (O.D) of each tube was measured at a wavelength of 490 nm using a UV spectrophotometer. The phenol and sulphuric acid methods were repeated using 0.2 ml of fruit samples. The optical densities of the sample solutions were determined. The total carbohydrates in the sample solution were calculated using the standard graph.

3.3.2.4.3 Estimation of protein (Lowrys' Method, 1951)

About 1g of the sample was weighed and ground well with a mortar and pestle in a 10 ml buffer. Centrifuge and use the supernatant for protein estimation. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ml) and water in a test tube. The Bovine Serum Albumin (BSA) range is 0.05 to 1mg/ml. The final volume in each test tube was 5 ml. From these different dilutions, 0.2ml protein solution was pipetted out to different test tubes, and 2 ml of alkaline copper sulphate reagent was added. The solution was mixed well and incubated at roomtemperature for 10 minutes. Then, 0.2 ml of Folin-Ciocalteau reagent to each test tube and incubated for 30 minutes. The optical density was recorded at 660 nm using a UV spectrophotometer. The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of the unknown sample was checked, and the concentration of the unknown sample was determined using the standard curve.

3.3.2.4.4 Estimation of Fats

About 1–5 g of the plant sample is massed into a polypropylene centrifuge bottle. Sodium acetate was added so that the total volume was 32 ml. Aliquots of methanol and chloroform were added, and the bottles were capped and shaken again for 30 minutes. Finally, an aliquot of water is added, and the bottles are shaken a final time for 30 minutes. Finally, an aliquot of water is added, and the bottles are shaken a final time for 30 minutes. Centrifuge tubes were used to hold 20 ml aliquots of the chloroform layer. These are then centrifuged for 10 minutes and allowed to set in a 25°C water bath for 15 minutes. The samples are then evaporated to dryness, placed in a hot air oven for 30 minutes, and cooled for at least 30 minutes. Finally, total lipid content was determined using the following formula:

$$\text{Total fat (g/100g wet weight)} = \frac{(W2-W1) \times VC \times 100}{(VA \times SW)}$$

Where W_2 is the weight of the glass tube and dried extract (g), W_1 is the weight of the empty dried glass tube (g), VC is the total volume of chloroform (ml), VA is the volume of extract dried (ml), and SW is the weight of sample assayed (g).

3.3.2.4.5 Estimation of Ascorbic acid

Reagents

Trichloro Acetic Acid (TCA) (4%), 2, 4-dinitrophenyl hydrazine reagent (DNPH) (2%) in 9N H_2SO_4, Thiourea (10%), Sulphuric acid (85%), Ascorbic acid solution: 100 μg/ml in 4% TCA.

Extraction

Ascorbic acid was extracted from 1g of the plant sample using 4% TCA, and the volume was made up to 10 ml with the same. The supernatant obtained after centrifugation at 2000 rpm for 10 minutes was treated with a pinch of activated charcoal, shaken vigorously using a vortex mixer and kept for 5 minutes. The charcoal particles were removed by centrifugation, and aliquots were used to estimate.

Procedure

About 1 ml of ascorbic acid was taken. The volume was made up to 2 ml with 4% TCA. Then, 0.5 ml of DNPH reagent was added to all the tubes, followed by two drops of 10% thio urea solution. The contents were mixed and incubated at 37°C for 3 hours, forming osazone crystals. The crystals were dissolved in 2.5 ml of 85% sulphuric acid in the cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid.

The tubes were cooled in ice, and absorbance was read at 540 nm in a spectrophotometer. The ascorbic acid concentration in the samples was calculated using the standard graph and expressed in terms of mg/g of sample.

3.3.2.4.6 Estimation of Anthocyanins

Reagents used

pH 1.0 buffer (0.025 M potassium chloride), pH 4.5 buffer (0.4 M sodium acetate), Ethanol, 0.1 M HCl.

Preparation of Sample

The fresh fruit pulp was extracted with 95% ethanol and HCl mixtures in a 1:2 ratio for 1 hr using a magnetic stirrer. The mixture was filtered, and the supernatant solution was used for further analysis.

Spectrophotometric Determination

The absorbance was measured after 30 min of preparation for the 2 test solutions of pH 1.0 and 4.5 using a spectrophotometer at 520 nm and 700 nm. Distilled water was used as the blank.

Calculations:

The anthocyanin pigment concentration (as cyanidin-3-glucoside equivalents) was calculated using the following equation:

$$\text{Anthocyanin concentration} = \frac{A \times MW \times DF \times 10^3}{e \times L}$$

Where, A= (A520nm-A700nm) pH(1.0)-(A520nm-A700nm)pH4.5, MW=449.2 g mol G^1 for cyanidin-3-glucoside; DF =Dilution factor; L = Path length (cm);

e =26,900 molar extinction coefficient, in L/mol cm for cyanidin-3-glucoside; 10^3 = Factor for conversion from g to mg.

3.3.2.4.7 Estimation of Minerals

The elements Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Phosphorous (P) were analysed by AAS (Pinnacle 900 H, Perkin Elmer). The samples were subjected to dry ashing at 500°C for 6 hours. The ash was dissolved in 1 N HCl, filtered and introduced into the AAS instrument. Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Selenium (Se), Chromium (Cr), Cobalt (Co), Cadmium, Lead and Mercury were analysed by ICP-MS (Thermo Scientific ICAP Qc). For analysis of minerals using ICP-MS, the samples were subjected to microwave digestion (Anton Paar, Multiwave 3000) and introduced into the instrument. The values are expressed as mean ± standard deviation on a fresh weight basis.

3.3.3 Leaf Essential Oil Profiling

3.3.3.1 Isolation of Essential Oil

The leaves of *S. travancoricum* were collected from eight populations in five different districts of Kerala. The fresh leaves were separated and washed thoroughly three times with tap water and finally rinsed with distilled water. The plant materials were chopped into small pieces (<1cm) by a sterile plant cutter.

Chopped fresh leaf sample materials were subjected to hydrodistillation using a Clevenger apparatus for about 3 hours to extract oil. The essential oils were measured directly in the extraction burette, and the quantity of essential oil (%) was calculated as volume (ml) per 100 g of plant material. The oils were collected in amber-coloured glass tubes, dehydrated using anhydrous sodium sulphate, sealed with parafilm, and stored at 4° C for further analysis.

3.3.3.2 GC-MS Analysis of Essential Oil

The essential oil sample was filtered through a 0.22 μm syringe filter before the analysis. About 1 μl of the filtered sample was analysed using GC-MS (QP-2010-Shimadzu) equipped with a Rxi-5Sil MS column of 30 m length, 0.25 mm diameter, and 0.25 μm thickness. The chromatographic conditions included helium gas (99.999%) used as the carrier gas with a constant flow of 1 ml/min at 250°C injection temperature, and a split injection mode was used. The oven temperature started at 60°C (isothermal for 2 min), with an increase of 3°C/min to 200°C for 5 min, then 10°C/min to 280°C for 5 minutes. EI mass spectra were measured at 70 eV, over the 50–500 amu mass range, and the ion source temperature was maintained at 200°C. The total GC running time was 66.66 minutes.

3.3.3.3 Identification of the Essential Oil Components

The essential oil components were identified by calculating their relative retention indices under temperature-programmed conditions for n-alkanes (C7 to C33). Individual compounds were identified by comparing their mass spectra with the database from the Library of the National Institute of Standard and Technology (NIST, 2018) and Wiley 8. A dendrogram was constructed to compare the chemical signatures of eight populations, employing principal component analysis using SPSS (v.29).

3.4.1 Molecular Characterization

3.4.1.1 Sampling

Eight populations of *Syzygium travancoricum* were collected from the southern Western Ghats. Twenty-four species of *Syzygium* were archived from NCBI (National Centre for Biotechnology Information) to understand the phylogenetic placement of populations of *S. travancoricum* (Appendix 6).

3.4.1 DNA isolation

The genomic DNA was extracted using NucleoSpin® Plant II Kit (Macherey-Nagel). About 100 mg of fresh leaf tissue was homogenized using liquid nitrogen, and the powdered tissue was transferred to a microcentrifuge tube. 400 µl of PL1 buffer was added and vortexed for 1 minute, followed by 10 µl of RNase solution. The homogenate was incubated at 65°C for 10 minutes. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow-through liquid was collected, and the filter was discarded. Later, 450 µl of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 minute, and discarded the flow-through liquid. This was followed by adding 400 µl of PW1 buffer to the column, centrifuging at 11000 x g for 1 minute, and discarding the flow-through liquid. Then 700 µl PW2 was added, centrifuged at 11000 x g, and the flow-through liquid was discarded. Finally, 200 µl of PW2 was added and centrifuged at 11000 x g for 2 minutes. The column was transferred to a new 1.7 ml tube, and 50 µl of buffer PE was added and incubated at 65°C for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4°C.

3.4.2 Agarose Gel Electrophoresis

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH 8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until the bromophenol dye front migrated to the bottom of the gel. The gels were visualized in a UV

transilluminator (Genei), and the image was captured under UV light using a Gel documentation system (Bio-Rad).

3.4.3 PCR Amplification

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (containing 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5 pM of forward and reverse primers (White *et al*., 1990).

Table 1. Details of primers used in the study

Target	Primer Name	Direction	Sequence (5′ → 3′)
ITS	ITS-5F	Forward	GGAAGTAAAAGTCGTAACAAGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC
matK	MATK-XF	Forward	TAATTTACGATCAATTCATTC
	MATK –NR1	Reverse	ACAAGAAAGGCGAAGTAT
rbcL	RBCL-AF	Forward	ATGTCACCACAAACAGAGACTAAAGC
	RBCL-724R	Reverse	TCGCATGTACCTGCAGTAGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

3.4.3.2 PCR amplification profile

ITS and rbcL

95 °C	-	30 sec	40 cycles
95 °C	-	5 sec	
58 °C	-	10 sec	
72 °C	-	15 sec	
72 °C	-	60 sec	
4 °C	-	∞	

matK

95 °C	-	30 sec	
95 °C	-	5 sec	
50 °C	-	10 sec	10 cycles
72 °C	-	15 sec	
95 °C	-	5 sec	
50 °C	-	10 sec	30 cycles
72 °C	-	15 sec	
72 °C	-	60 sec	
4 °C	-	∞	

3.4.3.3 PCR amplicons

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 μg/ml ethidium bromide. 1 μl of 6X loading dye was mixed with 5 μl of PCR products, and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei), and the image was captured under UV light using a Gel documentation system (Bio-Rad).

3.4.3.4 ExoSAP-IT Treatment

ExoSAP-IT (Thermo Fisher Scientific, 2021) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. 5 μl of PCR product was mixed with 2 μl of ExoSAP-IT and incubated at 37°C for 15 minutes, followed by enzyme inactivation at 80°C for 15 minutes (Applied Biosystems, 2021).

3.4.3.5 Sequencing using BigDye Terminator v3.1

The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufacturer protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	made up to 10 µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for 2 minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes for all the primers.

3.4.3.6 Post-Sequencing PCR Clean up

A master mix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction was prepared. About 12 µl of master mix, was added to each reaction containing 10 µl of reaction contents and was properly mixed. A master mix II of 2 µl of 3M sodium acetate at pH 4.6 and 50 µl of ethanol per reaction was prepared. About 50 µl of master mix II was added to each reaction, and the contents were thoroughly mixed by inverting followed by incubation at room temperature for 30 minutes. Then it was centrifuged at 14,000 rpm for 30 minutes. The supernatant was decanted and mixed with 100 µl of 70% ethanol. The filtrate was again centrifuged at 14,000 rpm for 20 minutes. The above step was repeated twice to ensure complete cleaning. Finally, the

supernatant was decanted, and the pellet was air-dried. The cleaned-up air-dried product was sequenced in ABI 3500 DNA Analyzer, Applied Biosystems RapidHIT ID System v. 1.3 (Thermo Fisher Scientific, 2021).

3.4.3.7 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems).

3.4.3.8 Sequence edit and alignment

The sequences were edited in Bioedit (Hall, 1999). The low-quality sequences at the 5' and 3' ends of the sequence were removed Geneious 9.1.6 (Wong *et al*., 2020). Multiple sequence alignment was performed using the MUSCLE algorithm which is inbuilt in MEGA X (Kumar *et al*., 2016). BLAST test was performed for the newly generated sequences in this study to check the homology and a threshold cut-off of 99 per cent identity was determined for the top match in each test (Altschul *et al.* 1990). After the alignment, the twenty-four DNA sequences (3 loci each) of eight populations were deposited in NCBI-GenBank, and the accession numbers are tabulated in Appendix 5. To create the phylogenetic dataset, twenty-five *Syzygium* species were obtained from GenBank for *matk*, *rbcl*, and *ITS,* and the details are given in Appendix 6.

3.4.4.2 Molecular Data

Parsimony analyses were performed on four data sets: DS1: *matk*; DS2: *rbcL*; DS3: *ITS*; DS4: concatenated *matk*, *rbcL*, *ITS*, and morphology) in PAUP 4.0b 10 (Swofford, 2002). The molecular data matrix and combined data matrices are summarized in Appendix 7 and 8, respectively. All characters were viewed as unordered and equal in weight.

Parsimony analysis

Parsimony analysis (using the command 'PSET') was performed independently for each dataset using tree-bisection-reconnection (TBR) branch-swapping, 1,000 random stepwise addition, holding only one tree at each step during stepwise addition, gaps were treated as missing, all characters were unordered and had equal weight.

Bayesian Phylogenetic analysis for concatenated dataset

Bayesian phylogenetic analysis was performed by combining the morphological, anatomical and molecular data, including eight populations of *S. travancoricum* and twenty-four *Syzygium* species that are obtained from Genbank in MrBayes 3.2.6. (Huelsenbeck & Ronquist, 2001). Best-fit model was analysed based on Bayesian Information Criterion (BIC) within MrBayes using the command called "nst=mixed". MCMC analysis was conducted for 5M generations using a sample frequency of 1000 trees. 25% burn-in discordance was enforced to omit the trees at the initial runs towards tree stabilization to attain high levels of posterior probability distribution. For the concatenated dataset, the data was partitioned, and set parameters were given as default except for the morphological dataset. Parameters and trees were summarized simultaneously to derive the tree attributes. The majority-rule consensus tree was viewed in figtree and the PP values were enabled in percentage on all branches for further interpretation.

3.5 Pharmacological Studies

3.5.1. Antimicrobial Assay

3.5.1.1 Test Organisms

Test organisms were collected from the National Collection of Industrial Microorganisms (NCIM) CSIR-NCL, Pune and maintained as reference stocks

in a deep freezer at -18°C to -20°C. The bacterial strains were revived, subcultured, and kept at 2–8°C in non-selective agar slants. These cultures were used for assessment.

3.5.1.2 Media preparation

For bacterial culture, Muller Hinton Agar (MHA) medium was used. MHA was prepared and sterilized for 15 minutes at 121°C. Following sterilization, about 20 ml of medium was poured into the sterile petri dishes and allowed to solidify. For fungal culture, Sabouraud Dextrose Agar (SDA) medium was used.

3.5.1.3 Inoculum

Bacterial pure cultures were used as the inoculum. Three to four similar bacterial colonies were chosen and transferred into approximately 5 ml of suitable broth, such as Tryptone Soya Broth (TSB - Himedia M 1263). It was then incubated for 8–12 hours at 37°C until light to moderate turbidity developed.

3.5.1.4 Procedure

The antimicrobial assay was carried out using the disc diffusion method. The oil was tested for antibacterial activity against four Gram-positive bacterial strains, *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus and Streptococcus mutans*, as well as four Gram-negative bacterial strains, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. About 30 µl of the essential oil was dissolved in Dimethyl Sulfoxide (DMSO) at a ratio of 1:2. The Whatman No.1 filter paper discs of 6 mm diameter were soaked with 10 µl of diluted essential oil and placed aseptically on the surface of agar plates at uniform intervals and incubated for 24 hours at 37°C. Oxoid Streptomycin Antimicrobial

susceptibility discs at 10 μg concentration were used as the positive control. Similarly, filter paper discs impregnated with 10 μl of the solvent DMSO were used as a negative control. The diameter of the discs, as well as the inhibition zones, was measured.

The antifungal effect of the oil was also investigated using the disc diffusion method as described earlier. A total of three fungal species (*Candida albicans*, *Aspergillus niger*, and *Penicillium chrysogenum*) were obtained from the National Collection of Industrial Microorganisms (NCIM) CSIR-NCL, Pune and cultured in Sabouraud Dextrose Agar (SDA) medium. Dilution of the oil in DMSO was carried out at a 1:2 ratio, and control discs soaked with 10μl of DMSO alone were used in conjunction with the test discs. Both assays were carried out in triplicate, and the results were calculated as the mean with the standard deviation of triplicates.

3.5.2. Antioxidant assay

The antioxidant activity of *S. travancoricum* plant extracts was determined using DPPH and Ferric Reducing Antioxidant Power (FRAP) Assays.

3.5.2.1 DPPH Assay

The free radical scavenging property of the extracts was determined according to the method described by Baliyan *et al*., 2022. A 100 ml stock solution of 0.2 mM DPPH (1,1-diphenyl-2- picrylhydrazyl) was prepared by weighing 7.83 mg DPPH and dissolving in 100 ml methanol. The leaf and bark extracts in water were used for the DPPH analysis. The plant extracts were serially diluted for 10, 20, 30, 40 and 50 μl concentrations. The experiments were done under complete darkness, and for this, test tubes were completely covered with aluminium foil and kept under darkness for 30 minutes. 1ml methanol

mixed with 1ml DPPH solution was used as the control. Absorbance was read at 517 nm in a UV spectrophotometer.

The 50% inhibitory concentration (IC_{50}) of each extract that can scavenge 50% of DPPH radicals was calculated by linear regression using the equation, $y = 1.8195x$, $R^2 = 0.9958$ for leaf extract and $y = 1.9761x$, and $R^2 = 0.9868$ for bark extract respectively.

Radical scavenging activity was expressed as the percentage of inhibition and was calculated using the following formula:

Scavenging activity (%) = [(AC - AS) / AC] x 100%

where AC is the absorbance of DPPH control, and AS is the absorbance of DPPH mixed with extract or gallic acid solution. The result was reported as IC_{50} values and gallic acid equivalents (μg GAE /ml).

3.5.2.2 Ferric Reducing Antioxidant Power Assay

The reducing power of the extracts was determined according to the method described by Oyaizu (1986), with some modifications. Leaf extracts of *S. travancoricum* in water, ethanol and the leaf essential oil were analyzed for their antioxidant properties using this method. The plant extracts at five different concentrations of methanolic extracts (20, 40, 60, 80, and 100 μg/ml) and L-ascorbic acid at the same concentrations was mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 minutes. Then, 2 ml of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% Ferric chloride ($FeCl_3$). In each case, the experiment was performed in triplicate.

Afterwards, the absorbances were measured spectrophotometrically at 750 nm using a UV-vis spectrophotometer and the absorbance values were recorded. FRAP values were obtained by comparing the absorbance values at 750 nm in test solutions with those containing ferrous ions in a known concentration of Ascorbic acid. They were expressed as mg/100 g Ascorbic equivalent (AAE) using the standard curve equation: $y = 0.0141x + 0.0429$, $R^2 = 0.9895$. The 50% inhibitory concentration (IC_{50}) of extracts was also calculated by linear regression.

3.5.3 In vitro cytotoxicity

3.5.3.1 Reagents used:

DMEM media	- Sigma Aldrich, USA D5648
Fetal Bovine Serum	- Gibco, US orgin-
0.25% Trypsin	- Invitrogen, USA 25200-056
MTT	- Sigma Aldrich M5655

3.5.3.2 Equipments Used:

Micropipettes	- F1 Thermoscientific USA
CO_2 Incubator	- Eppendorf, GERMANY
Phase Contrast Microscope Camera	- Olympus, JAPAN with Optika Pro 5
ELISA Reader	- ERBA, GERMANY
Culture Plates and Flasks	- NUNC, Thermoscientific USA
Image Magnification	- 10X

3.5.3.3 Procedure

L929 (Fibroblast) cell line was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's Modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in a

25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS (Foetal Bovine Serum), L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing: Penicillin (100 U/ml), Streptomycin (100 µg/ml), and Amphoteracin B (2.5 µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by an Inverted phase contrast microscope and followed by the MTT assay method.

3.5.3.4 Cells seeding in 96 well plates

Two-day-old confluent monolayer of cells was trypsinized, and the cells were suspended in 10% growth medium; 100 µl cell suspension (5 x 10^3 cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator.

3.5.3.5 Preparation of compound stock

1mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through a 0.22 µm millipore syringe filter to ensure sterility.

3.5.3.6 Cytotoxicity Evaluation

After 24 hours, the growth medium was removed, the freshly prepared sample solution was five times serially diluted by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µl of DMSO). Each concentration of 100 µl was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Non-treated control cells were also maintained.

3.5.3.7 Cytotoxicity Assay by Direct Microscopic observation

The entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD

camera), and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells, were considered indicators of cytotoxicity.

3.5.3.8 Cytotoxicity Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of the incubation period, the sample content in wells was removed, and 30 µl of reconstituted MTT solution was added to all test and cell control wells; the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed, and 100 µl of MTT Solubilization Solution (Dimethyl sulphoxide: DMSO, Sigma Aldrich, USA) was added, and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured using a microplate reader at a wavelength of 540 nm (Talarico *et al*., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of the Samples}}{\text{Mean OD of the control group}} \times 100$$

3.5.4 In vitro Anticancer Assay

B16 (Human skin cancer) cell lines were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS (Foetal Bovine Serum), L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing: Penicillin (100U/ml),

Streptomycin (100 µg/ml), and Amphoteracin B (2.5 µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by an inverted phase contrast microscope and followed by the MTT assay method.

3.5.4.1 Cells seeding in 96 well plates

Two-day-old confluent monolayer of cells was trypsinized, and the cells were suspended in 10% growth medium; 100 µl cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator.

3.5.4.1.1 Preparation of compound stock

1 mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through a 0.22 µm syringe filter to ensure sterility.

3.5.4.1.2 Anticancer Evaluation

After 24 hours, the growth medium was removed. Each freshly prepared compound in 5% DMEM were five times serially diluted by two-fold dilution (50 µg, 25 µg, 12.5 µg, 6.25 µg, 3.125 µg in 500µl of 5% DMSO) and each concentration of 100 µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Non-treated control cells were also maintained.

3.5.4.1.3 Anticancer Assay by Direct Microscopic observation

The entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera), and microscopic observations were recorded as images. Any

detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells, were considered indicators of cytotoxicity.

3.5.4.1.4 Anticancer Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS (Phosphate-buffered saline) until completely dissolved and sterilized by filter sterilization. After 24 hours of the incubation period, the sample content in wells was removed, and 30µl of reconstituted MTT solution was added to all test and cell control wells; the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed, and 100 µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added. The wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured using a microplate reader at a wavelength of 540 nm (Talarico *et al*., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of the Samples x 100}}{\text{Mean OD of the control group}}$$

3.5.4.2 Analysis of Cell Apoptosis by Flow Cytometry

Flow cytometry is a technology that simultaneously measures and analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence.

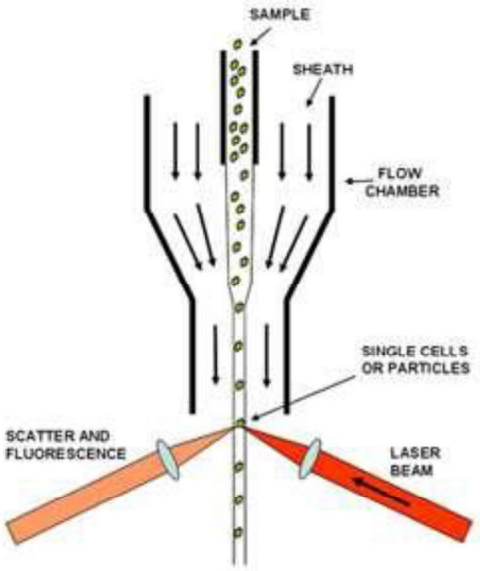


Fig. 3. Schematic representation of flow cytometric analysis of cells

3.5.4.2.1 Principle

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states such as Alzheimer's and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.

The FITC Annexin V/Dead Cell Apoptosis Kit (ThermoFisher Scientific, CA, USA) with FITC Annexin V and PI for flow cytometry provided a rapid and convenient assay for apoptosis. The kit contained recombinant annexin V conjugated to fluorescein (FITC annexin V), as well as a ready-to-use solution of the red-fluorescent propidium iodide (PI) nucleic acid binding dye. PI was impermeant to live and apoptotic cells but stained dead cells with red fluorescence, binding tightly to the nucleic acids in the cell. After staining a cell population with FITC annexin V and PI in the provided binding buffer,

apoptotic cells showed green fluorescence, dead cells showed red and green fluorescence, and live cells showed little or no fluorescence. These populations can easily be distinguished using a flow cytometer with the 488 nm line of an argon-ion laser for excitation.

Apoptosis, or programmed cell death, is an important and active regulatory pathway of cell growth and proliferation. Cells respond to specific induction signals by initiating intracellular processes that result in characteristic physiological changes. Among these are the externalization of phosphatidylserine (PS) to the cell surface, cleavage and degradation of specific cellular proteins, compaction and fragmentation of nuclear chromatin, and loss of membrane integrity (in late stages). Annexin V is a calcium-dependent phospholipid-binding protein with a high affinity for PS, a membrane component normally localized to the internal face of the cell membrane. Early in the apoptotic pathway, molecules of PS are translocated to the outer surface of the cell membrane where Annexin V can readily bind them. The Muse™ Annexin V and Dead Cell Assay utilizes Annexin V to detect PS on the external membrane of apoptotic cells. A dead cell marker is also used to indicate cell membrane structural integrity. It is excluded from live, healthy cells, as well as early apoptotic cells. Four populations of cells can be distinguished in this assay such as (i) non-apoptotic cells: Annexin V (-) and 7-AAD (-); (ii) early apoptotic cells: Annexin V (+) and 7-AAD (-); (iii) late stage apoptotic and dead cells: Annexin V (+) and 7-AAD (+); (iv) mostly nuclear debris: Annexin V (-) and 7-AAD (+).

3.5.4.2.2 Procedure

BI6 melanoma cell line was cultured as per standard procedures described earlier and treated with a compound with their IC_{50} concentration (57.26 μg/ml) and incubated for 24 hours. The cells were trypsinized after incubation

and 100 μl of cells in suspension were transferred to separate tubes. About 100 μl of the MuseTM Annexin V and Dead Cell Reagent were added to the tubes. The tubes were mixed thoroughly by pipetting up and down or vortexing at a medium speed for 3 to 5 seconds, then incubating for 20 minutes at room temperature in the dark. The cells were analyzed in a flow cytometer using Muse flow cytometry software. Cells were gated against untreated control cells and analyzed for apoptosis using Muse FCS 3.0 software.

3.5.4.3 In vitro ROS measurement using DCFDA

B16 (melanoma) was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and the antibiotic solution containing Penicillin (100 U/ml), Streptomycin (100 μg/ml), and Amphoteracin B (2.5 μg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). IC_{50} concentration of the sample (57.26 μg/ml) was added and incubated for 24 hours. Untreated control cells were also maintained.

3.5.4.3.1 Principle

Dichloro dihydro fluorescein diacetate. 2',7'- dichlorofluorescein diacetate (DCFDA; Sigma Aldrich), a fluorogenic dye that measures hydroxyl, peroxyl and other ROS activity within the cell. After diffusion into the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which ROS later oxidizes into 2', 7'- dichlorofluorescein (DCF), which emits green fluorescence upon excitation with a blue filter. Carboxy-H_2DCFDA is non-

fluorescent, but in the presence of ROS, when this reagent is oxidized, it becomes green fluorescent.

3.5.4.3.2 Procedure

The cells were washed with PBS, added 50 µl of DCFDA, and incubated for 30 minutes. After incubation, the excess dye was washed with PBS and fluorescence was imaged in a fluorescent microscope (Olympus CKX41 with Optika Pro5 CCD camera) the fluorescence was measured using a fluorimeter at 470 nm excitation and emission at 635 nm (Qubit 3.0, Life Technologies, USA) and expressed in arbitrary units.

3.5.4.4 Determination of Mitochondrial Membrane Potential by Flow Cytometry

Mitochondria are important cellular organelles that maintain crucial cellular energy balance, are a primary site of production of free radicals, and in addition, contain key regulators of cell death processes such as apoptosis. Mitochondria changes are thus highly sensitive indicators of cell health and stress. Cellular energy produced during mitochondrial respiration is stored as an electrochemical gradient across the mitochondrial membrane, and this accumulation of energy in healthy cells creates a mitochondrial membrane potential ($\Delta\Psi m$) that enables the cell to drive the synthesis of ATP. Loss of the mitochondrial inner transmembrane potential is often, but not always, observed to be associated with the early stages of apoptosis. The collapse of this potential is believed to coincide with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which then triggers the downstream events in the apoptotic cascade.

Mitochondrial membrane potential changes have been implicated in apoptosis, necrotic cell death, and caspase-independent cell death processes.

Depolarization of the inner mitochondrial membrane potential is thus a reliable indicator of mitochondrial dysfunction and cellular health, which has become increasingly important in studying apoptosis, drug toxicity and multiple disease states. The Muse™ MitoPotential Assay utilizes the MitoPotential Dye, a cationic, lipophilic dye to detect changes in the mitochondrial membrane potential and 7-AAD as an indicator of cell death. High membrane potential drives the accumulation of MitoPotential dye within the inner membrane of intact mitochondria, resulting in high fluorescence. Cells with depolarized mitochondria demonstrate a decrease in fluorescence and a downward shift. This parameter is displayed in the MitoPotential axis. A dead cell marker (7-AAD) is also used to indicate cell membrane structural integrity and cell death. It is excluded from live, healthy, and early apoptotic cells. Dead cells thus show increased fluorescence in the viability axis. Four populations of cells can be distinguished in this method such as: (i) (LL) Live cells with depolarized mitochondrial membrane: MitoPotential (-) and 7-AAD (-); (ii) (LR) Live cells with intact mitochondrial membrane: MitoPotential (+) and 7-AAD (-); (iii) (UR) Dead cells with depolarized mitochondrial membrane: MitoPotential (+) and 7-AAD (+); (iv) (UL) Dead cells with intact mitochondrial membrane: MitoPotential (-) and 7-AAD (+).

3.5.4.4.1 Reagent Preparation

BI6 cells were cultured as per standard procedures described earlier, treated with a compound with their IC_{50} concentration (57.26 g/ml), and incubated for 24 hours. Cells were trypsinized and subjected to flow cytometry as per the following procedures.

The working solution was prepared by diluting the Muse™ MitoPotential Dye 1:1000 in 1X assay buffer. After centrifugation, the cells were

resuspended in 1X assay buffer and added with 95 µl mitopotential working solution. It was further mixed thoroughly by vortexing and was incubated for 20 minutes in a 37°C CO_2 incubator. After incubation, 5 µl of Muse MitoPotential 7-AAD was added to each well, mixed thoroughly by vortexing for 3 to 5 seconds, and kept for incubation for another 5 minutes. The samples were loaded onto a flow cytometer (Millipore, USA), and the events were acquired after gating and correlated with controls.

3.5.4.5 Analysis of DNA content and Cell cycle analysis

Monitoring the ability of cells to proliferate is critical for assessing cell health during toxicity studies. The most accurate method of doing this is by directly measuring DNA synthesis. The basic principle of the MUSE cell cycle kit is a standard ethanol fixation and detergent permeabilization, which is sufficient to gain access to the DNA during the active cell cycle. The kit utilizes a premixed reagent which includes the nuclear DNA intercalating stains propidium iodide (PI), which discriminates cells at different stages of the cell cycle based on the differential DNA content in the presence of RNA*ase* to increase the specificity of DNA staining in each phase (G_0/G_1, S and G_2/M).

BI6 cells were cultured per standard procedures described earlier, treated with a compound with their IC_{50} concentration (57.26 µg/ml), and incubated for 24 hours. The cells were digested and transferred to a 12 x 75 mm polystyrene tube. The minimum recommended number of cells for fixation in a tube is 1×106 cells. The samples were then centrifuged at 3000 rpm for 5 minutes. The supernatant was removed without disturbing the pellet. After centrifugation, the cells appeared as visible pellets on the bottom of the tube.

The appropriate volume of PBS was added to each tube (i.e., 1ml of PBS per 1×106 cells), and the contents were mixed by pipetting several times. The cells

were centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded without disturbing the cell pellet, leaving approximately 50 µl of PBS per 1 × 106 cells. The pellet was resuspended in the residual PBS by repeated pipetting. The resuspended cells were finally added dropwise into the tube containing 1ml of ice-cold 70% ethanol while vortexing at medium speed. The tube was capped and incubated at -20°C.

3.5.4.5.1 Staining of Cell Cycle

After the overnight incubation, the samples were centrifuged at 3000 rpm for 5 minutes at room temperature. The supernatant was removed, and 250 µl PBS was added to the pellet. Then the centrifugation was done again at the same rpm and time. The pellet was taken after discarding the supernatant; 250 µl of cell cycle reagent was added and was incubated in the dark for 30 minutes. Finally, it was analyzed using a Flow Cytometer and Gating was performed with reference to untreated control cells.

3.5.5 Anti-inflammatory Assay

RAW 264.7 cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing Penicillin (100 U/ml), Streptomycin (100 µg/ml), and Amphotericin B (2.5 µg/ml). The cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The cells were grown to 60% confluency, followed by activation with 1 µl lipopolysaccharide (LPS). LPS-stimulated RAW cells were exposed to different concentrations (6.25, 12.5, 25 µg/ml) of sample solution and

incubated for 24 hours. After incubation, the anti-inflammatory assays were performed using the cell lysate.

3.5.5.1 Cyclooxygenase (COX) activity

The COX activity was assayed using the method of Walker and Gierse (2010). About 100µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/l, and haemoglobin 5 mM/l for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/l and terminated after 20 minutes of incubation at 37°C by the addition of 200µl of 10% trichloroacetic acid in 1 N hydrochloric acid. After centrifugation and the addition of 200µl of 1% thiobarbituric acid, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm, and percentage inhibition of COX activity was determined as per the following formula,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

3.5.5.2 Lipoxygenase (LOX) activity

The determination of LOX activity was done as per the method described by Axelrod *et al.* (1981). The reaction mixture comprised Tris-HCl buffer (pH 7.4), 50 µl of cell lysate, and sodium linoleate (200 µl) and was made up to 2 ml. The LOX activity was monitored as an increase of absorbance at 234 nm (Agilent Cary 60), which reflects the formation of 5-hydroxyeicosatetraenoic acid. The percentage inhibition of the enzyme was calculated as,

% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

3.5.5.3 Myeloperoxidase (MPO) activity

The cell lysate was homogenized in 50 mM potassium phosphate buffer and 0.57% hexadecyltrimethyl ammonium bromide (HTAB). The samples were centrifuged at 2000 g for 30 minutes at 4°C, and the supernatant was assayed for MPO activity. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/ml guaiacol and 0.0005% H_2O_2. The change in absorbance at 460 nm was measured. MPO activity was presented as units per ml of cell lysate. One unit of MPO activity (U) was defined as degrading 1 μM of peroxide per minute at 25°C.

$$\mathbf{U = (\Delta OD \cdot 4 \cdot Vt \cdot dilution\ factor) / (L \cdot £470 \cdot \Delta t \cdot Vs)}$$

Where ΔOD = density change; Vt = total volume (1.1 ml); L=light path (1 cm); £470 = extinction coefficient for tetraguaiacol (26.6 $mM^{-1}cm^{-1}$)

3.5.5.4 Inducible Nitric Oxide Synthase

Nitric oxide synthase activity was determined by the method described by Salter *et al*. (1996). The cell lysate was homogenized in 2 ml of HEPES buffer. The assay system contained 0.1 ml–2 μmol/l l-Arginine, 0.1 ml–4 μmol/l Manganese chloride, 0.1 ml–10 mmol/l 30 μg dithiothreitol (DTT), 0.1 ml–1 mmol/l NADPH, 0.1 ml–4 μmol/l tetrahydropterin, 0.1 ml 10 μmol/l oxygenated haemoglobin and 0.1 ml cell lysate. An increase in absorbance was recorded at 401 nm, and the enzyme activity was determined as per the following equation.

$$\mathbf{\%\ inhibition = ((Absorbance\ of\ control - Absorbance\ of\ test)/Absorbance\ of\ control) \times 100}$$

3.5.5.6 Estimation of Cellular Nitrite Levels

The level of nitrites was estimated by the method of Lepoivre *et al*. (1990). To 0.5 ml of cell lysate, 0.1 ml of 3% sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µl of the supernatant, 30 µl of 10% NaOH was added, followed by 300 µl of Tris-HCl buffer and mixed well. To this, 530 µl of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diaminedihydrochloride) was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a blank (Griess reagent). Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

3.5.6 Antidiabetic assay

The leaf extracts and leaf essential oil of *S. travancoricum* (STEO) were used for analyzing the antidiabetic properties. The leaf extracts included water extract (STWE) and ethanol extract (STEE).

3.5.6.1 Lipase inhibitory assay

Lipase inhibitory assay was carried out using the methodology described by Laya *et al*. (2023). About 20 µl of the sample at varying concentrations of (0–250 µg/ml) and 40 µl of pNPP (p-Nitrophenol palmitate) solution were filled in a 96 well microplate. Thereafter, the mixture was preincubated in a water bath at 37°C for 15 minutes. Then, 20 µl of the enzyme (10 units/ml) already prepared using the buffer (0.1 M, pH 6.8) was filled in each well before incubating in a water bath at 37°C for 40 minutes. After cooling at room temperature, the absorbance was measured at 410 nm using the

microplate reader. The control having α-amylase alone without any inhibitor represented 100% enzyme activity. The enzyme activity at various concentrations was measured as follows:

$$\textbf{Inhibition (\%)} = 100 \times (AC - AS)/(AC)$$

Where AS, AC is the Absorbance of Control and Absorbance of the sample.

3.5.6.2 α-amylase assay

α-amylase activity was tested using the methodology described by Sekhon-Loodu and Rupasinghe (2019). The plant extracts at varying concentrations (0-250 μg/ml) were used as test solutions for the analysis. The test solution, enzyme and soluble starch were dissolved in 20 mM sodium phosphate buffer containing 6 mM NaCl (pH 6.9). To each test solution was added 250 μl of pancreatic porcine α- amylase (1 U/ml, dissolved in the buffer at pH 6.9). The mixture was pre-incubated at 37°C for 15min before the addition of 250 μl of 0.5% starch. The mixture was then vortexed and incubated again at 37°C for 15 minutes, followed by 1 ml of dinitrosalicylic acid colour reagent, which was used for the termination of the reaction. The tubes were placed in a boiling water bath for 5 minutes, followed by cooling at room temperature. About 200 μl of the reaction mixture was taken into a 96-well plate, and the absorbance was read at 540 nm using the microplate reader. The control having α-amylase alone without any inhibitor represented 100% enzyme activity, and the enzyme activity at various concentrations was measured as follows:

$$\textbf{Inhibition (\%)} = 100 \times (AC - AS)/(AC)$$

Where AS, AC is the Action of control and action of the sample.

3.5.6.3 α-glucosidase inhibitory assay

α-glucosidase inhibitory activity was tested using the methodology described by Sekhon-Loodu and Rupasinghe (2019). The test extracts were taken at different concentrations varying from (0–250 μg/ml). The reaction mixture containing 20 μl extract at each concentration, 20 μl α-glucosidase and 60 μl of 10 mM potassium phosphate buffer (pH 6.8) were pre-incubated at 37°C for 15 minutes, followed by the addition of 20 μl of 5 mM p-nitrophenol-a-D-glucopyranoside substrate in a 96-well microplate. The mixture was then incubated at 37°C for the reaction to take place. After 15 minutes, 80 μl of stop solution containing 200 mM sodium carbonate was added. The absorbance was recorded at 405 nm using the microplate reader. The control having α-glucosidase alone without any inhibitor represented 100% enzyme activity, and the action of various extract concentrations was measured as follows.

$$\textbf{Inhibition (\%)} = 100 \times (AC - AS)/(AC)$$

Where AS, AC is the Absorbance of Control and Absorbance of the sample.

3.5.6.4 Statistical analysis

All experiments were performed in triplicates and the data is presented as a mean ± standard deviation.

3.6 Conservation Strategies

An ex situ conservation strategy was taken up *via* establishing green spaces at five locations distributed across different districts in Kerala, which focused on providing specific benefits to the community and the biodiversity, such as (i) to safeguard vital ecological processes and life-support systems necessary for human survival; (ii) to conserve the genetic resource from endangerment; (iii) to promote ethical and responsible use of genetic resources that benefit both rural populations and commercial enterprises; (iv) to preserve and evaluate the germplasm for futuristic research.

3.6.1 Study areas

The *ex situ* conservation was first conducted at Elavally Grama Panchayath, at a latitude of 10.58° N and a longitude of 76.09° E in the Chavakkad Taluk, Thrissur district, Kerala. This village is comprised of small islands like Kakkathuruth and Kaniyamthuruth, paddy fields, wetlands, and small hilly areas. Rising sea levels cause flooding and storm surges in this area during the monsoons, which is very dangerous for the community.

The second area selected was Panjal Grama Panchayath, at latitude of 10.7215° N and a longitude of 76.03055° E in the Thalappilly Taluk, Thrissur district, Kerala. This area is situated on the banks of Nila, Bharathapuzha. The third area was Varavur Grama Panchayath, at latitude of 10.43836° N and a longitude of 76.121933° E in the Thalappilly Taluk, Thrissur district, Kerala. This area is also near the bank of Bharathapuzha. The fourth area selected was Government College, Chittur, located on the banks of the Shokanashini River in the Chittur-Tattamangalam area with latitude of 10.677238° N and a longitude of 76.716339° E in Palakkad district, Kerala. The fifth area was Enadimangalam Grama Panchayath, at latitude of 9.73234° N and a longitude of 76.4854° E in the Adoor Taluk, Pathanamthitta district, Kerala.

3.6.2 Plant propagation methods

3.6.2.1 Seed germination

The seeds were collected from the ripe fruits of *S. travancoricum* populations in the Kalasamala sacred grove, Thrissur, from June to July 2017. The fruits were cleaned to remove the pulp and other trash and were shade-dried for 24 hours at room temperature. The dried seeds were sown in a germinating medium of coir pith, vermicompost, and sand at a 1:1:1 ratio in small poly bags.

3.6.2.2 Vegetative Propagation *via* stem cuttings

Healthy and disease-free secondary branches were randomly collected from the *S. travancoricum* populations during the same period. Semi-soft wood branches were chosen for preparing stem cuttings.

3.6.2.3 Rooting

The secondary branches of *S. travancoricum* trees were used as a source for the semi-softwood cuttings, which ranged in length from 10 to 15 centimetres and contained 3 to 4 nodes. The basal cut was made directly under the node without causing damage to the surrounding tissue. The cuttings that retained at least 2 centimetres of the plant's basal end were submerged in a solution of IBA (2000 ppm) in ethanol and distilled water at 1:1 proportion for 1 minute. The cuttings were then planted in polythene bags containing sand and clay in a ratio of 1:1 (w/w). Watering was done twice daily to the stem cuttings until the first shoots sprouted.

3.6.2.4 Planting of propagules

Forty healthy saplings were used for planting. The saplings were transplanted to the selected areas during the commencement of the northeast monsoon. First, the land was thoroughly ploughed to ensure easy water drainage. Then, small pits of appropriate size (50 cm x 50 cm x 50 cm) were dug out and preconditioned with moistened coir pith or coconut husk, which was finally covered with a thin layer of soil. The seedlings and rooted stem cuttings were planted into these pits and watered sufficiently. The growth of the saplings was monitored regularly to ensure adequate growth.

Chapter 4

Results

CHAPTER 4 RESULTS

Systematics of *Syzygium* Gaertn.

The genus *Syzygium* Gaertn. belongs to the Myrtaceae family and boasts more than 1200 species consisting mostly of woody trees and shrubs. The leaves are opposite, usually with pellucid dots and prominent intramarginal nerves, while the flowers come in both small and large sizes and appear in terminal, axillary or lateral cymes. Additionally, they have a corymbose inflorescence and flower in a centrifugal sequence, with a funnel-shaped calyx-tube. The terminal ends are five lobed, and they have either small or large fugacious or persistent lobes that can be distinct or obscure. A thickened staminal disc is absent, while the petals are tetra or pentamerous, round, concave, and usually fall off as calyptrate lids. The numerous stamens bend inward in the bud, and the ovary is bicarpellary or tricarpellary with several ovules per locule and a single style with a simple stigma. The fruits are berries, one-celled, calyx crowned, globoid, pyriform, or oblong in shape, while the seeds are globoid with sub-globoid embryos and fleshy cotyledons. The small radicle is hidden between the rough seed coat and the cotyledons.

4.1 Morphological Characterization of *S. travancoricum* Gamble

4.1.1 Diversity and Distribution of *S. travancoricum* Gamble

The tree was initially observed by Bourdillon (1894) in the wetlands of Travancore at a height of under 65 meters. In 1919, Gamble described it for both the Kew Bulletin and the Flora of the Presidency of Madras. More recently, Sasidharan (2004) rediscovered the tree in the Shendurney Wildlife Sanctuary, which is its type locality. He stated that this unique species is found exclusively in the Myristica swamp forests of the southern Western

Ghats region in Kerala. Additionally, the species has also been found in the freshwater Myristica swamps in the Siddapur taluk and Ankola taluk of Uttar Kannada district in Karnataka. The Myristica swamps are distributed throughout the forest ranges of Kulathupuzha, Anchal, and Shendurney Wildlife Sanctuary. In the present study, we located *S. travancoricum* populations in the swampy areas and sacred groves in various parts of southern Kerala, especially in the five districts, Thiruvananthapuram, Kollam, Thrissur, Kannur and Wynad (Fig. 1). In addition to Myristica swamps, numerous populations were found in the sacred groves of Thrissur and Kannur. However, compared to all other populations, the maximum number of trees were documented in the Kalassamala sacred grove, Thrissur, followed by the districts of Thiruvananthapuram, Kollam, Wynad and Kannur.

4.1.2 Phenology

S. travancoricum is a deciduous tree that grows in wetland areas, sacred groves and along riverbanks. Consequently, this species was least affected by a lack of water. As a result, they do not drop their leaves in the same manner as other deciduous trees, which accounts for the delayed beginning of leaf loss in January, followed by flushing in mid-February. The pattern of phenological occurrences differed between populations and geographical zones. The flowering response usually begins at the beginning of April and lasts until the end of May. The subsequent phase was fruiting, which lasted until the end of June. However, in the present study, the phenology of *S. travancoricum* differed in different geographical zones, especially in the Southern and Northern parts of Kerala. Hence, the phenology of this species could be influenced by climatic factors such as temperature, humidity, rainfall, and topographic factors.

4.1.3 Morphology

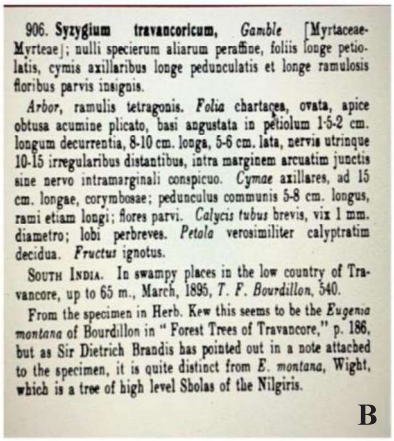
In the present research, *S. travancoricum* Gamble was described precisely using the morphological characters of the type specimens and original protologue. Furthermore, a thorough comparison was carried out with eight live populations that were collected from diverse areas in Kerala. (Fig. 1).

4.1.3.1 Morphological description of *S. travancoricum* from the type Materials and protologue

Gamble (1919) described and published the species *S. travancoricum* based on the collection of Bourdillon from the swampy places in the low country of Travancore at an altitude of up to 65 m from sea level. The type specimens (Fig. 4) possess long petioles of 2–3 cm, ovate leaves measuring 8–10 cm × 5–6 cm with a folded obtuse tip.

The leaves possess 10–15 irregularly spaced nerves on both sides, joined arcuately with the margin without visible intra-marginal nerves. The branchlets are quadrangular with axillary buds. The branches bear long pedunculate axillary cymose inflorescences which reach up to a size of 15–20 cm with a rachis length of 10 cm. The morphological characteristics observed in the type specimens completely corroborate with the protologue. In the protologue, the author states that this species is a medium-sized tree with long branches,

Syzygium travancoricum Gamble, Bull.Misc. Inform. Kew 1918(7): 240 (1918)
Protologue



906. **Syzygium travancoricum,** *Gamble* [Myrtaceae-Myrteae]; nulli specierum aliarum peraffine, foliis longe petiolatis, cymis axillaribus longe pedunculatis et longe ramulosis floribus parvis insignis.

Arbor, ramulis tetragonis. *Folia* chartacea, ovata, apice obtusa acumine plicato, basi angustata in petiolum 1-5-2 cm. longum decurrentia, 8-10 cm. longa, 5-6 cm. lata, nervis utrinque 10-15 irregularibus distantibus, intra marginem arcuatim junctis sine nervo intramarginali conspicuo. *Cymae* axillares, ad 15 cm. longae, corymbosae; pedunculus communis 5-8 cm. longus, rami etiam longi; flores parvi. *Calycis tubus* brevis, vix 1 mm. diametro; lobi perbreves. *Petala* verosimiliter calyptratim decidua. *Fructus* ignotus.

South India. In swampy places in the low country of Travancore, up to 65 m., March, 1895, *T. F. Bourdillon*, 540.

From the specimen in Herb. Kew this seems to be the *Eugenia montana* of Bourdillon in "Forest Trees of Travancore," p. 186, but as Sir Dietrich Brandis has pointed out in a note attached to the specimen, it is quite distinct from *E. montana*, Wight, which is a tree of high level Sholas of the Nilgiris.

B

Fig. 4. *Syzygium travancoricum* Gamble **A**. Lectotype © Botanical Survey of India, Coimbatore (K). **B.** Protologue

papery thin leaves with long petioles, leaf size 8–10 cm long and 5–6 cm broad with 10–15 irregularly spaced nerves on both sides, joined arcuately with the margin without visible intra-marginal nerves, ovate in shape and obtuse tip which is folded, base narrowed into petiole (1.5–2cm), long pedunculate axillary cymose inflorescences up to a size of 15 cm, common peduncle 5–8 cm long, with small flowers, short calyx tube (nearly 1mm in diameter), short lobes, petals calyptrate and deciduous, fruits small sized.

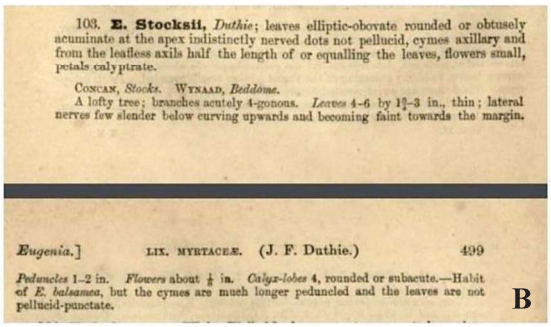
4.1.3.2 Morphological characters of collected sample populations

The morphological characteristics of live samples perfectly matched with the protologue and type materials of *S. travancoricum*, such as medium to large-sized trees; bark texture longitudinally fissured, bark greyish brown, blaze reddish brown, tetragonous branchlets, chartaceous, leaf ratio wider than long, leaf shape ovate-oblong, lamina size (area) 8–10 cm × 5–6 cm; leaf base narrow and decurrent, leaf margin entire undulate, leaf apex obtuse and folded, leaf arrangement opposite decussate, venation was parallel and regular and looped, stout petiole, petiole length 2–3 cm; the presence of groove on upper side petiole; lateral nerves 10–15 pairs cymose corymbose inflorescence in the terminal and axillary; long peduncle ranging between 8–10 cm; small creamy white flowers; pyriform hypanthium and coherent calyx. Petals are free, four-lobed; calyptrate; petal size 0.5–2.5 mm x 0.2–0.5 mm; globose flower bud; white coloured; sepals fused; triangular, green-coloured, and purple-coloured pointed tip; thick staminal disc present; white stamens; size 12.5–10.5 x 0.2–0.5 mm, bent inwardly in the bud; anther size 5–6 x 2.5–3 anther locules elongated, parallel and dehisces longitudinally, style colour is pale green, short, and simple; inferior ovary with two locules and many ovules per locule; axile placentation; fruit is a berry; deep purple-pink coloured; small fruits size 0.8–1.5 x 0.2–0.5 cm; fruit shape oblong-obtuse; single seeded; globose, brown, and smooth textured (Fig. 6–11).

4.1.3.3 Morphological description of *S. stocksii* from the type Materials and protologue

The type specimen (Fig. 5) possesses short petioles of 1–2 cm and elliptic leaves measuring 15–20 cm in length and 6–8 cm in breadth without a prominent folded obtuse tip. The leaves possess 18–20 irregularly spaced nerves on both sides, joined arcuately with the margin with distinct intramarginal nerves which faint towards the margins. The branchlets are quadrangular with axillary buds. The branches bear short pedunculate axillary cymose inflorescences which reach up to a size of 5–8 cm with a common peduncle of 2–5 cm long.

***Eugenia stocksii* Duthie, Fl. Brit. India [J. D. Hooker] 2(6): 498 (1879)**
Protologue


103. **E. Stocksii,** *Duthie*; leaves elliptic-obovate rounded or obtusely acuminate at the apex indistinctly nerved dots not pellucid, cymes axillary and from the leafless axils half the length of or equalling the leaves, flowers small, petals calyptrate.

CONCAN, *Stocks*. WYNAAD, *Beddôme*.

A lofty tree; branches acutely 4-gonous. *Leaves* 4–6 by 1½–3 in., thin; lateral nerves few slender below curving upwards and becoming faint towards the margin.

Eugenia.] LIX. MYRTACEÆ. (J. F. Duthie.) 499

Peduncles 1–2 in. *Flowers* about ½ in. *Calyx-lobes* 4, rounded or subacute.—Habit of *E. balsamea*, but the cymes are much longer peduncled and the leaves are not pellucid-punctate.

B

Fig. 5. *Syzygium stocksii (Duthie)* Gamble **A.** Lectotype © The Board of Trustee of the RBG, Kew **B.** Protologue

The morphological characteristics observed in the type specimen completely corroborate with the characters mentioned in the protologue. In the protologue, the author states that this species is characterized by large-sized trees with long branches which are acutely tetragonous, indistinctively nerved, dots, not pellucid, curving upwards and becoming faint towards the margins, with short petioles, leaf size 15–20 cm long and 6-8 cm broad, elliptic-obovate with rounded or obtusely acuminate tip, short pedunculate axillary cymose inflorescences up to a size of 5-8 cm, common peduncle 2–5 cm long, with small flowers, short calyx tube (nearly

1mm in diameter), short lobes, petals calyptrate and deciduous, fruits small sized.

However, the samples collected from Pookode (Wynad) and Paliyeri Mookambika Kavu (Kannur) areas showed distinct morphological characters that resembles to *S. stocksii* concerning the blaze colour, which was greyish brown, comparatively large-sized leaves 15–20 cm in length and 8–10 cm in breadth; elliptic to obovate leaf shape rather than ovate, obtuse leaf base, obtusely acuminate leaf tip without oblique ends, short and thin petiole without a prominent groove, the number of lateral nerves ranged between 18–20, and prominent intra marginal veins which fainted towards the margins (Fig. 4–9). The inflorescence seen in these populations was comparatively shorter, having a 5–10 cm size with a peduncle length of 2–5 cm. Each inflorescence possessed 20–200 blooms. The flowers were small and actinomorphic, with triangular, deciduous bracts without purple pointed tips. The flowers were complete, hermaphroditic, and cyclic. The corolla had four imbricate and deciduous petals. The petals were 0.32 to 0.64 mm wide and 4.2 to 6.84 mm long. About 160–200 stamens formed a ring around the calyx tube entrance in the androecium. Anthers were white and longitudinally dehiscing, while filaments were yellow. The filament length ranged from 6.5 to 12.57 mm, and the anthers were 0.32 to 0.43 mm in size. The ovary was inferior, bi-carpellary, bilocular, syncarpous, with axile placentation, and had many ovules. The ovary was 1.8–3.52 mm long. The stigma was simple, white and 2.83–4.46 mm long. After thoroughly analysing the morphology of eight populations, the present research identified distinct morphological characteristics to determine the species' distinctiveness among *S. travancoricum* populations. As we could not identify overlapping characters in the sample populations, we consider these two populations distinct and reinstate *S. travancoricum* here. The

combination of morphological and genetic traits can further be utilised to validate our findings for determining species distinctiveness between more *Syzygium* populations and reconstructing these taxa's phylogeny. A few morphological traits, such as blaze colour, leaf size, leaf shape, leaf base, leaf tip, petiole size, the number of lateral nerves in the leaf blade, the size and position of the inflorescence, the length of the peduncle, and the nature of sepals are found to be diagnostic in distinguishing the two species.

Specimens examined:

INDIA. **Kerala,** Kollam Dist.: Aickad, 4 Apr. 1988, C. N. Mohanan CAL0000029238

INDIA. **Kerala,** Kollam Dist.: Kodumon, 1 Mar. 1979, C. N. Mohanan CAL00000214404

INDIA. **Kerala,** Wayanad Dist.: Lakkidi, 20 Mar. 1984, R. T. Balakrishnan CALI162932

INDIA. **Kerala,** Wayanad Dist.: Pookode, 10 Apr. 2001, A. K. Pradeep CALI162930

INDIA. **Kerala,** Wayanad Dist.: Pookode, 10 Apr. 2001, A. K. Pradeep CALI162931

INDIA. **Kerala,** Travancore, 6 Mar. 1994, T. F. Bourdillon MH00002090

INDIA. **Kerala,** Koncan, 26 Feb. 2004, P. Lakshminarasimhan K000260039

INDIA. **Kerala**, Thiruvananthapuram Dist.: JNTBGRI Palode, 24 Apr. 2019, *P.S.Smitha* 320 STC03055

INDIA. **Kerala**, Thiruvananthapuram Dist.: Idinjaar, 24 Apr. 2019, *P.S. Smitha* 321 STC02988

INDIA. **Kerala**, Kollam Dist.: Shendurney, 24 Apr. 2019, *P.S. Smitha* 322 STC03084

INDIA. **Kerala**, Thrissur Dist.: Kalassamala, 26 Apr. 2019, *P.S. Smitha* 323 STC03075

INDIA. **Kerala**, Wynad Dist.: Pookode, 27 Apr. 2019, *P.S. Smitha* 324 STC03013

INDIA. **Kerala**, Wynad Dist.: MSSRF, 27 Apr. 2019, *P.S. Smitha* 325 STC03093

INDIA**. Kerala**, Kannur Dist.: Konginichalkavu, 28 Apr. 2019, *P.S. Smitha* 326 STC03077

INDIA. **Kerala**, TBGRI, Palode, 30 Apr. 2019, *P.S. Smitha* 327 STC03037

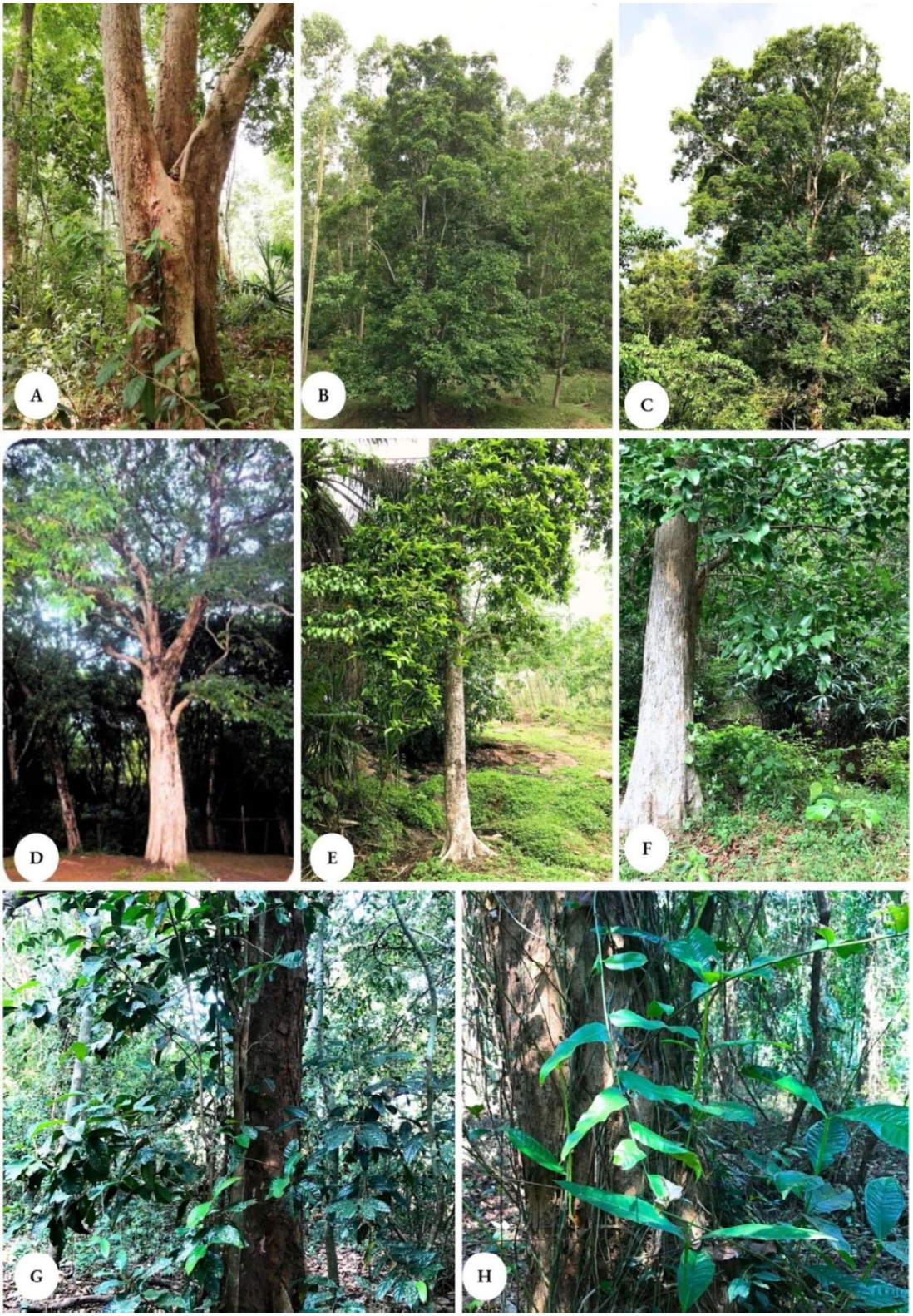


Fig. 6. ***Syzygium travancoricum*** **–** Habit. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

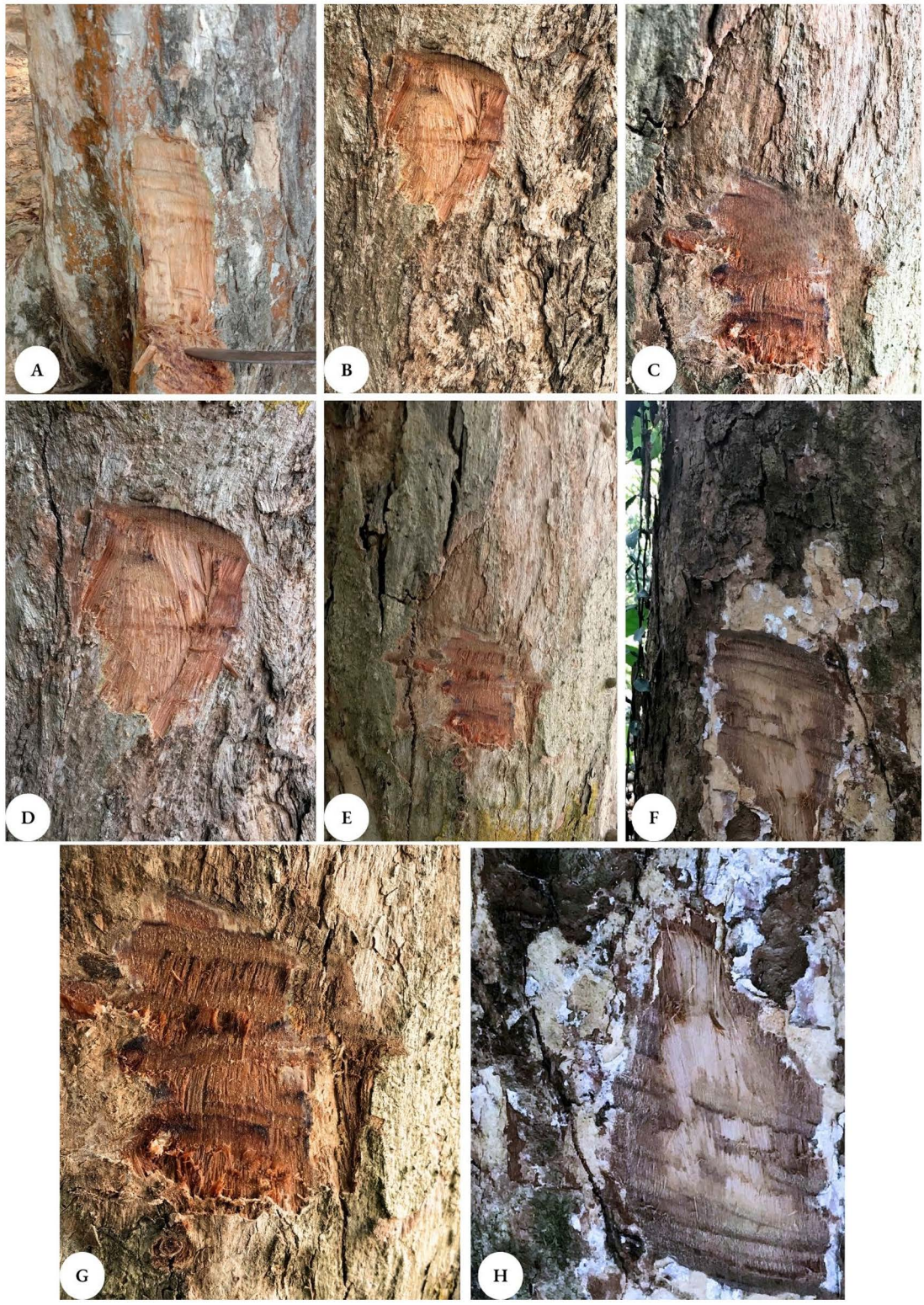


Fig. 7. ***Syzygium travancoricum*** **-** Blaze. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

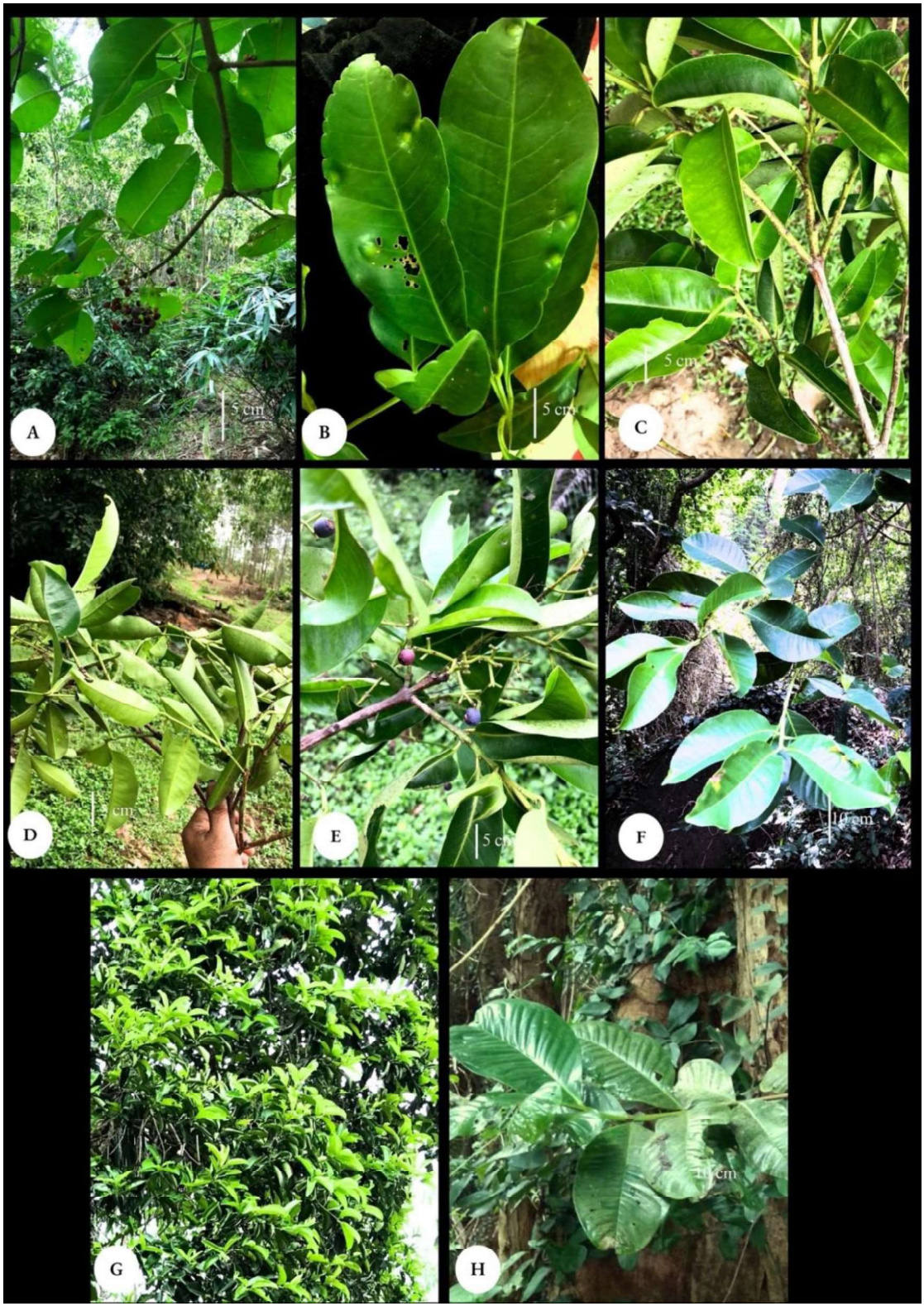


Fig. 8. ***Syzygium travancoricum*** **-** Leaf. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

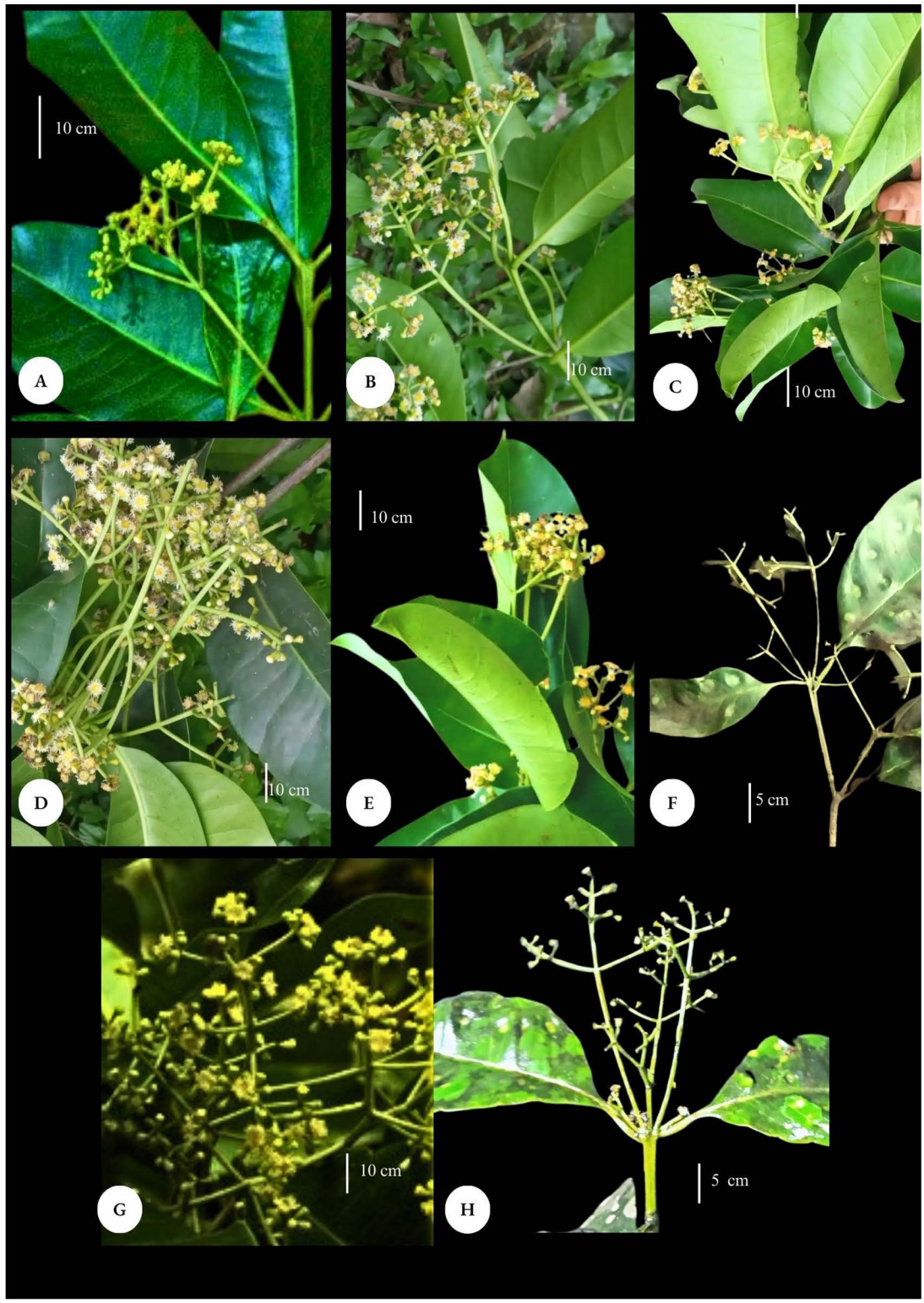


Fig. 9. ***Syzygium travancoricum*** **-** Inflorescence. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

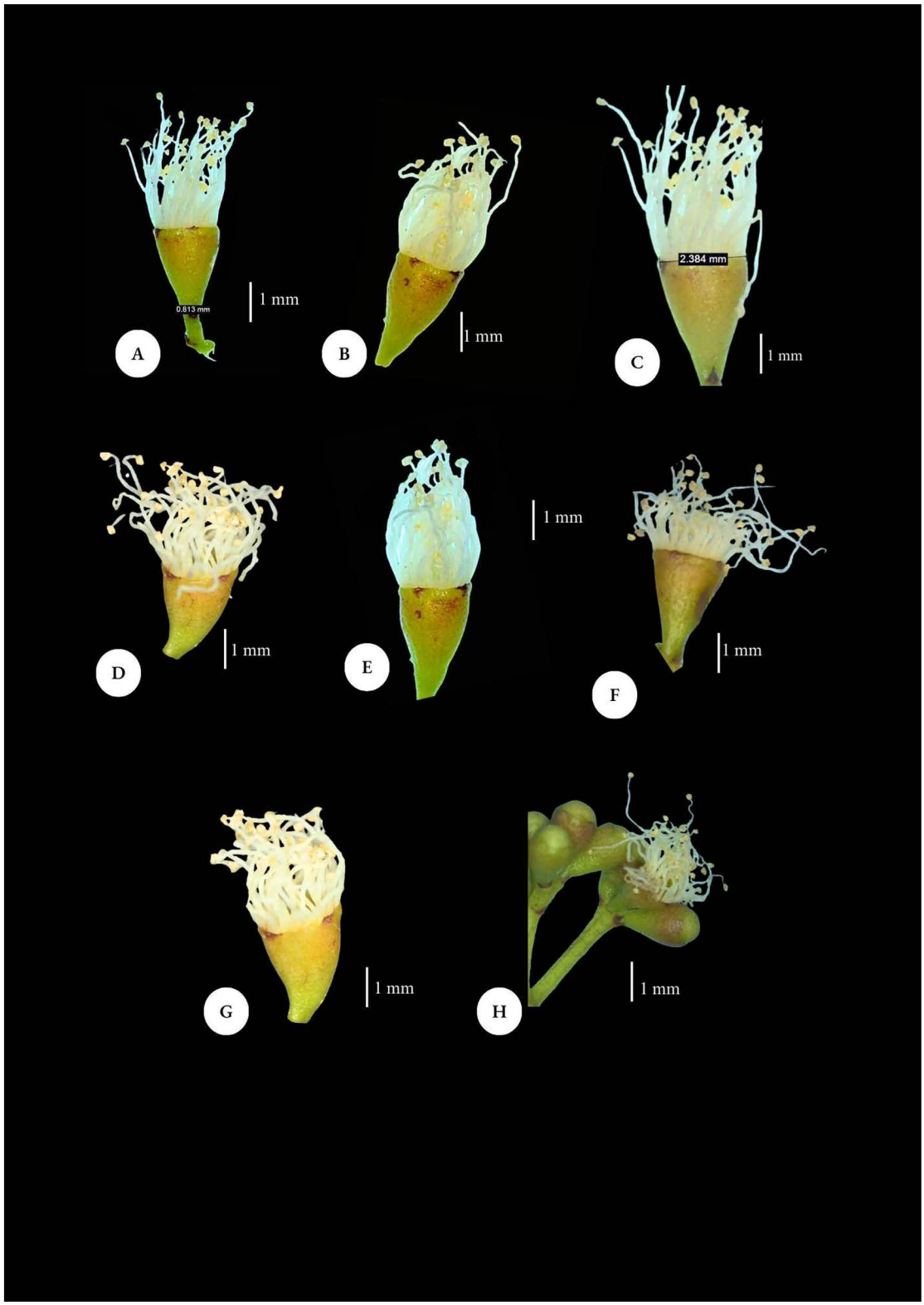


Fig. 10. ***Syzygium travancoricum*** **-** Flower. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

Fig. 11. ***Syzygium travancoricum*** **-** Fruits. **A.** JNTBGRI**; B.** Idinjaar**; C.** Kollam**; D.** Thrissur**; E.** MSSRF**; F.** Pookode**; G.** Konginichalkavu**; H.** Paliyeri Mookambika Kavu

4.2 Anatomical and Histochemical Characterization

The observations of the anatomical features of *S. travancoricum* samples are presented in Fig. 12–16. The images highlight the secretory structures and anatomical characteristics of the bark, stem, petiole, and lamina.

4.2.1 Bark

Cross-sections of the bark revealed periderm with phellem, phellogen, phelloderm and phloem (Fig. 12). The phelloderm region comprised approximately ten to twelve layers of parenchymatous cells (Figure 12A-H), with abundant stone cells, and was represented as a conspicuous feature of all the samples analyzed. The periderm appeared curved with discontinuous layers of radially flattened phelloderm. The discontinuous nature of the phelloderm may be due to irregular patches of sclereids and druses. However, the phellem comprised of compactly packed rectangular cells arranged in radial rows. Sclerenchymatous fibres surrounded the secondary phloem which may be the remnants of deteriorated primary phloem. Extra stelar regions, including the secondary phloem, contained druses in all samples (Figures 12A, B, C, D, E and G). However, prismatic crystals were present predominantly instead of druses in samples collected from Pookode and Paliyeri Mookambika Kavu (as observed in 12F and H). The phellogen layer was continuous and apparent in all the samples examined.

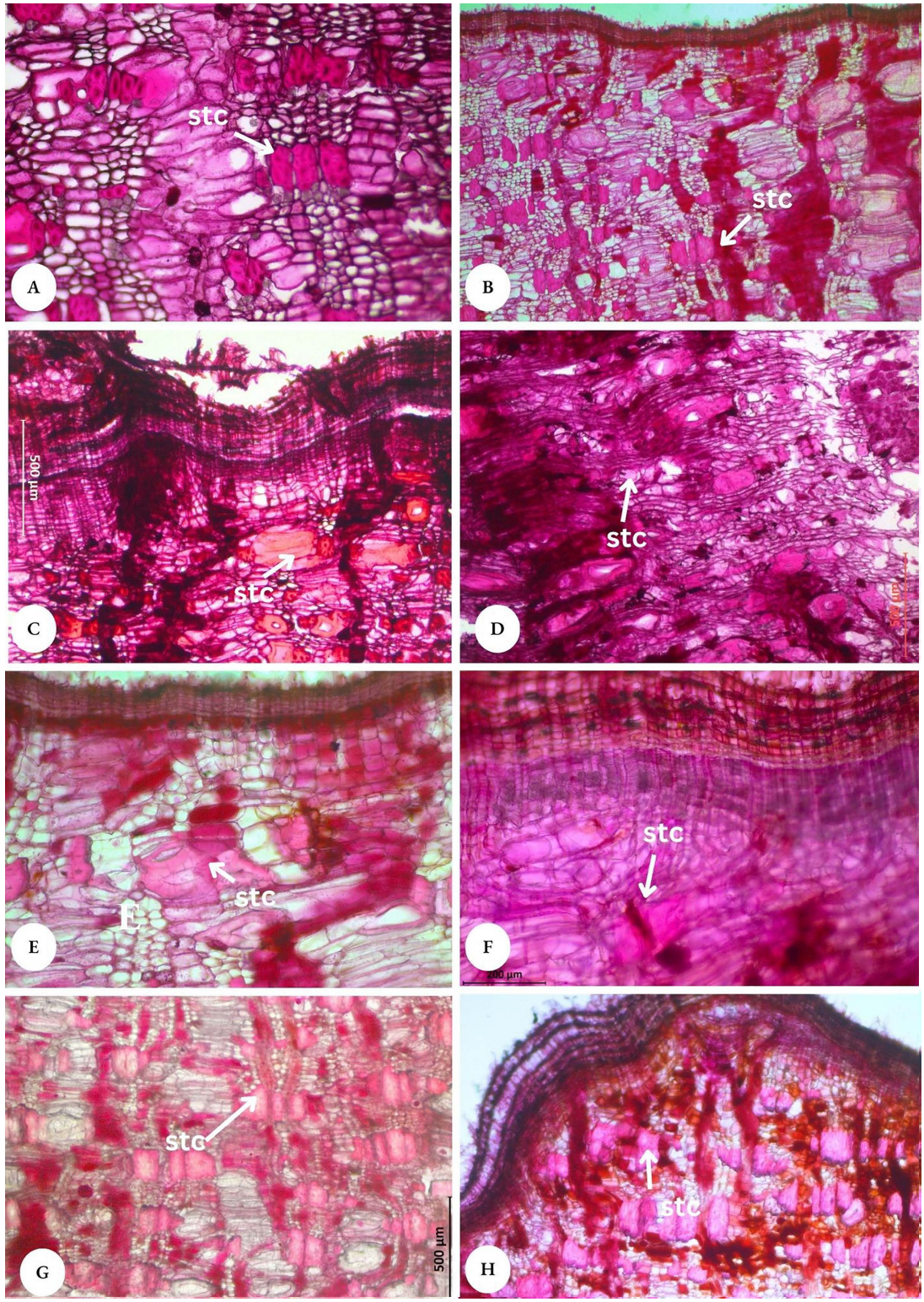


Fig. 12. ***Syzygium travancoricum*** - Bark T.S. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu

Bars: A: 200 µm, B-H: 500 µm

4.2.2 Stem

The secondary stem exhibited a quadrangular outline with a well-defined periderm in cross-section. The periderm comprised continuous layers of radially flattened phellem, a narrow band of phellogen, and a parenchymatous phelloderm. The phelloderm consisted of isodiametric parenchyma cells mixed with lignified cells, stone cells, druses, and starch depositions (Fig. 13). The druses were scattered around the vascular system, phloem and pith region. Druses were prominent over prismatic crystals in all samples except Pookode and Paliyeri Mookambika Kavu, which possessed prismatic crystals rather than druses (Fig. 13a. F and H). The vasculature of the stem was of open and bicollateral type.

The phloem was externally and internally located to the xylem, forming a continuous ring. The internal phloem contained a continuous band of stone cells and druses. In contrast, Pookode and Paliyeri Mookambika Kavu samples possessed discontinuous bands of stone cells with scattered prismatic crystals. Moreover, the arrangement of internal phloem and stone cells appeared wavy or cross-shaped in all samples except Pookode and Paliyeri Mookambika Kavu, where no such arrangement pattern was observed. The central pith region comprised parenchyma, lignified cells, stone cells, druses, and starch grains in all samples. However, it was worth noting that stone cells and lignified cells were absent in the samples collected from Pookode and Paliyeri Mookambika Kavu (Fig.13b. F and H).

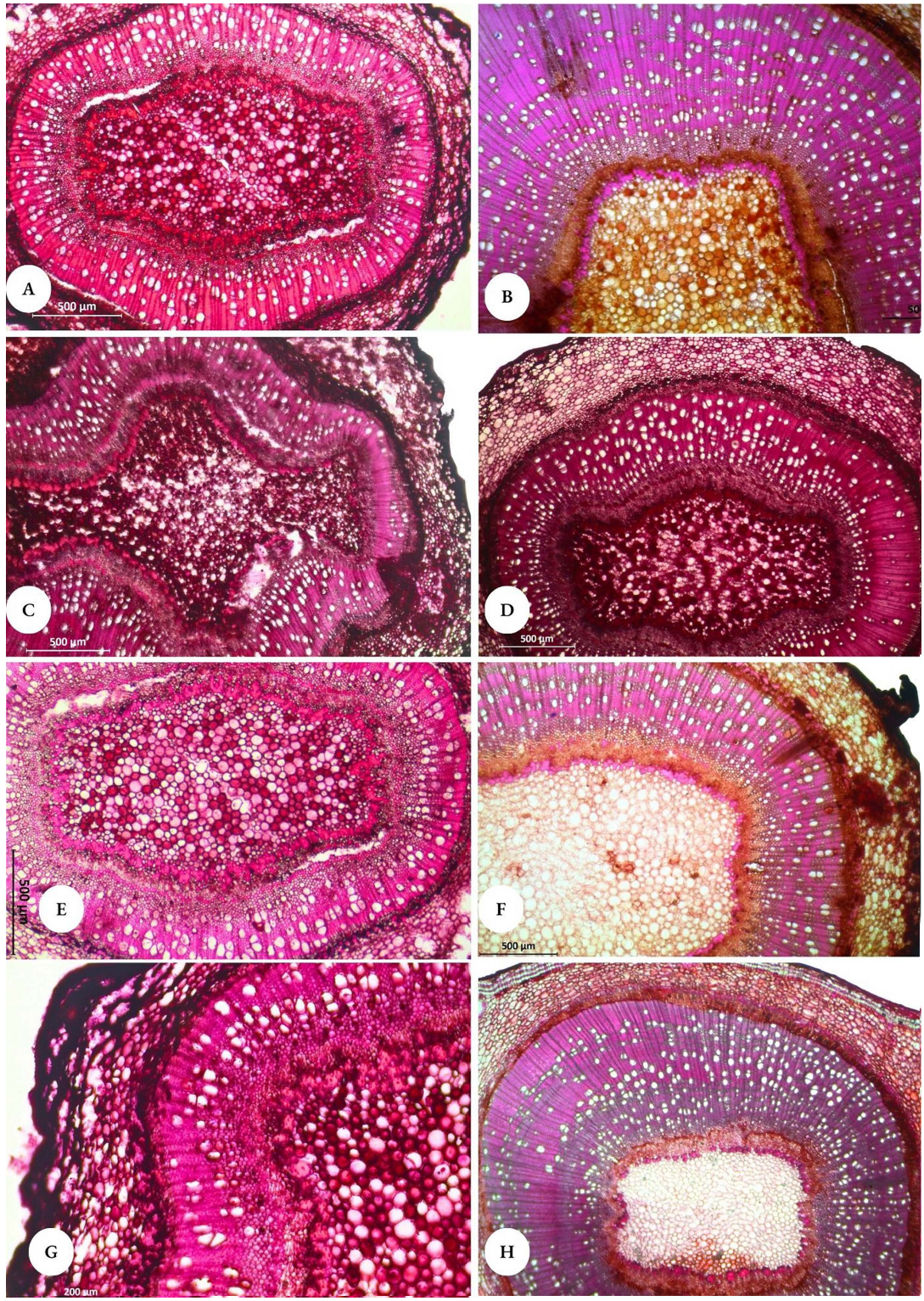


Fig. 13a. ***Syzygium travancoricum*** - Stem T.S. **A.** JNTBGRI**; B.** Idinjaar**; C.** Kollam**; D.** Thrissur**; E.** MSSRF**; F.** Pookode**; G.** Konginichalkavu**; H.** Paliyeri Mookambika Kavu

Bars: A-H: 500 µm G: 200 µm

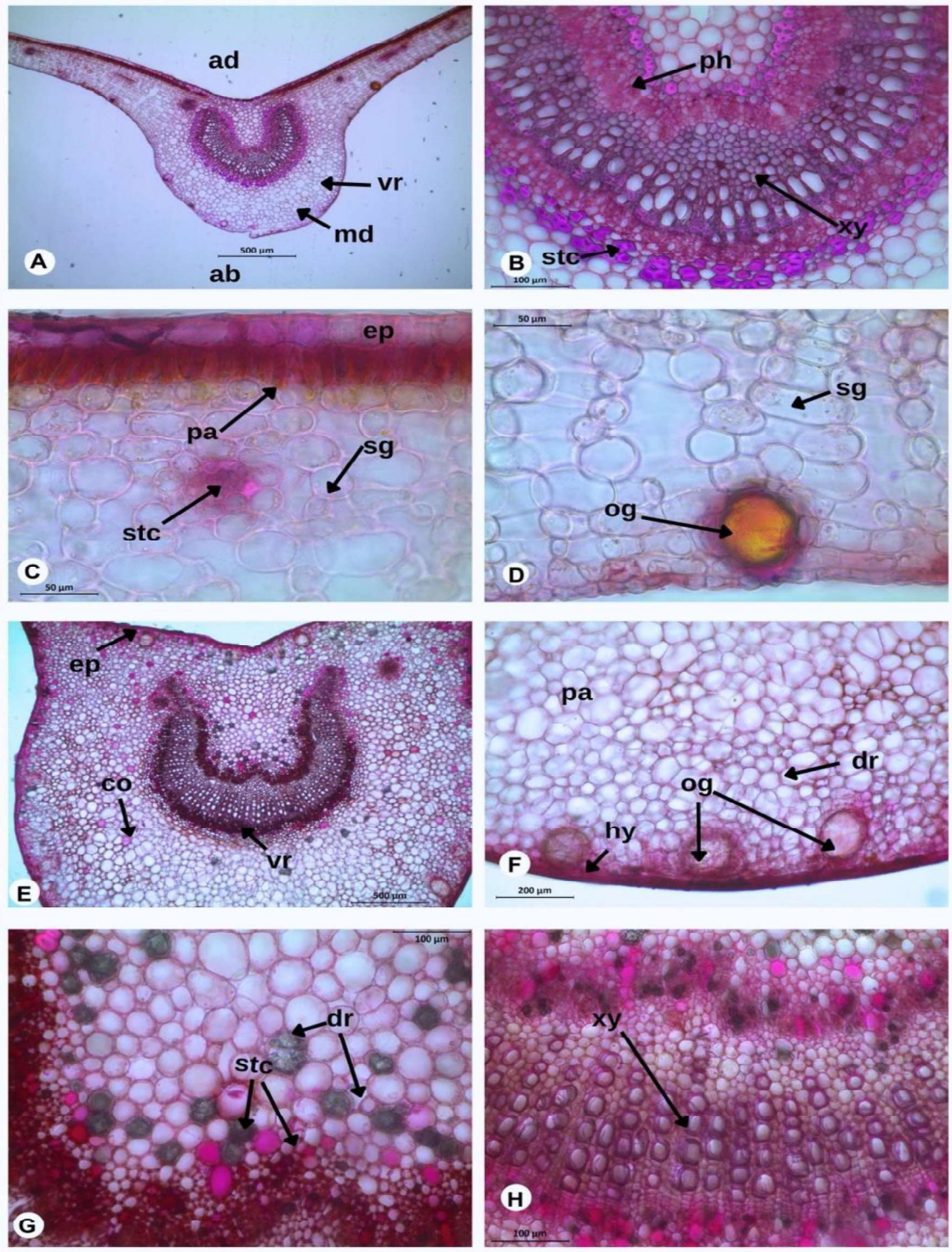


Fig. 13b. ***Syzygium travancoricum*** **–** Distribution of starch grains, sclereids, druses and oil glands in lamina and petiole **A.** Lamina - general view; **B**. Midrib; **C.** Starch grains, sclereids and druses; **D.** Oil glands; **E.** Petiole - General view; **F.** Oil glands; **G.** Stone cells and druses; **H.** Vascular system. pa: parenchyma; co: cortex; dr: druses; hy: hypodermis; ph: phloem; xy: xylem; stc: stone cells; og: oil glands. **Bars:** A, E: 500 µm; B, G, H: 100 µm; F: 200 µm; C, D: 50 µm.

4.2.3 Lamina

The cross-section of the lamina revealed a concave to convex midrib and two narrow side wings. The adaxial surface of epidermal cells was straight with an arc-shaped abaxial surface. The upper and lower epidermis consisted of single-layered radially flattened parenchymatous cells protected by a thin cuticle (Fig. 15). However, the adaxial epidermis was broader than the abaxial epidermis. Stomata were observed to be of both paracytic type in all samples collected (Fig. 14A) except Pookode and Paliyeri Mookambika Kavu populations which had anisocytic stomata (Fig. 14B). The mesophyll layer was composed of single-layered palisade parenchyma at the adaxial region and four to five layers of spongy parenchyma at the abaxial region.

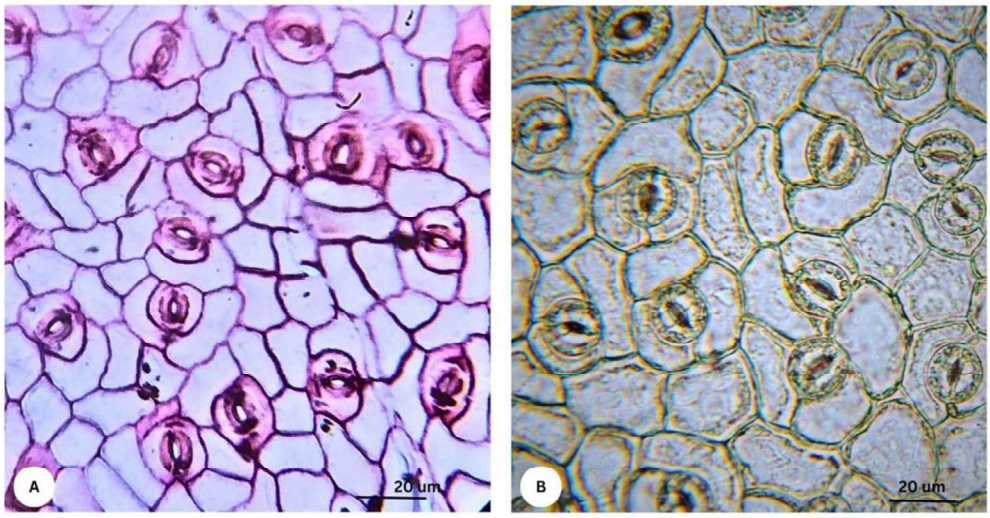


Fig. 14. ***Syzygium travancoricum*** **-** Stomata **A.** Paracytic **B**. Anisocytic
Bars: 20 μm

The leaf anatomy revealed a closed type of vascular system. The vascular system at the midrib region appeared 'U-shaped' with straight arms. It comprised a larger bicollateral vascular bundle extending from the abaxial face to the adaxial face with outer and inner patches of phloem (Fig. 15). There was a continuous sheath of sclerenchyma around the vascular bundle. The phloem region showed an abundance of sclereids and druses. Druses were

also present in the midrib parenchymatous and mesophyll layers. Oil glands were found dispersed in the mesophyll layers. The oil glands seemed bigger than the surrounding cells and bordered with small parenchyma cells. The palisade and spongy mesophyll tissue also had abundant oil glands and druses (Figure 15C, D).

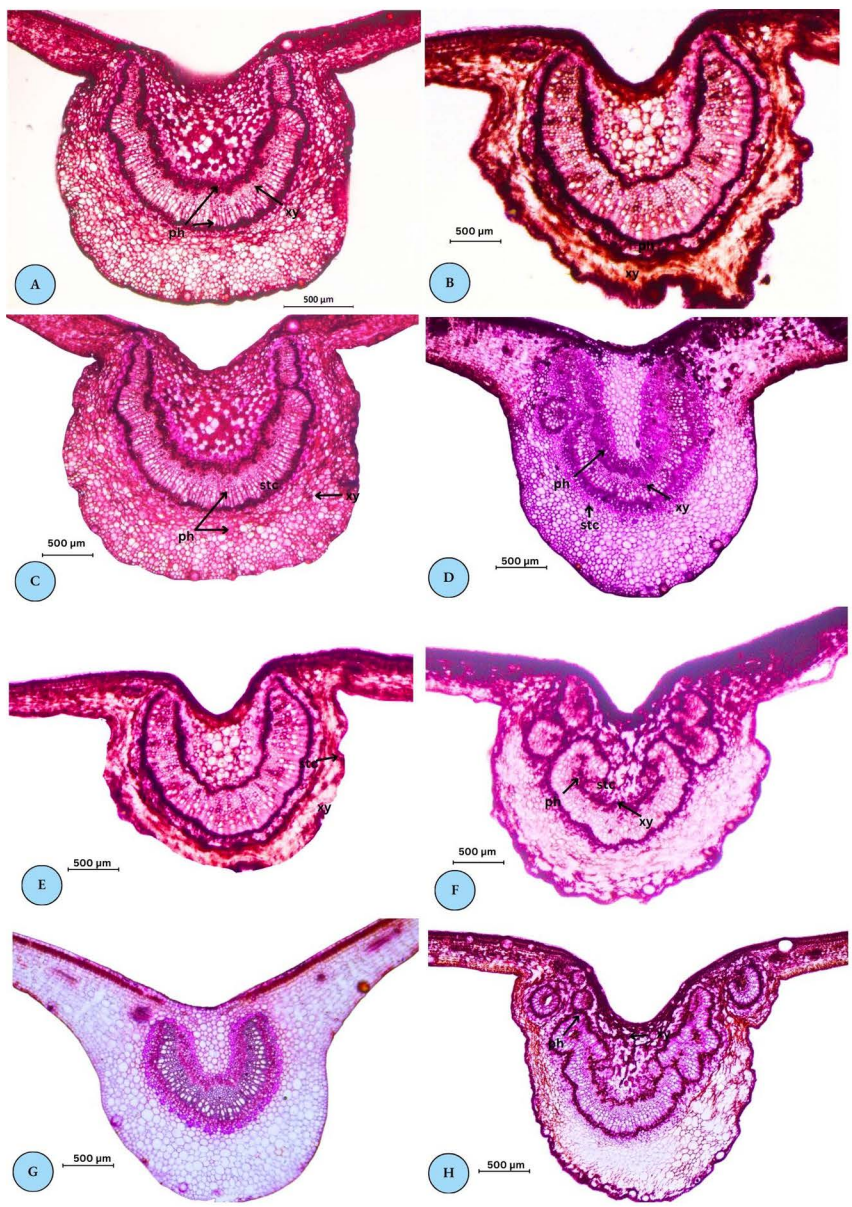


Fig. 15. ***Syzygium travancoricum*** - Lamina T.S. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu
Bars: A-H: 500 µm

The leaf anatomy of the Pookode and Paliyeri Mookambika Kavu samples differed from other samples regarding midrib vasculature. In both samples, the midrib vasculature displayed a unique 'U-shaped' pattern with incurved margins, which was straight in other samples. Moreover, both samples also exhibited another distinct feature, i.e., presence of adaxial phloem partitions, which was absent in other samples.

4.2.4 Petiole

The petiole was observed to have a cylindrical shape in its transverse section (Fig. 16). The adaxial surface had a V-shape and extended outward. In contrast, the abaxial surface had a U-shape. The cortical zone was found to have numerous essential oil cavities, mainly in the abaxial hypodermal region. The vasculature of the petiole had an open-type and U-shape, with intra and extra xylary phloem. The cortical region had more than eight layers of parenchyma cells. Additionally, the cortical region contained an abundance of druses, sclereids, and tannin depositions. The petiole anatomy of Pookode and Paliyeri Mookambika Kavu samples differed from other samples regarding midrib vasculature. In both samples, the midrib vasculature displayed an incurved margin, while it was straight in the other samples.

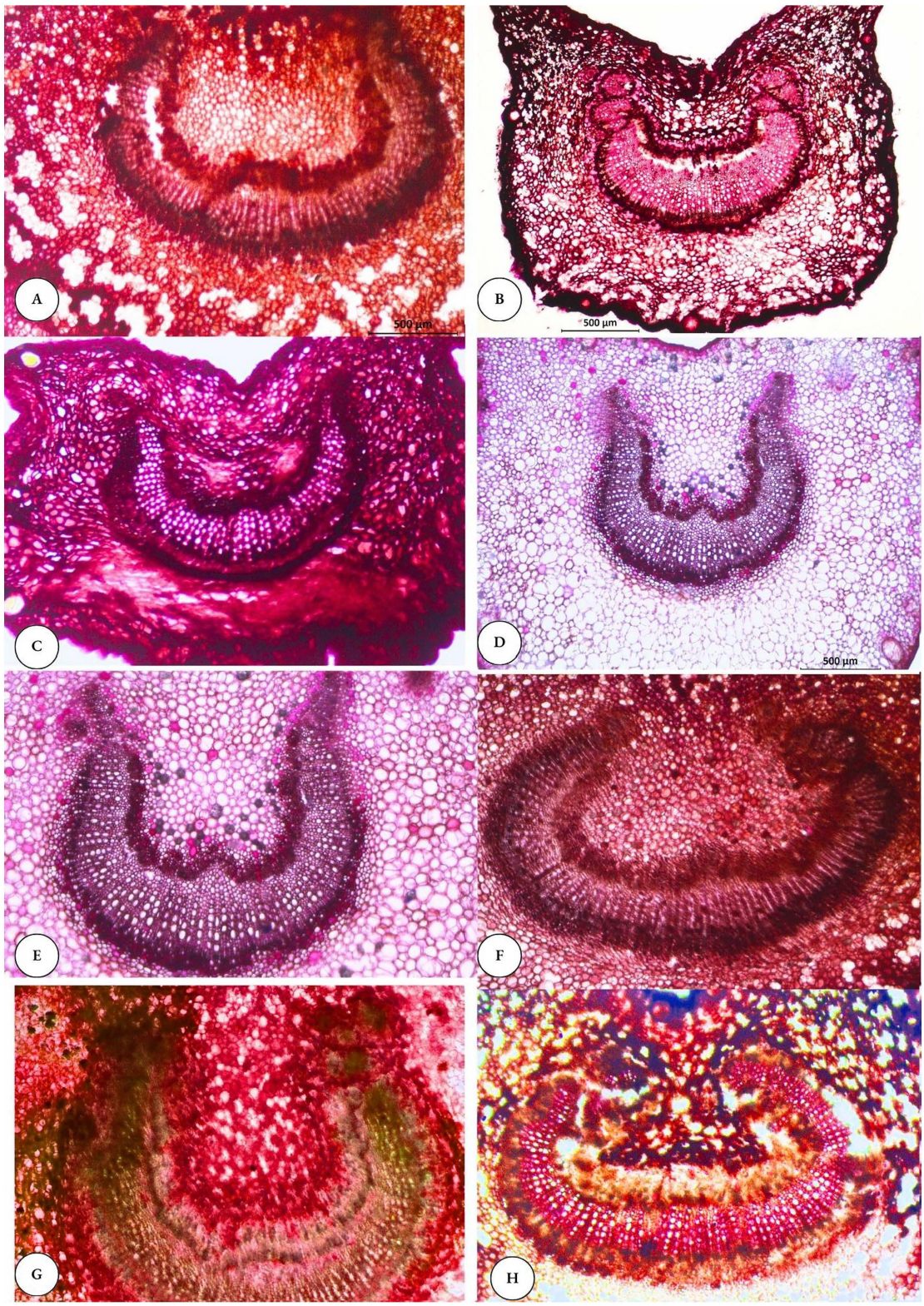


Fig. 16. ***Syzygium travancoricum*** **-** Petiole T.S. **A.** JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.**Thrissur; **E.** MSSRF; **F.** Pookode; **G.** Konginichalkavu; **H.** Paliyeri Mookambika Kavu
Bars: A-H: 500 µm

4.2.5 Comparative Morphology and Anatomy

The eight populations exhibited similar reproductive features, yet some morphological traits such as blaze colour, leaf ratio, leaf shape, leaf base, petiole size, the number of lateral nerves in the leaf blade, the position of the inflorescence, the size of the peduncle, size of the inflorescence, and fruit colour proved useful in distinguishing the populations. Based on the comparative morphological and anatomical analysis, the populations were differentiated into two species, *S. travancoricum* and *S. stocksii* (Table 2). The samples collected from JNTBGRI, Idinjaar, Kollam, Thrissur, MSSRF and Konginichalkavu populations shared core characteristics of *S. travancoricum* with those stated by Gamble in the protologue of *S. travancoricum* and the type material of *S. travancoricum* deposited at the Royal Botanical Gardens Kew herbaria (K000821396). Specifically, *S. travancoricum* exhibited a reddish brown coloured blaze, ovate-oblong leaves measuring between 8–10 cm x 5–6 cm, a narrow and decurrent leaf base, apex acuminate and folded, prominent lateral nerves consisting of 10–15 pairs, and petiole size of 2–3 cm, stout and grooved above, characteristically long peduncles, inflorescence occurred both as axillary and terminal cymes and the fruits were small sized, deep pinkish to purple coloured.

On the other hand, *S. stocksii* had a greyish-brown blaze colour and elliptic-obovate leaves, which were much larger in size, ranging from 15–20 x 8–10 cm, with 18–20 pairs of lateral nerves and short petiole of 0.5–1 cm, inflorescence arose as axillary cymes from leafless axils and was short sized with a peduncle size ranging between 2.5–5 cm. The fruits were comparatively large-sized and had a deep purplish-black colour on ripening. The plant had actinomorphic flowers with triangular, deciduous bracts, and each inflorescence had 20–200 blooms. Despite being hermaphrodite, the flower has a long anther-stigma distance. The ovary annexed the floral

receptacle, which appeared to be pyriform with a shallow cavity. The calyx had four sepals, and the corolla was comprised of four to five imbricate and deciduous petals. The ovary was inferior, bicarpellary, bilocular, and syncarpous, with axile placentation and many ovules. The flowers showed a white style with a stigma almost completely obscured by stamens. Hence, morphological investigation clearly demonstrated the distinctiveness among *S. travancoricum* populations to distinguish them as two species.

While all samples shared similar bark anatomy, variations in lamina, petiole, and stem anatomies were observed. Specifically, leaf samples from JNTBGRI, Idinjaar, Kollam, Thrissur, MSSRF, and Konginichalkavu populations displayed a prevalence of paracytic stomata. Lamina anatomy revealed a high abundance of druses and sclereids. At the same time, the midrib vasculature of the lamina and petiole displayed a distinct 'U shape' with straight margins and no adaxial phloem partitions. The distribution of sclerenchyma cells around the vascular tissues in the midrib region of the lamina showed continuity, with the adaxial palisade parenchyma being uniseriate and continuous. In contrast, leaf samples from Pookode and Paliyeri Mookambika Kavu displayed anisocytic stomata, a prevalence of prismatic crystals, U-shaped midrib vasculature with incurved margins and adaxial phloem partitions, discontinuous patches of sclereids around the midrib-vascular tissues, and a uniseriate discontinuous layer of adaxial palisade parenchyma. The stem T.S exhibited a terete outline and a rectangular pith that was predominantly parenchymatous.

Table 2. Comparative morphological differences between *Syzygium travancoricum* and *Syzygium stocksii*

Sl. No	Characters	*S. travancoricum*	*S. stocksii*
1	Habit	Medium tree	Large tree
2	Blaze colour	Reddish brown	Greyish brown
3	Leaf ratio	Wider than long	2 times longer than wide
4	Leaf shape	Ovate oblong	Elliptic obovate
5	Lamina size (area)	8–10 x 5–6 cm	15–20 x 8–10 cm
6	Leaf base	Narrow, decurrent	Obtuse
7	Leaf margin	Entire undulate	Entire undulate
8	Leaf apex	Obtuse folded	Obtusely acuminate
9	Petiole size	Stout	Thin
10	Petiole length	2–3 cm	0.5–1 cm
11	Petiole shape	presence of groove on upper side	no groove on upper side
12	No. of lateral nerves	10–15 pairs	18–20 pairs
13	Inflorescence position	terminal and axillary	axillary
14	Peduncle length	8–10 cm	2.5–5cm
15	Petal shape	4–5 lobed calyptrate	4–5 lobed calyptrate
16	Flower bud shape	dome	pyriform
17	Sepal shape	triangular, green coloured with purple tips	triangular, green coloured, rounded
18	Anther size	5–6 x 2.5–3 mm	3.5–4.5 x 2.5–1.5 mm
19	Fruit size	0.5–1.0 x 0.2–0.3 cm	1.0–1.5 x 0.2-0.5 cm
20	Fruit colour	Pinkish-purple	Purplish-black
21	Fruit shape	Sub-globose	Sub-globose

Table 3. Comparative anatomical differences between *Syzygium travancoricum* and *Syzygium stocksii*

Sl. No.	Characters	*S. travancoricum*	*S. stocksii*
1.	Lateral vein orientation	Parallel, regular and looped	Parallel, regular and looped
2.	Lateral vein loop	Present	Present
3.	Stomata	Paracytic	Anisocytic
4.	Epidermis abaxial anticlinal cell wall	Straight	Straight
5.	Lamina cuticular ornamentation	Smooth	Smooth
6.	Leaf crystals	Druses predominant; Prismatic crystals absent/infrequent	Prismatic crystals predominant; druses absent or infrequent
7.	Midrib adaxial surface cross-section	Grooved	Grooved
8.	Midrib abaxial surface cs	Convex	Convex
9.	Midrib adaxial phloem partition	Absent	Present
10.	Midrib vascular shape	Arc with straight margins	Arc with incurved margins
11.	Midrib sclerenchyma	Continuous	Discontinuous
12.	Lamina adaxial cell shape (cs)	Equal width and height	Equal width and height
13.	Lamina abaxial cell shape (cs)	Equal width and height	Equal width and height
14.	Adaxial palisade layer	Single layer	Single layer
15.	Adaxial palisade layer-arrangement	Continuous	Discontinuous
16.	Foliar sclereids	Present	Present
17.	Twig cross-section (mature)	Quadrangular	Terete
18.	Pith	Angular	Rectangular

4.2.5.1 Phylogenetic Analysis using morphological and anatomical characters

The phylogram represented UPGMA tree of *S. travancoricum* populations (red highlighted) from southern Western Ghats indicating the phenetic relationships at the inner nodes. However, two populations, Pookode and Paliyeri Mookambika Kavu (green highlighted), diverged out into another distinct clade, showing close relatedness to *S. stocksii* (Fig. 19).

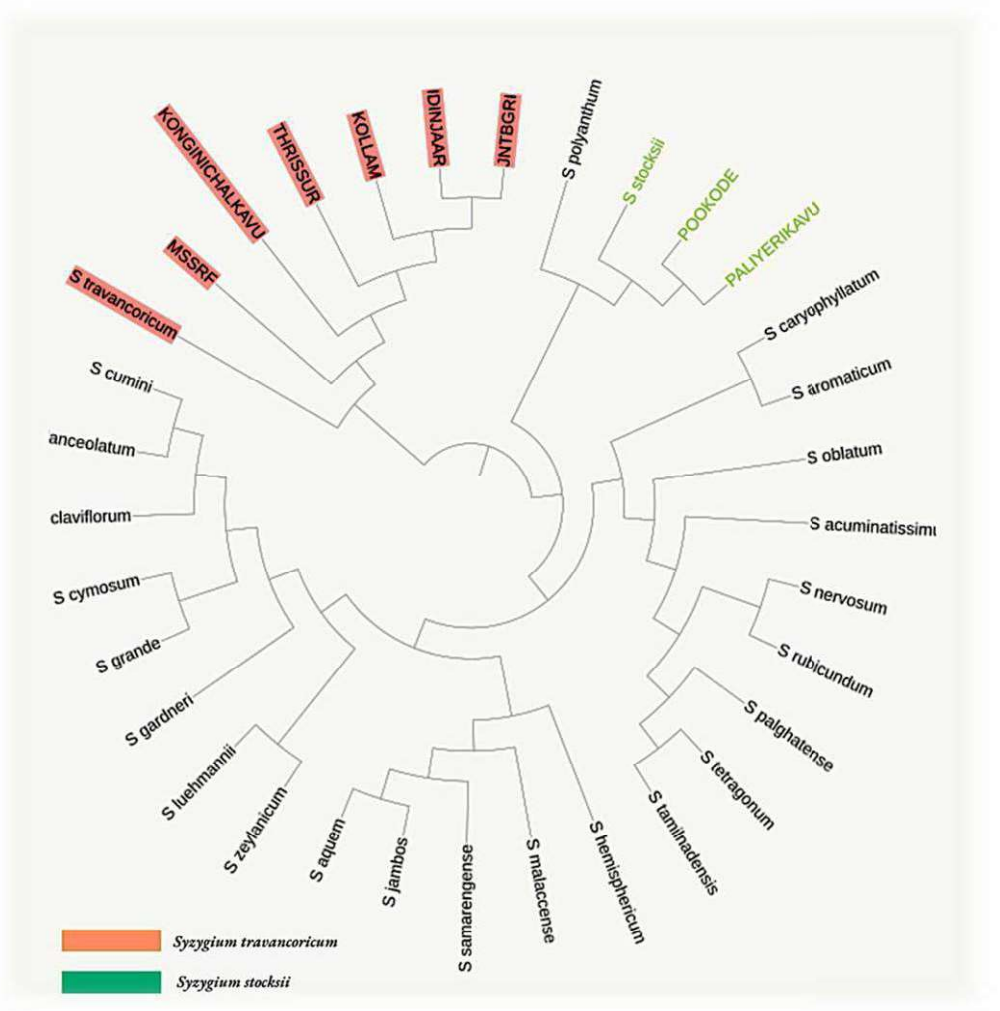


Fig. 17. UPGMA Phylogram of *Syzygium travancoricum* based on morphological and anatomical characters

4.2.6 Histochemical Analysis

Through histochemical analysis, it was discovered that *S. travancoricum* populations contain valuable secondary metabolites such as tannin, lignin, starch, and essential oils in various plant parts. Interestingly, oil cavities were not only present in the leaves but also in the petiole and stem. Moreover, the study also identified that many secretory substances were tested positive in the histochemical analysis, indicating their accumulation within the cells. It is evident that these substances contribute significantly to the distinct aroma of the leaves.

Extensive lignin deposition was observed in the mechanical tissues and xylem walls of *S. travancoricum.* This was distinguishable by the pinkish-purple hue when treated with Phloroglucinol S reagent in the cross-sections of the bark, lamina, stem, and petiole. Moreover, Sudan Red revealed the appearance of orange-pink oil glands dispersed throughout the cortical and hypodermal region of the lamina, petiole, and stem (Figure 17C, 17G, 18C , 18E). Ferric chloride treatment showed greyish-black coloration, indicating the presence of tannin in different plant parts. Tannin was found in the phellem and secondary cortex of bark, as well as in the hypodermal, cortical, and pith regions of stem and petiole, and the hypodermal and midrib region of leaves (Figure 17B, 17F, 18B, 18F). This finding suggests that tannin and lignin can accumulate in specific secretory structures and ground tissue. Iodine solution treatment revealed that starch was the abundant reserve food material in almost every part of the plant, especially in the mesophyll cells of leaves, cortex, and pith regions of stem and petiole, which appeared deep blue (Figure 17A, 17E, 18A, 18G). Thus, an extensive histochemical analysis of eight *S. travancoricum* populations revealed that all major phytochemical constituents, such as tannin, lignin, starch, and essential oils, were invariably present in all populations.

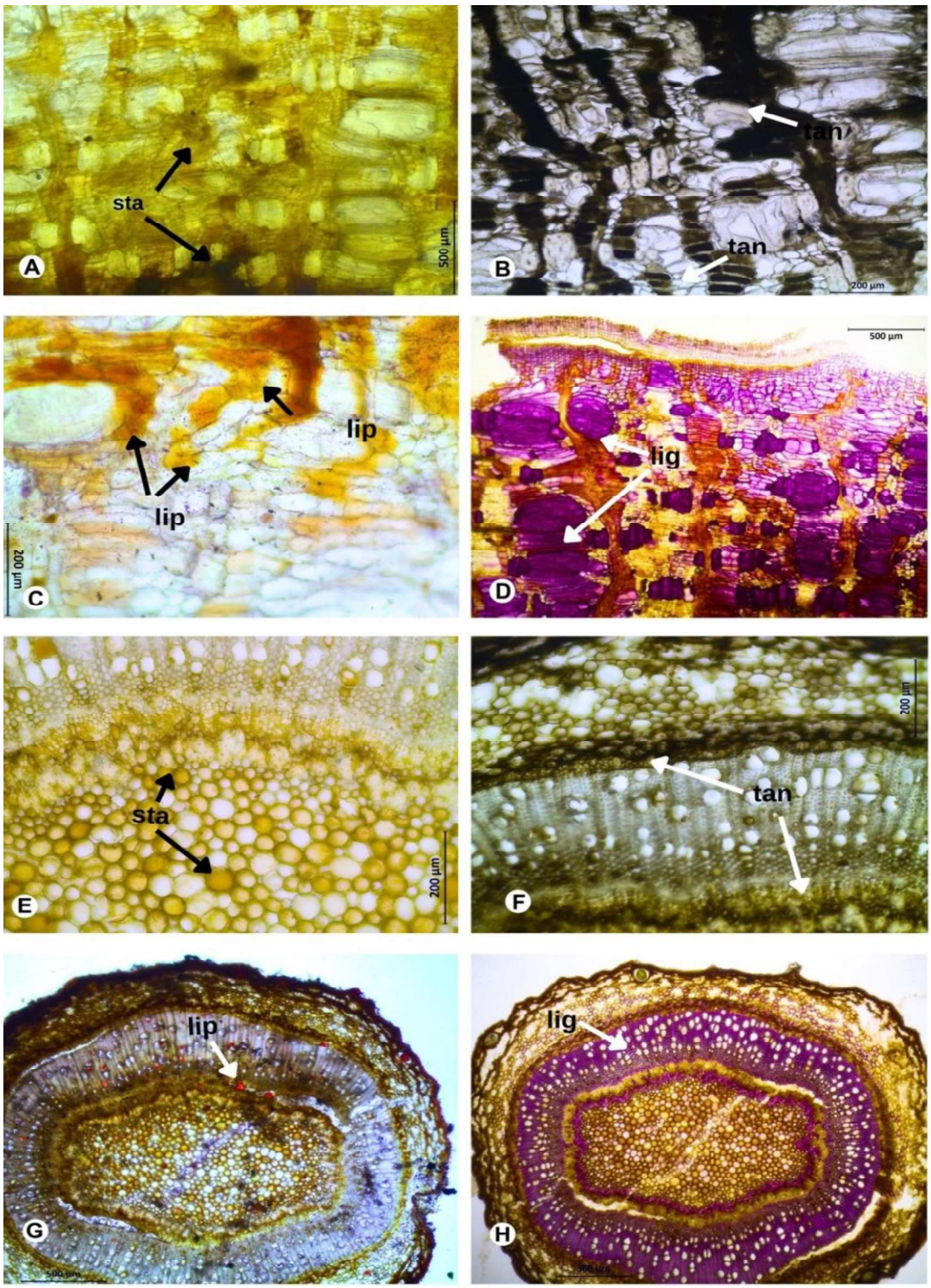


Fig. 18. ***Syzygium travancoricum*** **-** Histochemical analysis of bark and stem

A. E. Starch **B, F.** Tannin **C, G.** Lipid **D, H.** Lignin

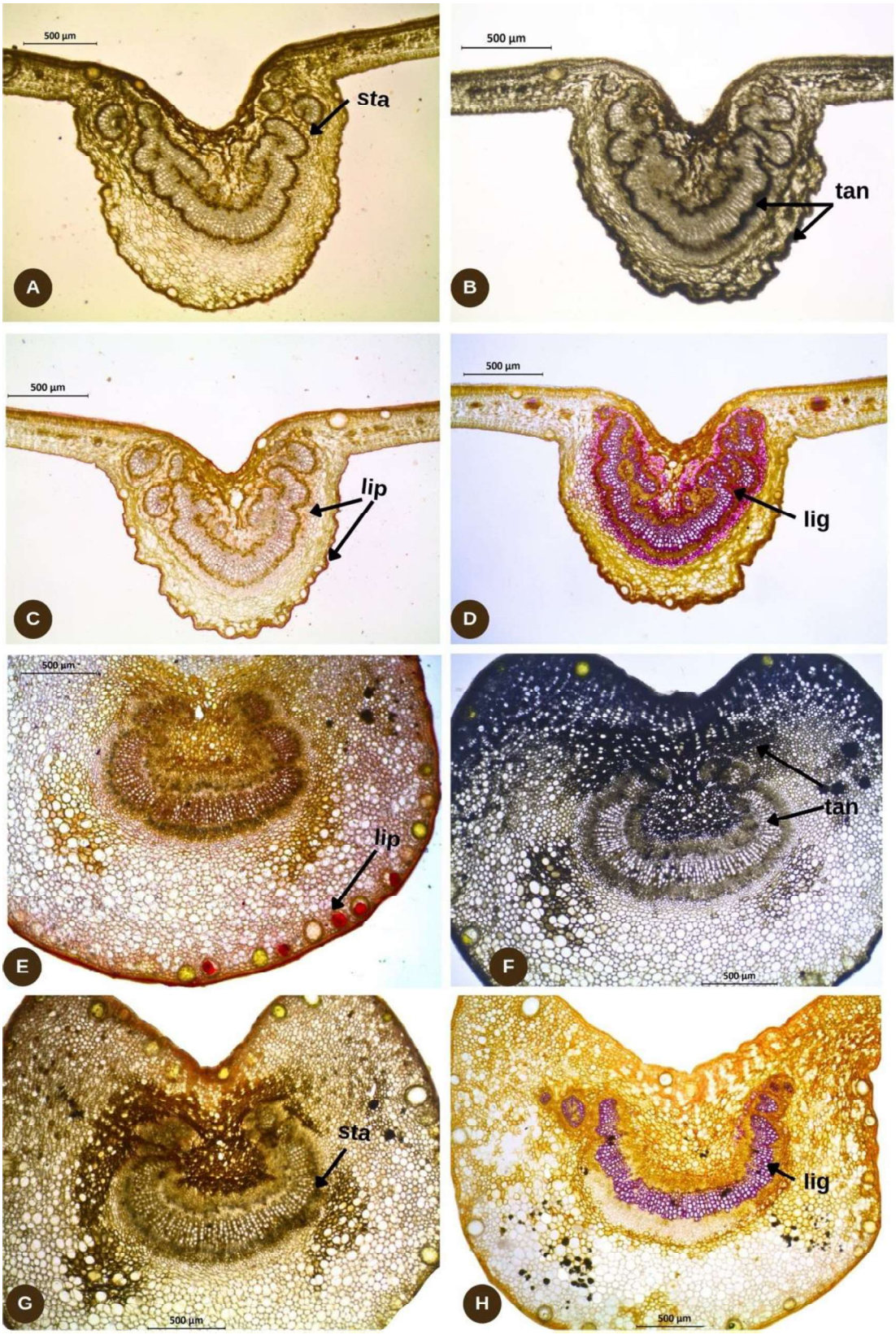


Fig. 19. ***Syzygium travancoricum*** **-** Histochemical analysis of petiole and leaf **A, G.** Starch **B, F.** Tannin **C, E.** Lipid **D, H.** Lignin

4.3 Phytochemical characterization

4.3.1 Qualitative analysis

The preliminary phytochemical analysis of water and ethanol extracts of *S. travancoricum*, revealed that the plant possesses a diverse range of phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids, phenols, and terpenoids. Our findings have revealed that the leaves and bark of the plant are rich sources of these secondary metabolites, albeit in varying concentrations depending on the specific plant part. Significantly, the water extract exhibited a higher concentration of these chemical constituents compared to the ethanol extract, thus highlighting the importance of solvents used in phytochemical extraction.

Table 4. Qualitative analysis of *Syzygium travancoricum* leaf and bark extracts

Compounds	Test	*S. travancoricum*			
		Water extract		Ethanol extract	
		Leaf	Bark	Leaf	Bark
Steroids	Liebermann Burchard test	+++	+++	++	++
Terpenoids	Salkowski test	+++	++	++	++
Phenols	Ferric chloride test	+++	++	++	++
Tannins	Gelatin test	+++	++	++	++
Flavonoids	Braymer's test	+++	++	++	++
	Shinoda test	+++	++	++	++
	Lead acetate test	++	++	++	++
Coumarins	NaOH test	-	-	-	-
Alkaloids	Mayer's test	+++	++	++	++
	Wagner's test	++	++	++	++
	Dragendorff's test	++	++	++	++
Saponins	Froth test	+++	++	++	++
Carbohydrates	Molisch's test	+++	+++	++	++
Reducing sugars	Fehling's test	++	+	++	+
Protein	Ninhydrin test	+++	++	++	+
	Biurette test	+++	++	++	+

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (–) sign.

4.3.2 Quantitative analysis

4.3.2.1 Estimation of total phenol

Table 5 compares the total amount of phenolic compounds found in the leaf and bark extracts of *S. travancoricum*. The study revealed that water extracts of *S. travancoricum* contained the highest concentration of phenolic compounds compared to ethanol extracts. Furthermore, the leaves had a significantly higher phenolic compounds (240.2 mg GAE/g) compared to the bark extracts, confirming the abundance of phenols in leaves as opposed to barks (Table 5).

Table 5. Total phenolic compounds in leaf and bark extracts of *Syzygium travancoricum*

Plant part	Concentration mg GAE/g Equivalent		
	Average OD	Ethanol extract	Water extract
Leaf	0.840±0.02	224.4	240.2
Bark	0.640±0.02	171.8	180.3

Values are mean ± SD (n=3)

4.3.2.2 Estimation of total flavonoid

In Table 6, we see a clear comparison of the flavonoid content found in leaf and bark extracts of *S. travancoricum*, measured in milligrams of quercetin equivalent per gram (mg QUE/g). The water extracts of both leaf and bark demonstrated the highest flavonoid content, with 78 mg QUE/g and 40 mg QUE/g respectively, surpassing the ethanol extracts. Notably, the leaves boasted a higher flavonoid content compared to the bark.

Table 6. Total flavonoids in leaf and bark extracts of *Syzygium travancoricum*

Plant part	Concentration mg QUE/g Equivalent		
	Average	Ethanol extract	Water extract
Leaf	0.27±0.02	36.2	78.0
Bark	0.38±0.01	14.7	40.4

Values are mean ± SD (n=3)

4.2.2.3 Proximate Analysis

The proximate analysis of *S. travancoricum* fruit samples at different ripening stages clearly indicated that these fruits are an abundant source of essential nutrients, such as vitamins, minerals, phenols, anthocyanins, and carbohydrates. The total phenolic compound was also very high, specifically in the raw fruits (480 mg/100g). The minerals present in the fruit include Sodium, Potassium, Calcium, Magnesium, and Phosphorus. In particular, Potassium and Calcium were found to be highly concentrated, ranging from 137.55–170.39 mg/100g and 40.75–47.99 mg/100g, respectively, compared to other minerals (Table 7). The concentration of other minerals, such as Magnesium, Sodium, and Phosphorus, varied between the different ripening stages. Furthermore, the fruit also contained various trace microelements like Iron, Copper, Nickel, Chromium, Zinc, and Cobalt. Based on these findings, it is clear that *S. travancoricum* fruit is a valuable addition to any balanced diet.

Table 7. Proximate Analysis of fresh fruits of *Syzygium travancoricum*

Sl. No.	Proximate Composition	Fruits		
		Raw	Semi-ripened	Ripened
1	Total mineral content (%)	1.36±0.02	1.30±0.01	1.03±0.02
2	Total carbohydrates (g/100g)	14.80±0.18	13.90±0.05	13.88±0.05
3	Total protein (mg/100g)	10.46±0.04	9.50±0.04	9.42±0.02
4	Total fat (%)	0.46±0.01	0.44±0.02	0.37±0.03
5	Total phenolic compounds (mg/100g)	480.30±0.16	400.30±0.12	340.23±0.10
6	Total anthocyanin (mg/100g)	15.60±0.08	36.60±0.01	54.81±0.04
7	Ascorbic acid (mg/100g)	18.56±0.12	16.60±0.05	8.15±0.06
8	Sodium (mg/100g)	14.63±0.01	14.60±0.02	12.33±0.01
9	Potassium (mg/100g)	170.39±0.03	172.1±0.03	137.55±0.02
10	Calcium (mg/100g)	47.99±0.03	40.4±0.02	40.75±0.02
11	Magnesium (mg/100g)	11.54±0.01	10.62±0.01	10.88±0.01
12	Phosphorus (mg/100g)	14.17±0.02	14.2±0.02	13.66±0.02
13	Iron (mg/100g)	2.05±0.01	2.1±0.02	1.34±0.01
14	Copper (mg/100g)	0.23±0.01	0.2±0.02	0.23±0.02
15	Nickel (mg/100g)	0.23±0.01	0.2±0.01	0.25±0.02
16	Chromium	0.19±0.01	0.2±0.02	0.19±0.01
17	Zinc (mg/100g)	0.06±0.01	0.1±0.01	0.04±0.02
18	Selenium (mg/100g)	ND	ND	ND
19	Aluminium (mg/100g)	0.24±0.04	0.19±0.01	0.20±0.01
20	Cobalt (mg/100g)	0.50±0.03	0.45±0.01	0.42±0.02
21	Mercury (mg/100g)	ND	ND	ND
22	Lead (mg/100g)	ND	ND	ND
23	Arsenic (mg/100g)	ND	ND	ND
24	Cadmium (mg/100g)	ND	ND	ND

Values are mean ± SD (n=3)

4.3.3 Essential Oil Profiling

The essential oils from eight populations of *S. travancoricum* were analyzed, and their phytocompounds were detected through GC-MS. A dendrogram was constructed for chemotaxonomical comparison, and it was found that each species had its own specific key compounds. Furthermore, the number

of compounds between species also varied. Such variations can be an important tool for species identification.

Hydrodistillation of *S. travancoricum* leaves yielded pale yellow-coloured essential oil (0.08% v/w) (0.8 ml/1.0 kg leaves). The chemical composition of the oil was analyzed using GC-MS, and the major peaks of compounds can be observed in Fig. 22 and 23. The essential oil was found to be a highly complex mixture of monoterpenoids, diterpenoids, and sesquiterpenoids (Table 8 and Fig. 20), revealing a total of 34 phytochemical compounds (Table 8).

The sesquiterpenoid compounds were the major contributors, constituting over 60% of the total oil content. The most dominant sesquiterpenoids included β-caryophyllene and caryophyllene epoxide, while β-geraniol, α-farnesene, and α-selinene were also identified. Monoterpenoids were the second most abundant and comprised 40% of the essential oil. The primary monoterpenoids identified were α-pinene, β-pinene, isomers of ocimene, myrcene, β-limonene, and α-terpineol. Diterpene was represented by +-cembrene, and moderate quantities of phenol was present. Additionally, trace amounts of the diglyceride compound 1,3 diolein were detected. The concentration of non-oxygenated sesquiterpenoids in the essential oil constituent of *S. travancoricum* was higher than that of oxygenated sesquiterpenoids. Monoterpenoids were predominantly acyclic, followed by bicyclic and monocyclic. The essential oil of *S. travancoricum* contained unique chemical compounds such as viridiflorol, +-cembrene, 6 epi-shyobunol, 1, 3 diolein, and calerene epoxide (Table. 8, Fig 24a and 24b). The chemical profile of *S. travancoricum* populations showed variations in the composition and concentration of major chemical compounds. To gain a more comprehensive understanding of the chemical composition profile of the essential oil, it is necessary to investigate samples from different locations and harvest times.

Table 8. Leaf essential oil GC-MS profile of *Syzygium travancoricum* populations

Compounds	Populations															
	TBG		IDJ		KOL		TCR		POO		MSSRF		KON		PAL	
	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT
α-Pinene	9.9	10.02	30.5	10.05			1.4	10.07	6.2	6.37	15.6	10.02	16.8	6.37	22.6	6.37
β-Pinene					1.5	30.74	0.8	7.71	10.3	7.74			36.3	7.73	34.6	7.75
α-Ocimene			8.9	10.60	14.1	10.09.			0.8	9.95			3.6	9.93	4.5	9.95
β-Ocimene	8.1	10.41	13.8	10.41	32.7	10.52	0.6	10.17	3	10.35	53.1	10.42	18.8	10.34	20.1	10.36
Cis-Epoxy-Ocimene					1.2	14.38										
β-Myrcene			1.5	8.25											0.9	8.19
β-Limonene									1.0	9.60			2.9	9.59	4	9.60
α-Terpineol									0.6	16.5			3.1	16.48	0.6	16.50
α-Humulene	9.2	27.8	5.2						0.8	27.69	4.2	27.79	1.1	27.67		
α-Caryophyllene					5.4	27.83	2.9	27.3								
β-Caryophyllene	44.3	26.46	25.4	26.40	24.8	26.53	7.0	25.98	2.4	26.28	21.9	26.39	4.5	26.26	3.2	26.29
β-Geraniolene													0.7			
Para Cymene													0.6	9.44		
Cymene															0.8	9.45
(-)Trans-Pinocarveol															0.5	14.20
2-Camphanyl Acetate															0.5	20.63
β-Elemene					0.6	25.23										
α-Farnesene			1.6	29.98	0.8	29.99							0.6	29.88		
α-Selinene	2.6	29.49			1.9	29.50	3	29.09	1.5	29.39	1.2	29.49	1.2	29.38	0.5	29.41
β-Selinene	1.7	29.12			1.8	29.14			11.9	29.04			1.4	29.01	0.8	29.05
Nerolidol (+-)	4.6	32.20	8.4	32.19			12.3	31.85	4.1	2.9			4.4	32.08	0.8	32.15
α-Copaene							1.1	24.14	1.4	24.45						
α-Gurjunene									1.5	28.59						
β-Caryophyllene Epoxide	10.4	32.99	4.6	32.96	10.2	33.07	38.7	32.63	8.4	32.87	3.9	32.98	3.2	32.86	4	32.89
Humulene Epoxy II					1.1	33.99	6.2	33.59	1.5	33.89						

Compounds	Populations															
	TBG		IDJ		KOL		TCR		POO		MSSRF		KON		PAL	
	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT	A%	RT
Aromadendrene	4.4	27.19			1.1	27.19	4.2	26.78	1.8	36.24						
(-)-Spathulenol	3.7	32.80			1.5	32.87							0.8	32.69		
Juniper camphor									4.8	35.62						
Viridiflorol							0.7	31.68	7.8	41.10						
Calarene Epoxide									9.9	43.32						
(+)-Cembrene									1.9	37.78						
6-Epi-Shyobunol									3.5	38.56						
1,3-Diolein									1.7	59.75						
2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4-Hydroxybenzyl) Phenol									13.2	66.18						

	Total Characterization %							
Acyclic monoterpenoid	8.1	22.7	48.8	5.9	3.8	53.1	22.4	25.4
Monocyclic monoterpenoids	**-**	-	0.8	-	1.5	-	5.9	4.6
Bicyclic monoterpenoids	9.9	30.5	-	2.2	-	-	-	-
Diterpenoids	-	-	-	-	1.9	-	-	-
Non-oxygenated sesquiterpenes	62.4	40.6	34.3	41.7	27.1	27.3	14.5	7.6
Oxygenated sesquiterpenes	18.5	4.6	15.1	49.8	57.8	3.9	4.0	4.0
Total identified area percentage	98.9	99.9	100.0	99.6	99.4	99.8	99.9	99.7
Ratio (Non-oxygenated sequiterpenes/Oxygenated sesquiterpenes)	**3.4**	**8.8**	**2.3**	**0.8**	**0.5**	**7.0**	**3.6**	**1.9**
Yield of essential oils (%) (v/w)	**0.4**	**0.6**	**0.8**	**0.6**	**0.5**	**0.4**	**0.3**	**0.4**

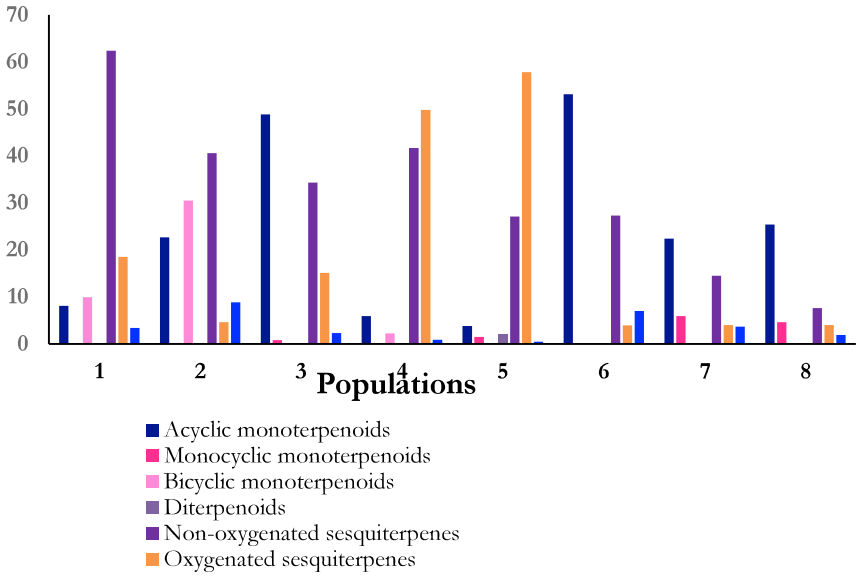


Fig. 20. Leaf essential oil GC-MS profile of *Syzygium travancoricum* populations

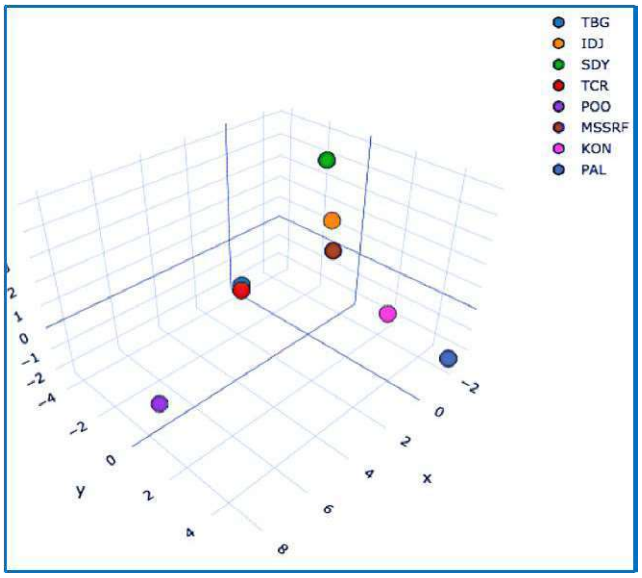


Fig. 21. Principal Component Analysis based on leaf essential oil constituents of *Syzygium travancoricum* populations. **TBG**: JNTBGRI; **IDJ**: Idinjaar; **SDY**: Shendurney (Kollam); **TCR**: Thrissur; **POO**: Pookode; **MSSRF**: MSSRF, Wynad; **KON**: Konginichalkavu; **PAL**: Paliyeri Mookambika Kavu

The present study utilised principal component analysis (PCA) to examine the relatedness of eight populations of *S. travancoricum* in terms of the chemical composition of leaf essential oil. The results revealed substantial variations in the phytochemical profile. Simultaneously, it revealed certain shared characteristics among some populations, allowing for the classification of the

populations into three principal components, PCA1, PCA2 and PCA3, based on their chemical constitution (Fig. 21). The populations from JNTBGRI, Thrissur and MSSRF showed more relatedness. In contrast, the populations from Paliyeri Mookambika Kavu and Pookode were distinct from other populations regarding their chemical constituents.

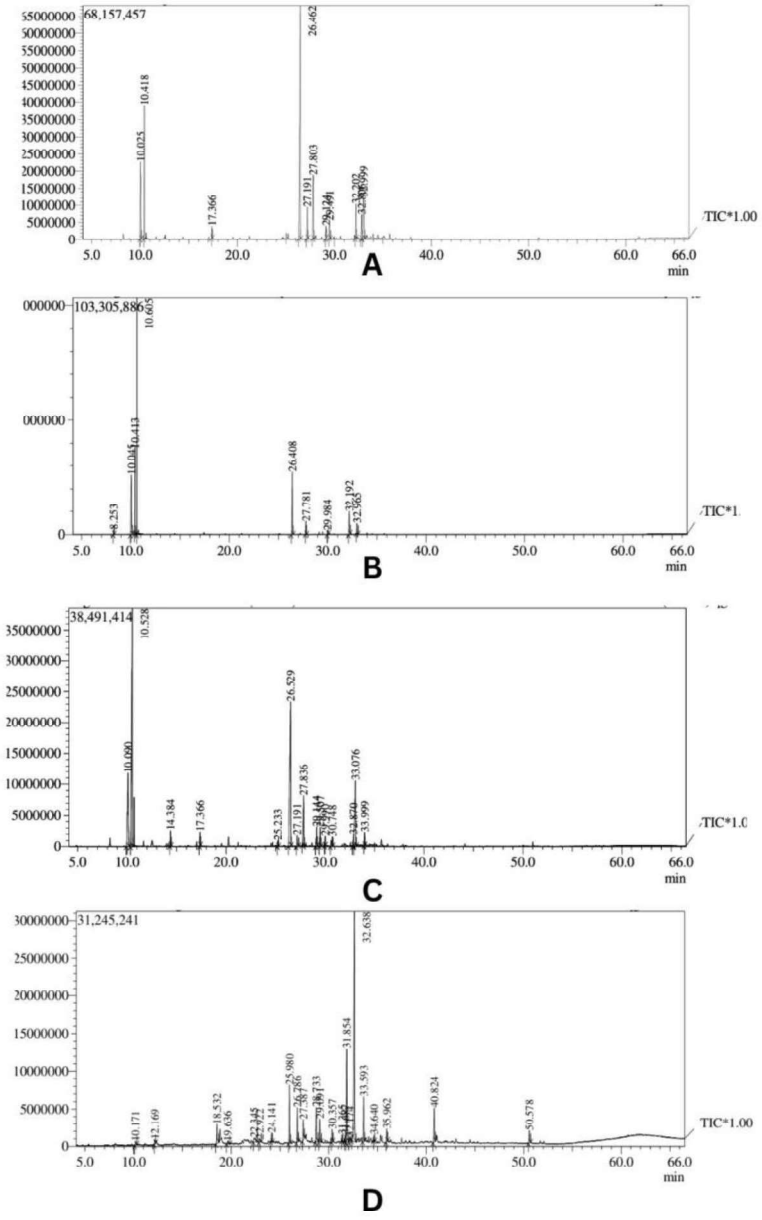


Fig. 22. GC-MS chromatogram of *Syzygium travancoricum* leaf essential oil. **A**. JNTBGRI; **B.** Idinjaar; **C.** Kollam; **D.** Thrissur

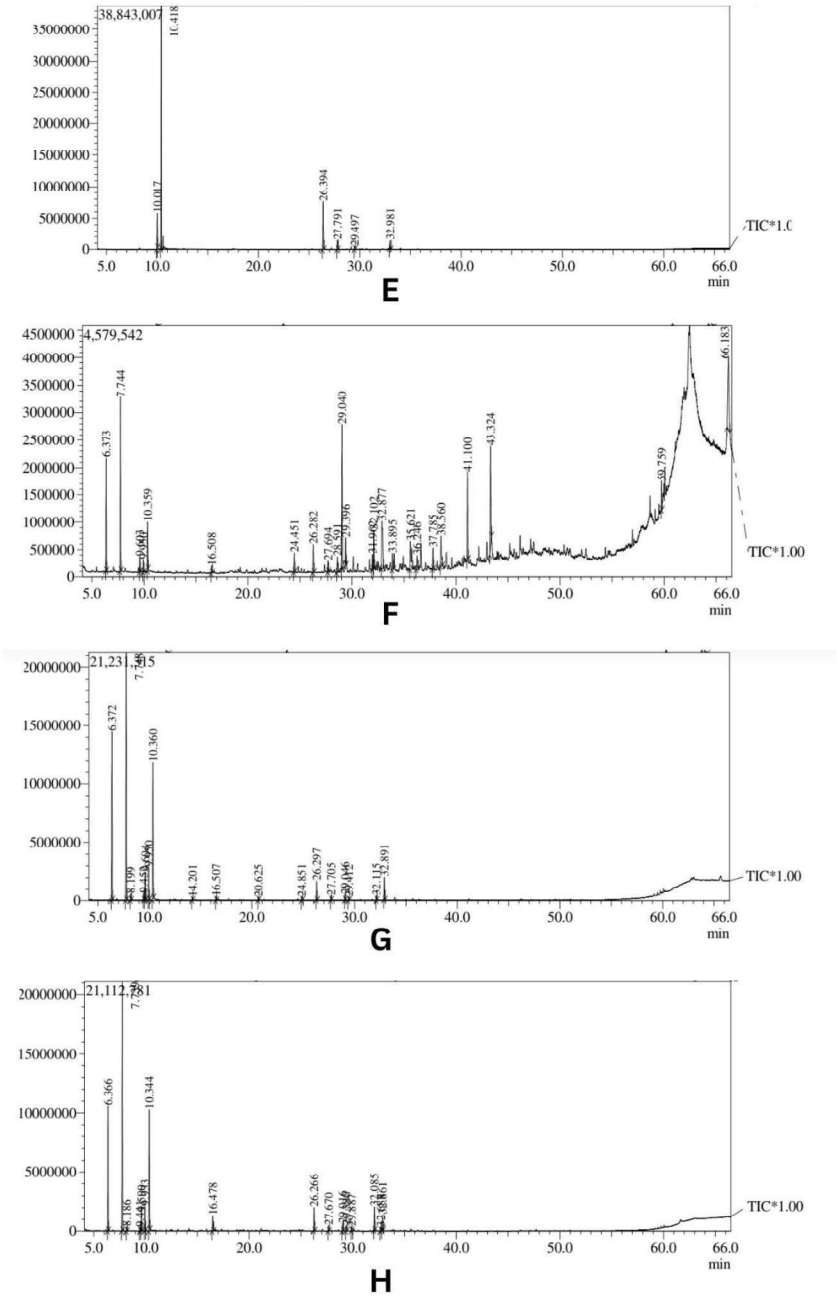


Fig. 23. GC-MS chromatogram of *Syzygium travancoricum* leaf essential oil. **E**. MSSRF; **F.** Pookode; **G**. Konginichalkavu; **H**. Paliyeri Mookambika Kavu

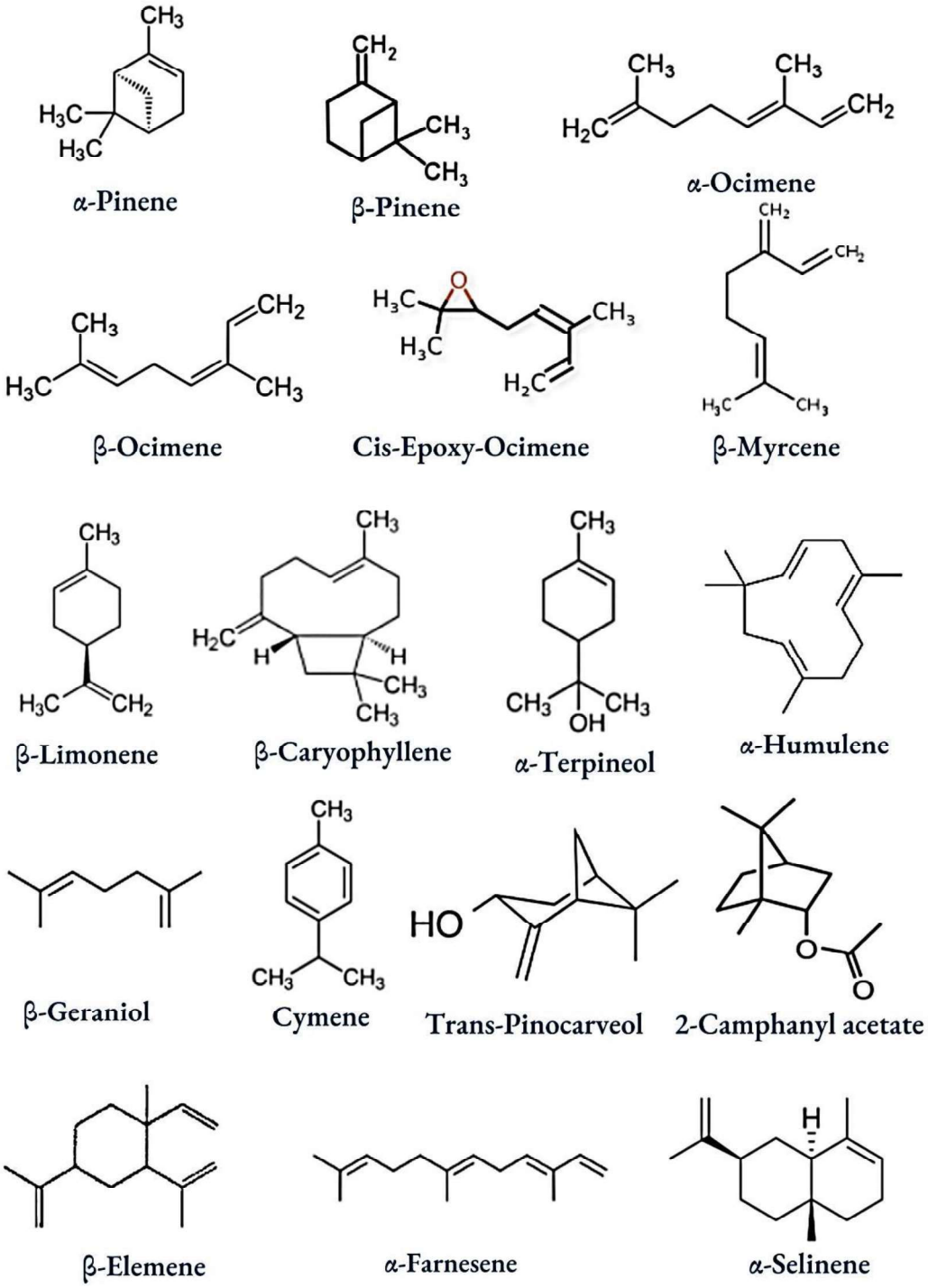


Fig. 24a. Schematic representation of phytochemical compounds identified in *Syzygium travancoricum* leaf essential oil using GC-MS

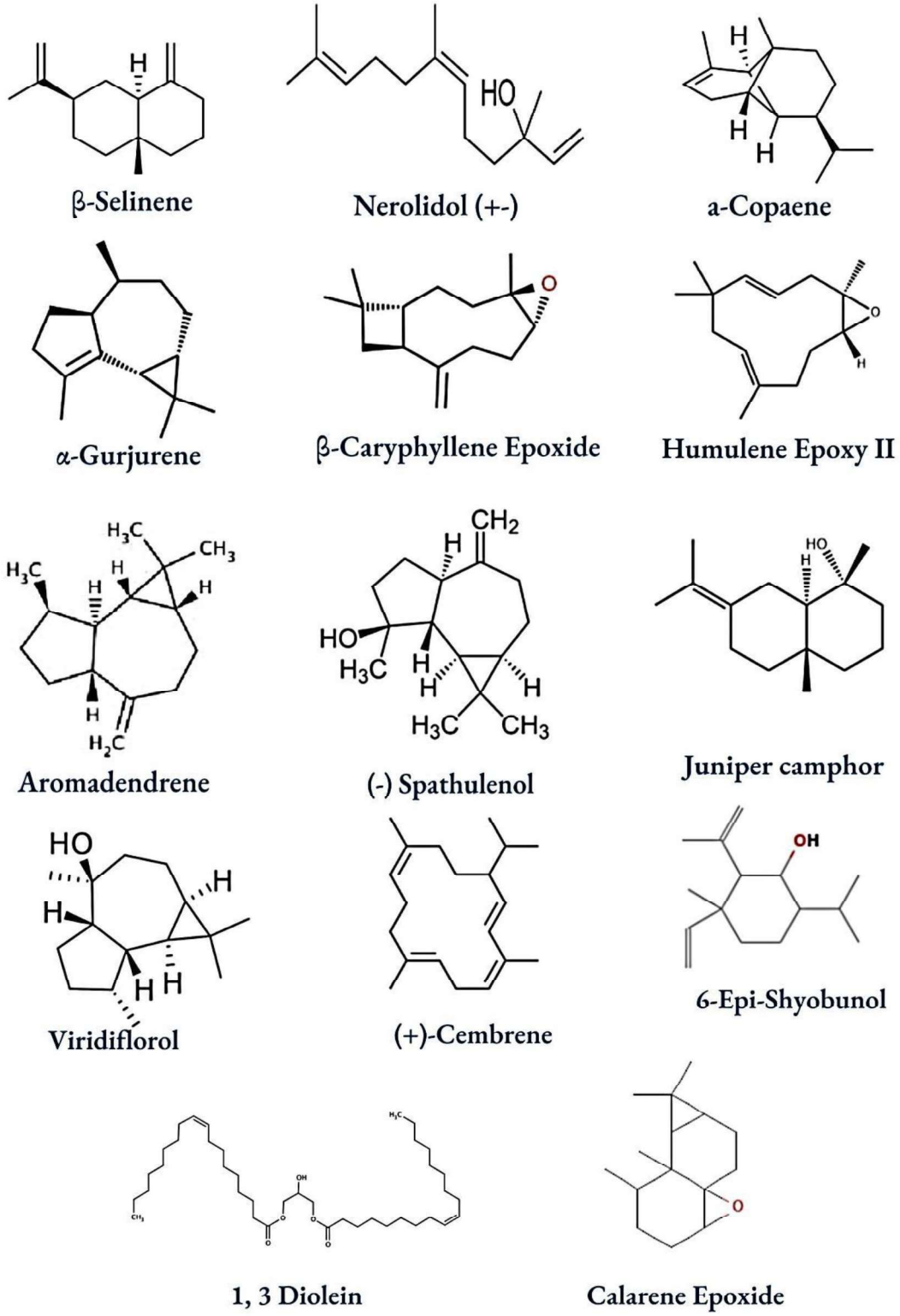


Fig. 24b. Schematic representation of phytochemical compounds identified in *Syzygium travancoricum* leaf essential oil using GC-MS

4.4 Molecular Data

Eight populations of *S. travancoricum* were analyzed with twenty-four known *Syzygium* species that were retrieved from NCBI to construct a phylogenetic tree towards understanding the phylogenetic placement of populations of *S. travancoricum*. The analysis utilized DNA sequences from twenty-four *Syzygium* species for the *ITS* and *matK* loci. In comparison, only 18 *Syzygium* species were included for the *rbcL* locus due to the lack of available sequences (Appendix 6).

4.4.1 Bayesian Phylogeny

Phylogenetic tree based on molecular data showed three different lineages with respect to *S. travancoricum* (Fig. 25). The sequence data of *S. travancoricum* retrieved from NCBI GenBank indicates lineage 1 that separates the rest of populations which are analysed in this study at 100% PP. Two populations of *S. travancoricum* collected from the Northern part of Kerala *i.e.* Pookode and Paliyeri Mookambika Kavu (lineage 2) showed sister relationships with the rest of the populations at 73% PP (lineage 3).

The phylogenetic tree based on the concatenated dataset using both molecular and morphological data sets showed two distinct monophyletic lineages at 100% PP, where the two populations of Pookode and Paliyeri Mookambika Kavu were strongly found together as clade 1 and the rest of the populations clustered together along with the sequence data of *S. travancoricum* retrieved from NCBI at 100% PP (Fig. 26). Hence the Clade 1 is identified in this study as *S. stocksii* and Clade 2 is identified as *S. travancoricum*. There is no other topological conflict observed between the above two datasets that differentiated based on either inclusion or exclusion of morphological data.

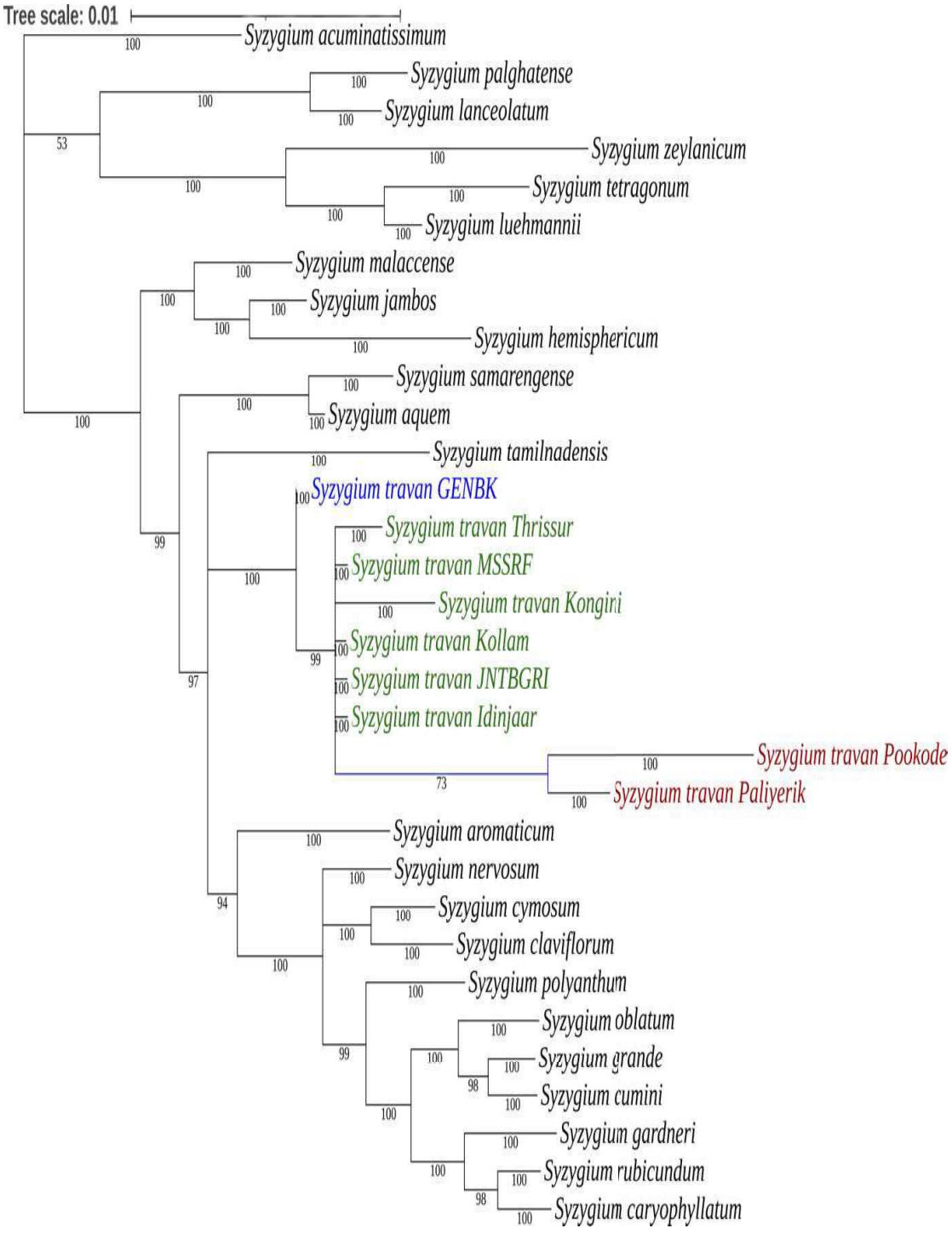


Fig. 25. Phylogenetic tree of *Syzygium travancoricum* using DNA barcodes

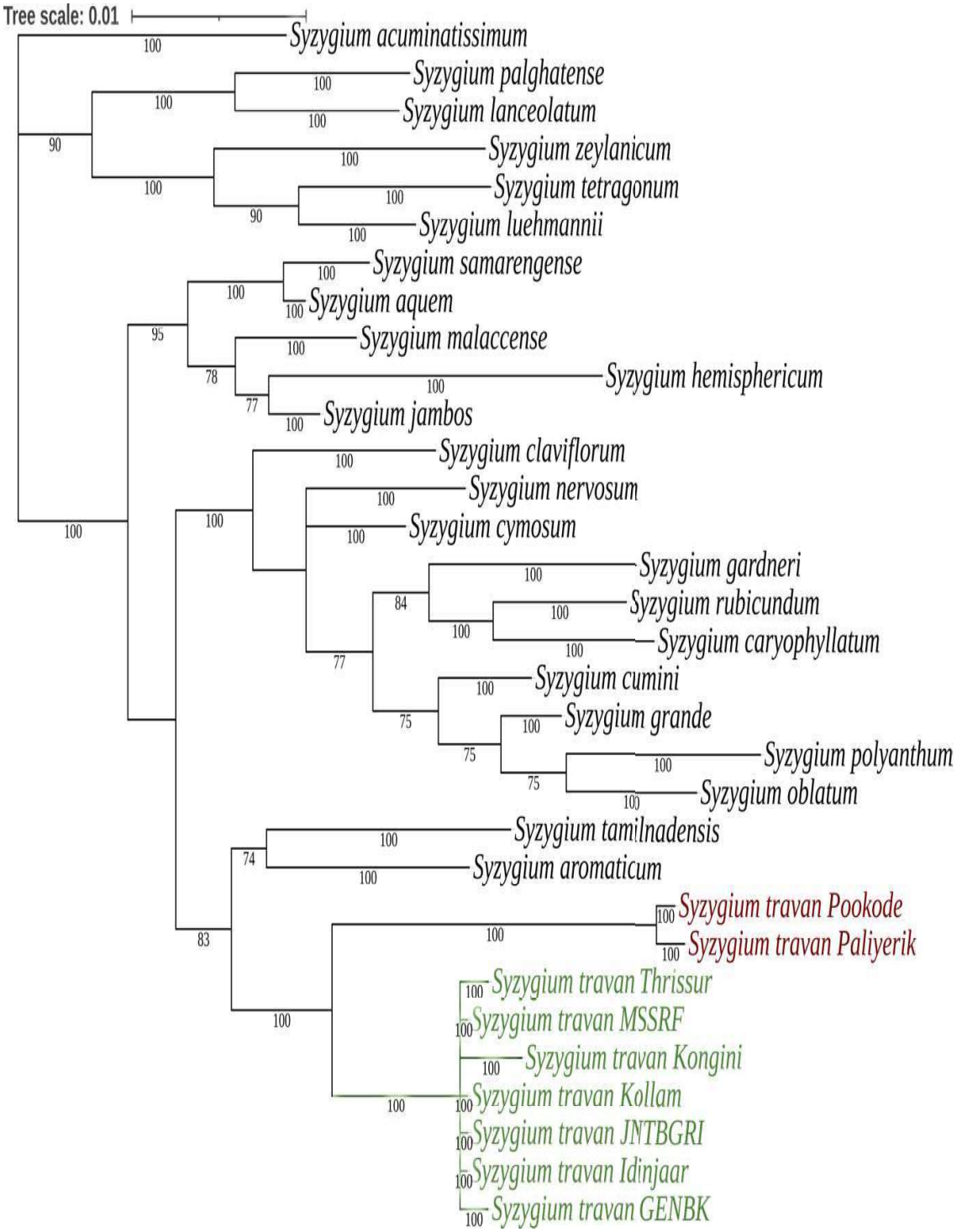


Fig. 26. Phylogenetic tree of *Syzygium travancoricum* using combined morphological and molecular data

4.5 Pharmacological studies

4.5.1 Antimicrobial Assay

The essential oil of *S. travancoricum* was found to be highly effective against both Gram-positive and Gram-negative bacteria as well as fungal strains in the antimicrobial study conducted. The essential oil in DMSO at a 1:2 dilution exhibited remarkable inhibition against *B. subtilis*, *B. cereus*, *E. coli*, and *S. mutans*, surpassing that of the control (Table 9, Fig. 27 & 28). However, it demonstrated only moderate activity against *P. aeruginosa*, *S. aureus*, *S. typhimurium*, and *K. pneumoniae*.

Table 9. Antibacterial activity of *Syzygium travancoricum* leaf essential oil

Tested bacteria	Zone of inhibition (mm)		
	Leaf oil in DMSO (10 µl)	**Positive Control** Streptomycin **(10 µg/disc)**	**Negative Control** Solvent **(10 µl)**
Gram-positive bacteria			
Bacillus cereus	16.2±0.94	22.1±0.58	ND
Bacillus subtilis	10.0±0.31	23.3±0.58	ND
Staphylococcus aureus	9.4±0.36	20±0	ND
Streptococcus mutans	11.2±0.35	8.3±0.58	ND
Gram-negative bacteria			
Escherichia coli	13.2±0.04	13.2±0.34	ND
Salmonella typhimurium	9.2±0.31	16±0	ND
Klebsiella pneumoniae	9.7±0.34	22.7±0.58	ND
Pseudomonas aerugenosa	8.6±0.17	13.3±0.58	ND

Values are given as mean ± SD of triplicate experiment.
Diameter of inhibition zones (mm), including disc size 6 mm
Standard antibiotics: Streptomycin (10µg/disc); ND - not detected

Additionally, the oil displayed outstanding inhibition against *C. albicans* at a 1:2 dilution compared to the control, and moderate activity against *A. niger*. However, no activity was observed against *P. chrysogenum* (Table 10, Fig. 29).

Table 10. Antifungal activity of *Syzygium travancoricum* leaf essential oil

Tested fungi	Zone of inhibition (mm)		
	Sample (10 µl)	**Positive Control** Ampicillin (10 mcg/disc)	**NegativeControl** DMSO (10 µl)
Aspergillus niger	8.2±0.18	11.3±0.18	ND
Candida albicans	15.34±0.21	14±0.58	ND
Penicillium chrysogenum	ND	11.2±0.35	ND

Values are given as mean ± SD of triplicate experiment
Diameter of inhibition zones (mm) including disc size 6 mm
Standard antibiotics: Clotrimazole (10 mcg/disc); ND - not detected

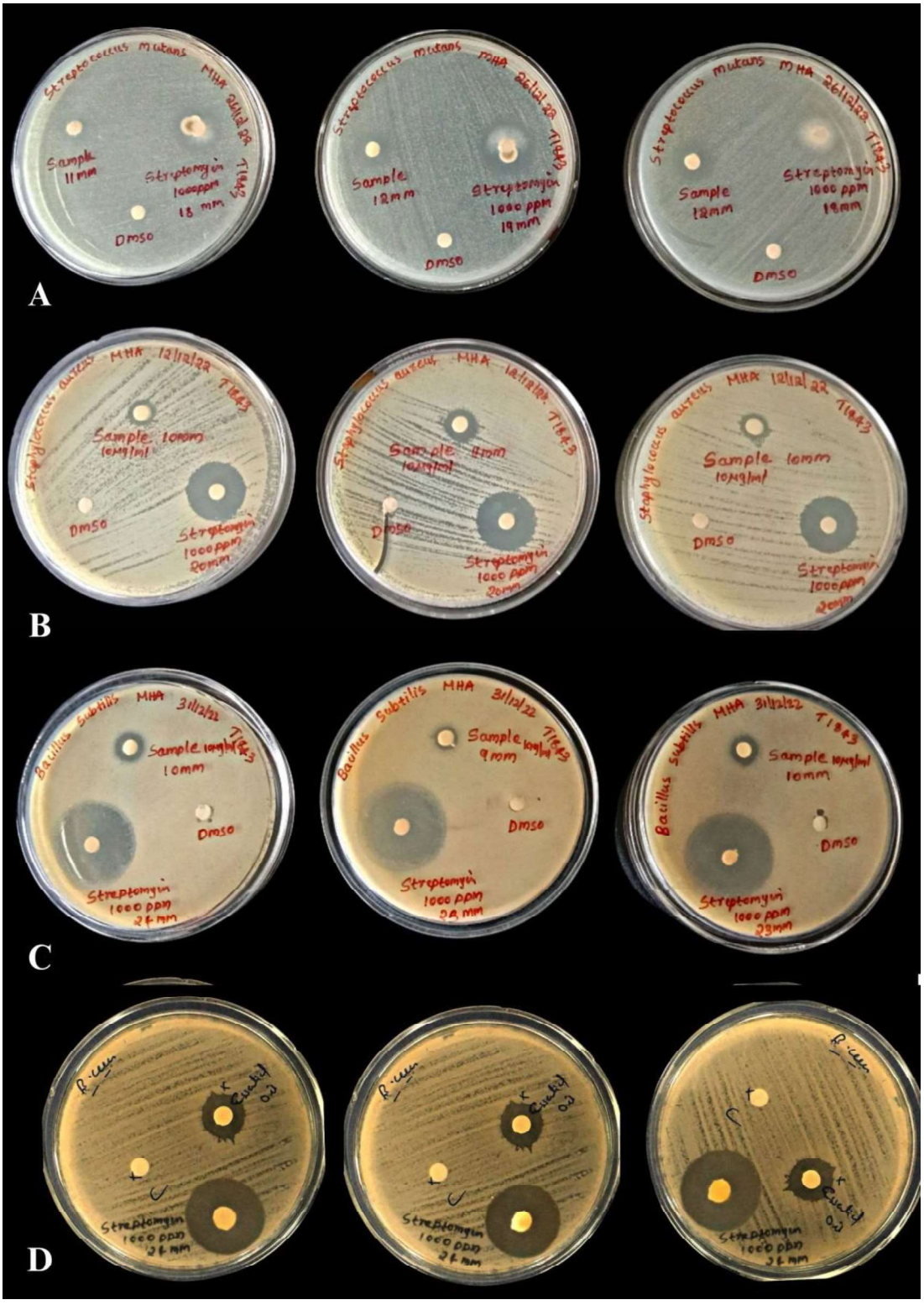


Fig. 27. Antibacterial activity of *Syzygium travancoricum* leaf essential oil against Gram-positive bacteria. **A.** *Streptococcus mutans*; **B.** *Staphylococcus aureus*; **C.** *Bacillus subtilis*; **D.** *Bacillus cereus*

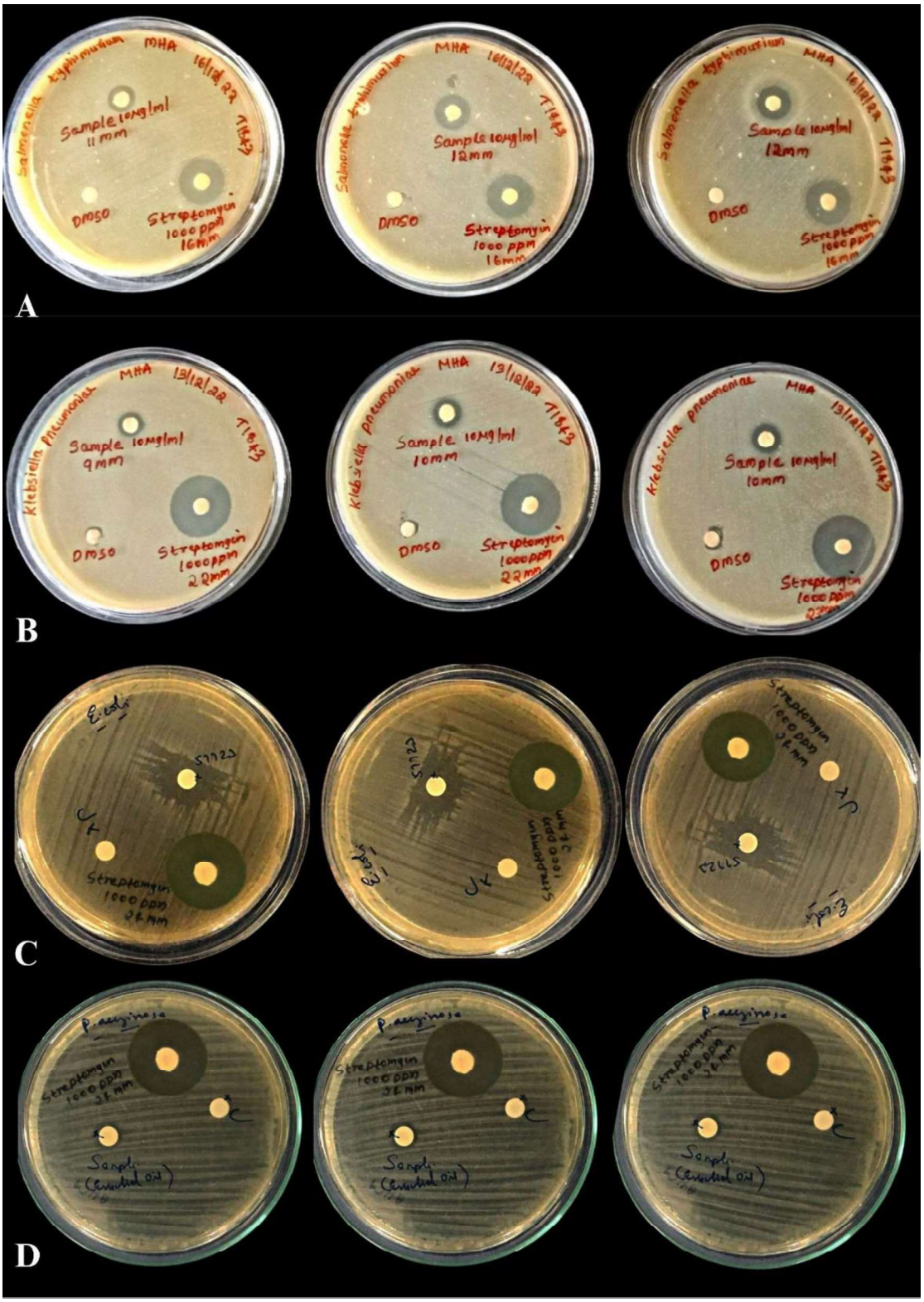


Fig. 28. Antibacterial activity of *Syzygium travancoricum* leaf essential oil against selected Gram-negative bacteria. **A.** *Salmonella typhimurium*; **B.** *Klebsiella pneumoniae*; **C.** *Escherichia coli*; **D.** *Pseudomonas aeruginosa*

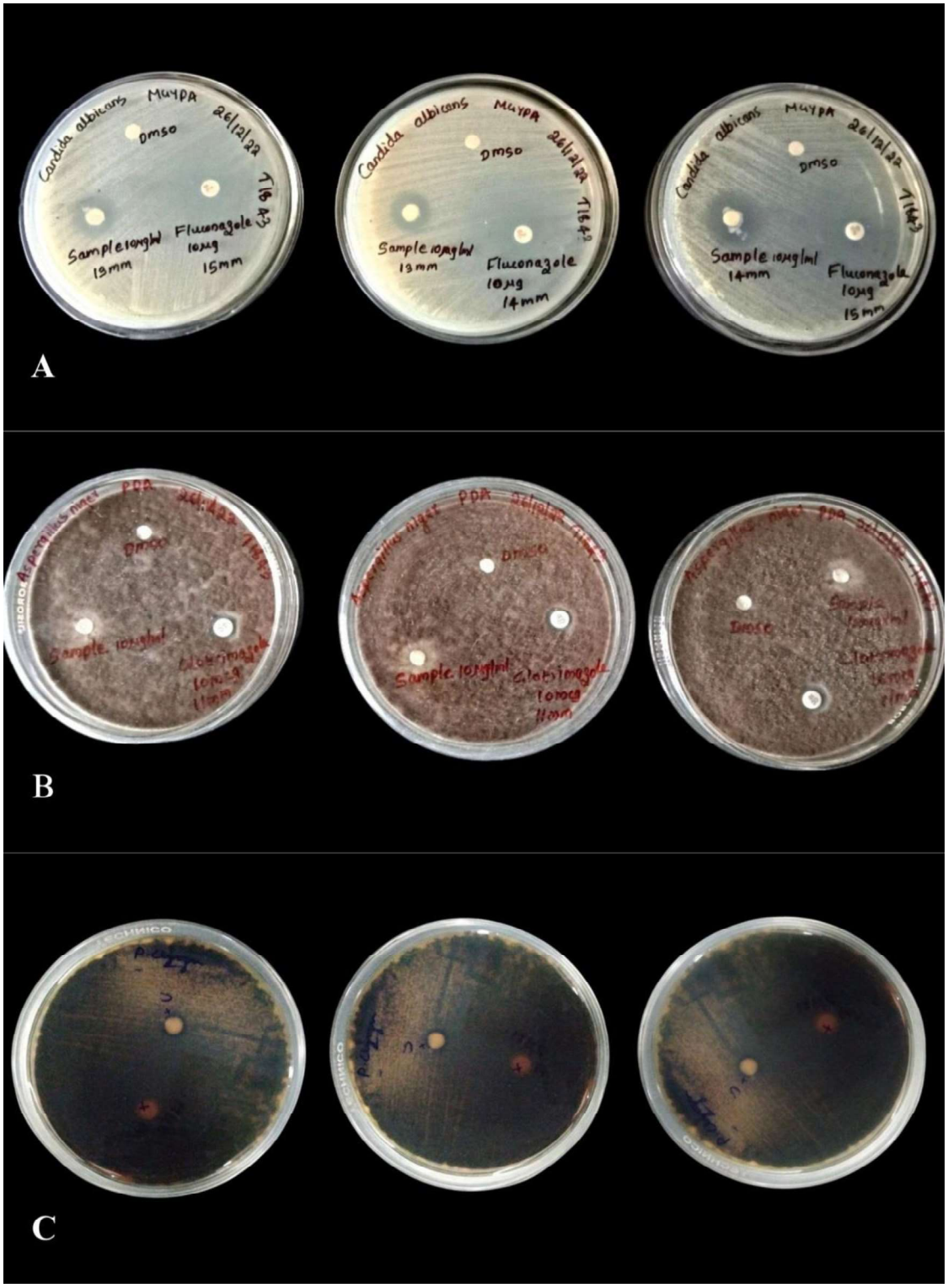


Fig. 29. Antifungal activity of *Syzygium travancoricum* leaf essential oil against selected fungal strains. **A.** *Candida albicans*; **B.** *Aspergillus niger*; **C.** *Penicillium chrysogenum*

4.5.2 Antioxidant Assay

4.5.2.1 DPPH Assay

S. travancoricum was found to possess high levels of antioxidants based on the DPPH assay. The leaf and bark extracts displayed the most significant antioxidant potential, exhibiting IC_{50} values of 27.48 µg/ml and 25.30 µg/ml, respectively. The presence of phenols in the leaves and bark is likely responsible for this. Fig. 30 illustrates the standard graph of Gallic acid. Table 11 and Fig. 31 portrays the antioxidant potential of the leaf and bark extracts using DPPH assay.

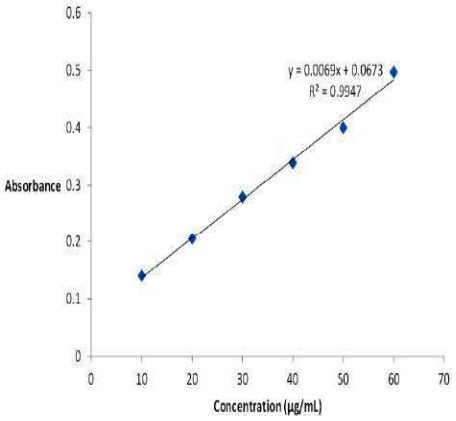


Fig. 30. Calibration curve of Gallic acid standard

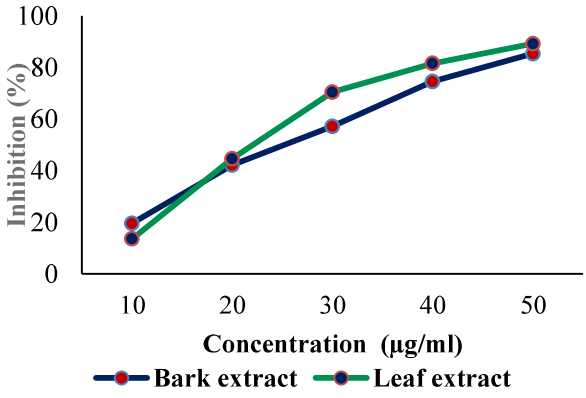


Fig. 31. DPPH assay of *Syzygium travancoricum* leaf and bark extracts

Table 11. Antioxidant activity of *Syzygium travancoricum* Gamble plant extracts using DPPH Assay

Sample Concentration (µg/ml)	Leaf Extract	IC_{50} (µg /ml)	Bark Extract	IC_{50} (µg /ml)
10	19.59±0.01		13.55±0.02	
20	42.16±0.08	**27.48**	44.68±0.04	**25.30**
30	57.19±0.02		70.48±0.01	
40	74.60±0.01		81.56±0.01	
50	85.37±0.05		89.25±0.01	

Values are given as mean ± SD of triplicate experiment
[a]Expressed as µg AAE/100mg

4.5.2.2 FRAP Assay

The ferric-reducing power of *S. travancoricum* extracts was determined by FRAP assay. The results are expressed as ascorbic acid equivalents mg AAE/100 mg extract, mean ± SD of three replications. The results showed a dose-response relationship between inhibition percentage and concentration of leaf extracts. The water extract demonstrated the highest ferric-reducing potential compared to ethanol and essential oil (Fig. 26). The water, ethanol and essential oil extracts showed EC_{50} values of 61.17 µg/ml, 72.43 µg/ml and 114.09 µg/ml, respectively. The results indicated that the FRAP assay is a valuable tool for determining the antioxidant activity of *S. travancoricum* extracts (Table 12, Fig. 32).

Table 12. Antioxidant activity of *Syzygium travancoricum* leaf extracts using FRAP assay

Sample Concentration (µg/ml)	STEO[a]	EC_{50}	STEE[a]	EC_{50}	STWE[a]	EC_{50}
20	4.57±0.01		18.85±0.01		13.36±0.02	
40	11.43±0.01		33.27±0.02		25.92±0.02	
60	21.97±0.02	**114.09**	48.83±0.02	**72.43**	35.44±0.01	**61.17**
80	33.53±0.01		62.47±0.02		57.81±0.01	
100	46.70±0.01		82.87±0.01		71.38±0.02	

Values are given as mean ± SD of triplicate experiment

[a]Expressed as µg AAE/100mg

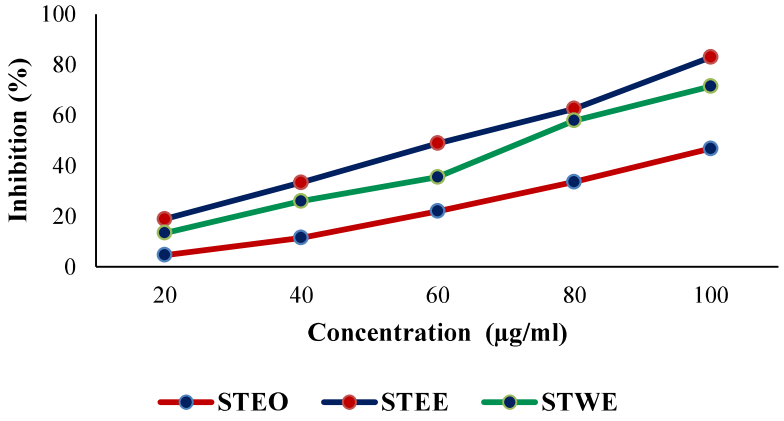


Fig. 32. FRAP assay of *Syzygium travancoricum* leaf extracts

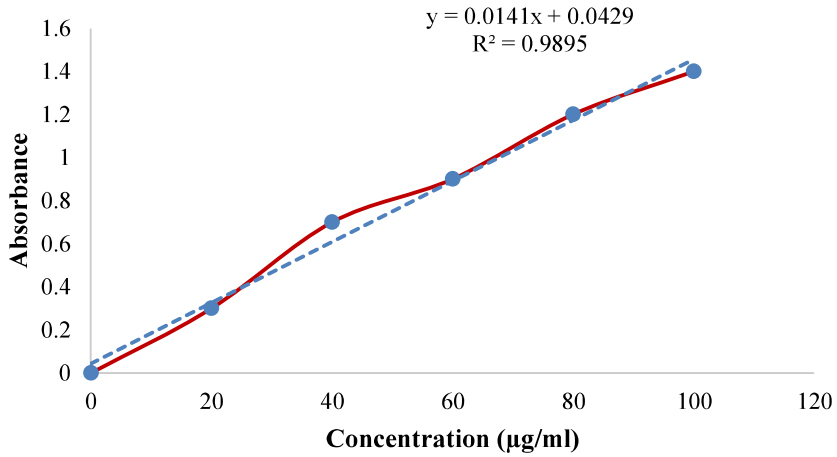


Fig. 33. Standard graph of Ascorbic acid

The results from Figures 31 and 32 unequivocally demonstrate that each extract possessed antioxidant properties with varying degrees of inhibition percentages. The concentration of the extract was directly proportional to the percentage of DPPH radical inhibition and ferric-reducing power. The IC_{50} values were precisely determined by using the regression equation of the curves, which indicates the concentration required to inhibit DPPH by 50%. The EC_{50} values for *S. travancoricum* leaf extracts showed that the antioxidant activities was higher in, water extracts followed by ethanol extract and essential oil extracts. The abundance of phenolic compounds in the ethanol and water extracts could be confidently claimed as the possible reason for this remarkable antioxidant property compared to essential oil.

4.5.3 Cytotoxicity Assay

The data presented in Table 13 and Fig. 34 demonstrate that an increase in extract concentration significantly reduces the percentage of viable cells in the L929 fibroblast cell line when assessing the cytotoxic properties of the leaf essential oil of *S. travancoricum* using an in vitro MTT assay. The LC_{50} value of 65.09 suggests that the essential oil has moderate cytotoxicity. Notably, the 100 µg/ml concentration exhibited the highest level of cytotoxicity among the five concentrations tested. The morphological profile of the treated and

control cells (Fig. 35) clearly demonstrated that the cytotoxicity increased with increase in oil concentration according to a dose-dependent manner.

Table 13. In vitro cytotoxic effects of *Syzygium travancoricum* leaf essential oil on L929 fibroblast cell line

Sample Concentration (µg/ml)	Average OD	Percentage Viability	LD_{50}
Standard	1.07±0.01	100.00	
6.25	1.04±0.01	97.64	
12.5	0.99±0.004	92.46	
25	0.85±0.01	80.20	**65.09**
50	0.70±0.001	65.98	
100	0.22±0.004	20.43	

Values are given as mean ± SD of triplicate experiment

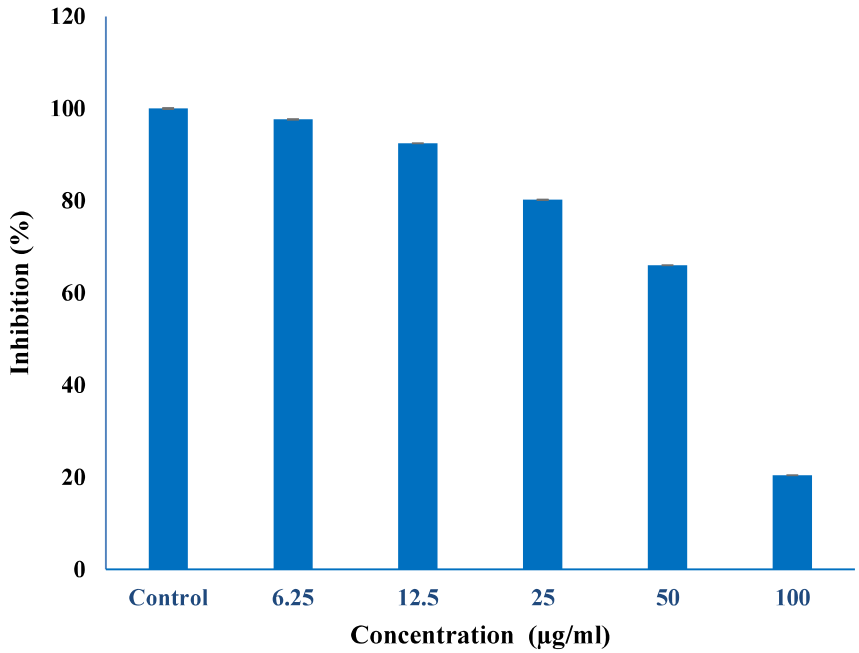


Fig. 34. In vitro cytotoxic effects of *Syzygium travancoricum* leaf essential oil on L929 fibroblast cell line

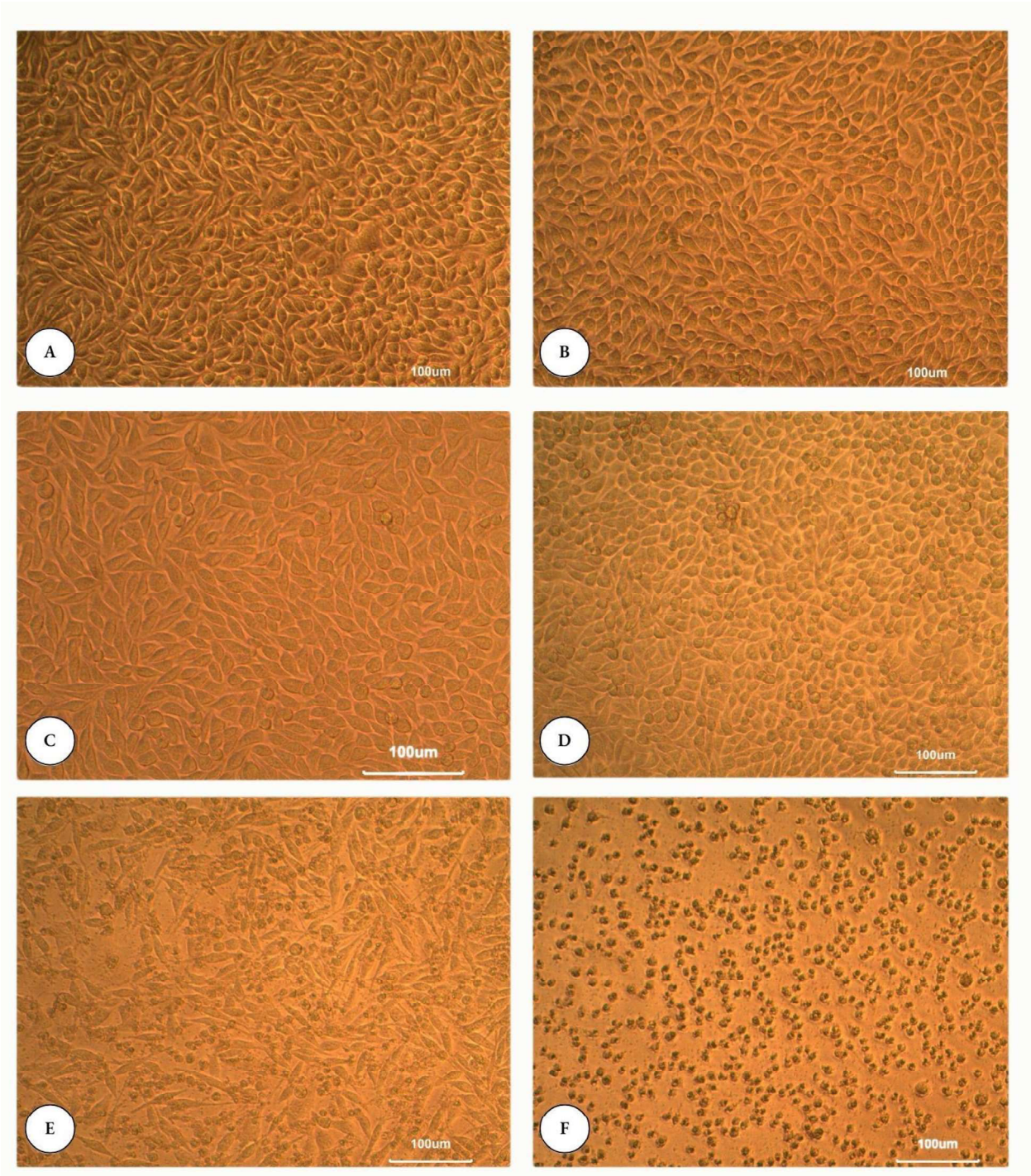


Fig. 35. Morphological profile of L929 fibroblast cell line treated with *S. travancoricum* essential oil at different concentrations after 24 hours. **A.** Control; **B.** 6.25 µg/ml; **C.** 12.5 µg/ml; **D.** 25 µg/ml; **E.** 50 µg/ml; **F.** 100 µg/ml

4.5.4 In vitro Anticancer Activity

4.5.4.1 MTT Assay

The anticancer activity of *S. travancoricum* essential oil was studied in vitro, and the results were presented in Table 14 and Fig. 36. The findings revealed a dose-dependent emergence of standard apoptotic features and changes in morphology, including cellular shrinkage and detachment. Membrane blebbing, chromatin condensation, nuclear fragmentation, and reduction were observed in the treated B16 cells compared to the control cells, which maintained a typical intact morphology (Fig. 37). These results confidently suggest the potential anticancer properties of *S. travancoricum* essential oil.

Table 14. In vitro anticancer activity of *Syzygium travancoricum* leaf essential oil against B16 melanoma cells using MTT assay

Sample Concentration (µg/ml)	Average OD	Percentage Viability	IC_{50} value (µg/ml)
Control	1.15±0.05	100	
3.25	1.06±0.05	92.73	
6.25	0.94±0.04	82.30	**57.26**
12.5	0.87±0.05	75.91	
25	0.79±0.05	69.17	
50	0.65±0.04	56.98	

Values are given as mean ± SD of triplicate experiment

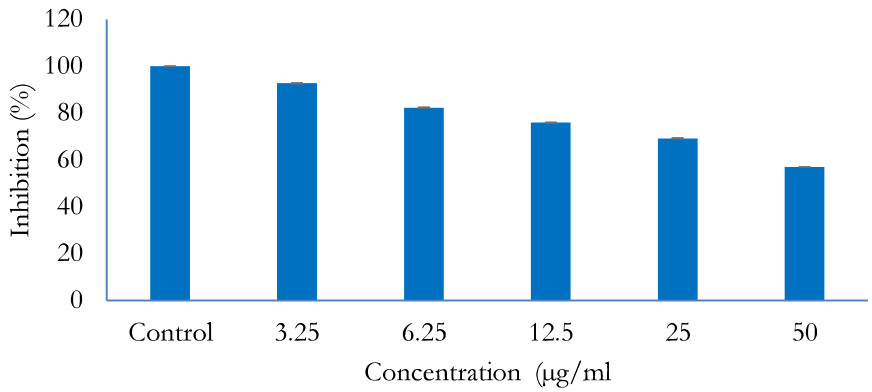


Fig. 36. *In vitro* anticancer activity of *Syzygium travancoricum* leaf essential oil against B16 melanoma cells using MTT assay

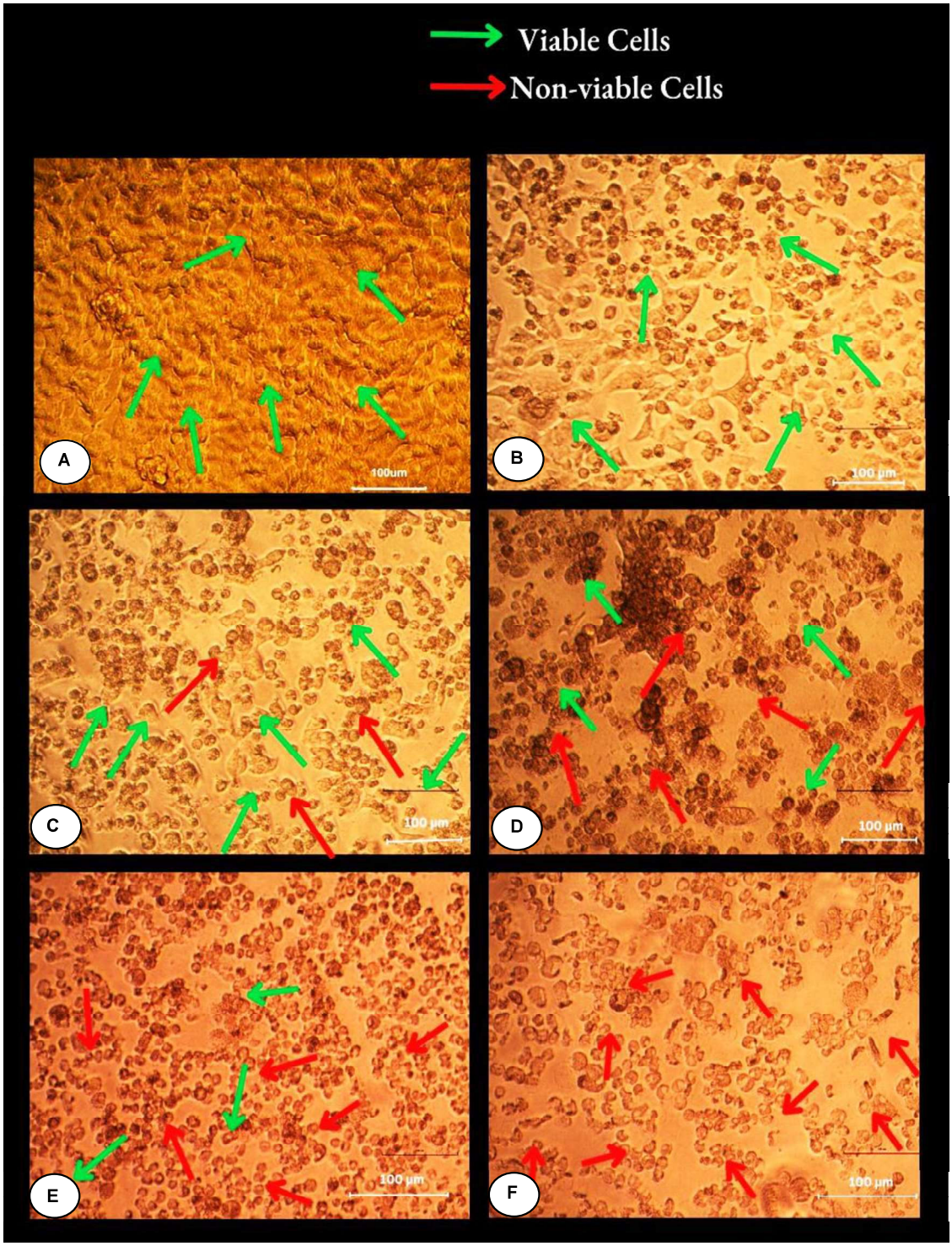


Fig. 37. Morphological Profile of B16 cells after treated with *Syzygium travancoricum* leaf essential oil at different concentrations after 24 hours. **A.** Control; **B.** 3.25 µg/ml; **C.** 6.25 µg/ml; **D.** 12.5 µg/ml; **E.** 25 µg/ml; **F.** 50 µg/ml

4.5.4.2 Apoptosis by flow cytometry

Table 15. Apoptosis profile of untreated B16 melanoma cells

Cell Type	Cell Conc. (Cells/ml)	% Gated
Live (LL)	3.14E+06	96.84
Early Apoptotic (LR)	0.00E+00	0.00
Late Apop./Dead (UR)	9.83E+04	3.03
Debris (UL)	4.27E+03	0.13
Total Apoptotic	9.83E+04	3.03

Table 16. Apoptosis profile of B16 melanoma cells treated with *Syzygium travancoricum* leaf essential oil

Cell Type	Cell Conc. (Cells/ml)	% Gated
Live (LL)	3.82E+06	85.19
Early Apoptotic (LR)	1.44E+04	0.32
Late Apop./Dead (UR)	6.50E+05	14.49
Debris (UL)	0.00E+00	0.00
Total Apoptotic	6.64E+05	14.81

The results revealed that *S. travancoricum* leaf essential oil effectively induced cell death among B16 melanoma cells via apoptosis.Tthe apoptotic cells showed green fluorescence, dead cells showed red and green fluorescence and live cells showed little or no fluorescence (Fig. 36).

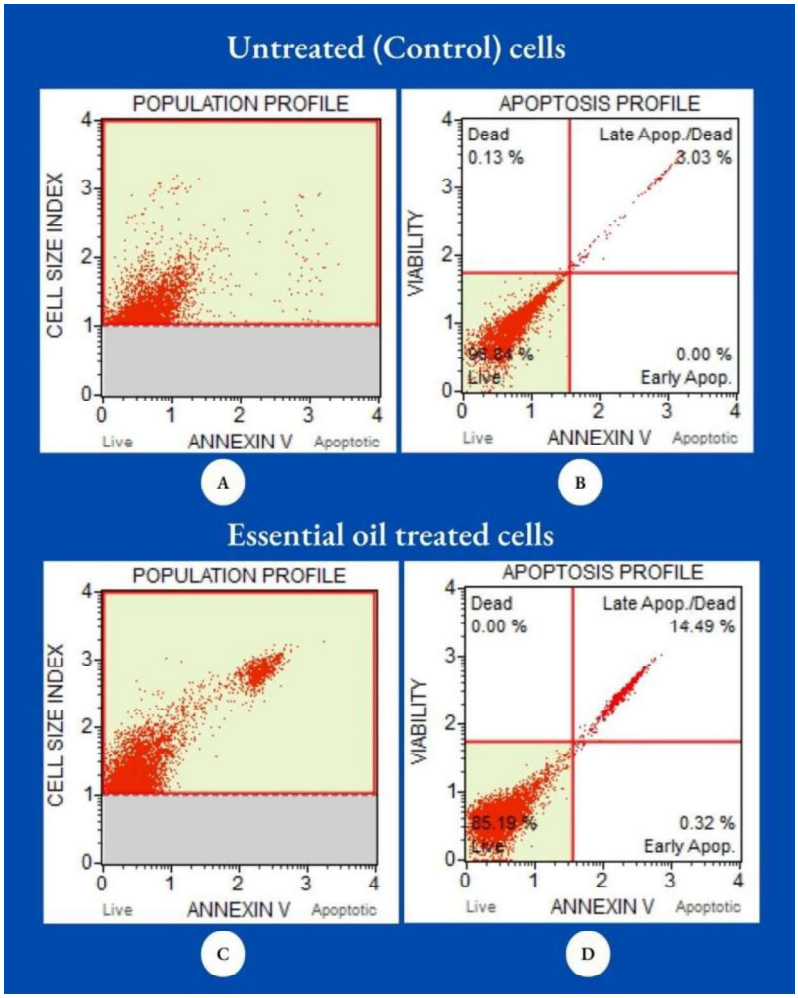


Fig. 38. Apoptosis profiles of B16 melanoma cells treated with *Syzygium travancoricum* leaf essential oil for 24 h.

Four populations of cells were distinguished in this assay such as non-apoptotic cells: Annexin V (-) and 7-AAD (-); early apoptotic cells: Annexin V (+) and 7-AAD (-); late-stage apoptotic and dead cells: Annexin V (+) and 7-AAD (+); mostly nuclear debris: Annexin V (-) and 7-AAD (+).

After treatment with *S. travancoricum* essential oil at its IC_{50} value, the ratio of late apoptotic or necrotic B16 cells increased significantly. Comparing control and treated cells, in control, the ratio of living cells to late apoptotic cells did not change significantly, and the ratio of early apoptotic cells was zero (Table 15, Fig. 38 A, B). Without treatment, most B16 cells were alive, and the apoptotic/dead ratio was 3.03%, as depicted in the figure. However, exposure

to *S. travancoricum* essential oil elevated the proportion to 14.49% apoptotic. In contrast, the ratio of apoptotic/dead cells in control cells was unaltered, and over 90% of cells were alive (Table 16, Fig. 38C, D). These findings unequivocally demonstrated that *S. travancoricum* essential oil affects the viability of B16 cancer cells and induces apoptosis.

4.5.4.3 ROS activity

A higher mitochondrial membrane potential generally results in increased adenosine triphosphate (ATP) production and increased reactive oxygen species (ROS) production. Hence the ROS production in untreated and treated B16 cancer cells using *S. travancoricum* essential oil at its IC_{50} value was examined. According to the results (Table 17, Fig. 39), using ST essential oil at its IC_{50} concentration on B16 melanoma cells could trigger the production of reactive oxygen species (ROS), resulting in cell death through apoptosis. The treated cells displayed a threefold increase in ROS production compared to the control cells, indicating that the essential oil can stimulate intracellular ROS production in cancer cells. These findings suggest that the essential oil treatment may cause both ROS-dependent and independent cell death in human B16 melanoma cells at its IC_{50} concentration.

Table 17. Comparison of ROS activity in *Syzygium travancoricum* leaf essential oil-treated and untreated (control) B16 cells

Sample code	Fluorescence Intensity (AU)
Control	293.23
Sample	668.74

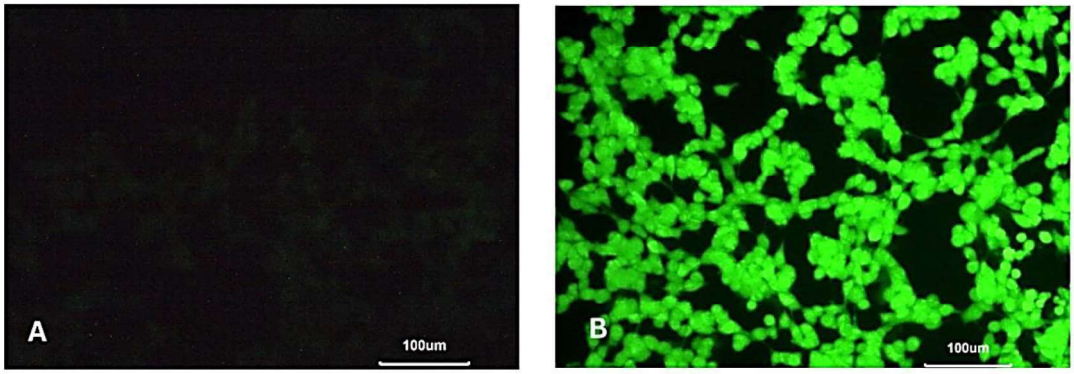


Fig. 39. Comparison of ROS activity in *Syzygium travancoricum* leaf essential oil-treated and untreated (control) B16 melanoma cells: **A**. Control cells; **B**. Treated cells

4.5.4.4 Mitochondrial Membrane Potential

The potential of *S. travancoricum* essential oil to induce cellular apoptosis was assessed by analyzing its effect on the mitochondrial membrane potential of B16 cells. The level of intracellular ROS was measured by observing the intensity of a fluorescent derivative known as 2′,7′-dichlorofluorescein (DCF), which is produced by cellular redox reactions from a non-fluorescent substance called DCFH-DA. The results indicated a significant reduction in the mitochondrial membrane potential of B16 cells treated with the essential oil, as compared to the control group (Table 18 & Table 19). This strongly suggests that the essential oil has the capability to disrupt the metabolic activity of mitochondria in B16 cells, as depicted in Fig. 40.

Table 18. Mitochondrial potential of untreated B16 melanoma cells

Cell Type	Cell Conc. (Cells/ml)	% Gated
Live (LR)	2.58E+06	92.31
Depolarized/Live (LL)	2.56E+00	0.92
Depolarized/Dead (UL)	0.00E+04	0.00
Dead (UR)	1.89E+03	6.77
Total Depolarized	2.56E+04	0.92
Total Cell Concentration	**2.79E+06/ml**	

Dilution Factor: 1.00

Table 19. Mitochondrial potential of *Syzygium travancoricum* leaf essential oil-treated B16 melanoma cells

Cell Type	Cell Conc. (Cells/ml)	% Gated
Live (LR)	4.21E+06	46.59
Depolarized/Live (LL)	2.48E+06	27.45
Depolarized/Dead (UL)	3.28E+05	3.63
Dead (UR)	2.02E+06	22.33
Total Depolarized	2.81E+06	31.08
Total Cell Concentration	**9.03E+06/ml**	

Dilution Factor: 1.00

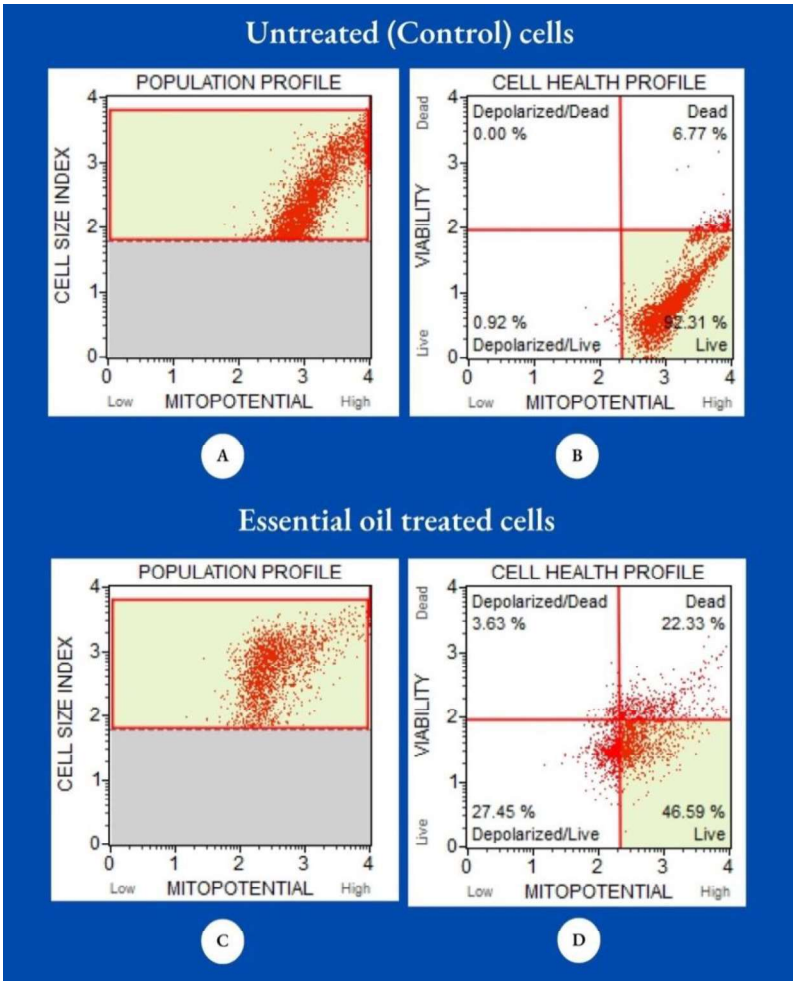


Fig. 40. Comparison of Mitochondrial potential of untreated and *Syzygium travancoricum* leaf essential oil treated B16 melanoma cells **A.** Population profile of control cells; **B.** Cell health profile of control cells; **C.** Population profile of Treated cells; **D.** Cell health profile of treated cells.

4.5.4.5 Analysis of DNA content and Cell cycle analysis

To determine whether the antiproliferative activity was associated with specific changes in cell cycle distribution, the cell cycle progression was evaluated by directly measuring the DNA synthesis in each phase of the cell cycle for 30 hours after treating the B16 cell line with the essential oil of *S. travancoricum*. Upon treatment of the B16 cell line with essential oil of *S. travancoricum*, there was a significant increase in the proportion of cells in the G0/G1 phase (75.6%), demonstrating G0/G1 cell cycle arrest. Additionally, the distribution of treated cells in the S phase remained consistent with control cells, while the proportion of cells in the G2/M phase decreased significantly. These results were confirmed through cell cycle distribution analysis, which showed a reduced accumulation of cells in both G0/G1 and G2/M, demonstrating the occurrence of S-phase cell cycle arrest after 24 hours of essential oil treatment. Notably, the proportion of B16 cells in the G2/M phase decreased to 4.1%, which was almost half that of the control group. (Fig. 41; Table 20 and Table 21).

Table 20. Cell cycle analysis of untreated B16 melanoma cells using flow cytometry

	G0/G1	S	G2/M
%Gated	69.3	18.0	8.1
Mean	2211.2	4206.2	5871.6
%CV	32.6	10.1	9.7

Table 21. Cell cycle analysis of *Syzygium travancoricum* leaf essential oil treated B16 cells using flow cytometry

	G0/G1	S	G2/M
%Gated	75.6	18.6	4.1
Mean	1982.5	4122.9	5888.2
%CV	34.0	9.1	9.9

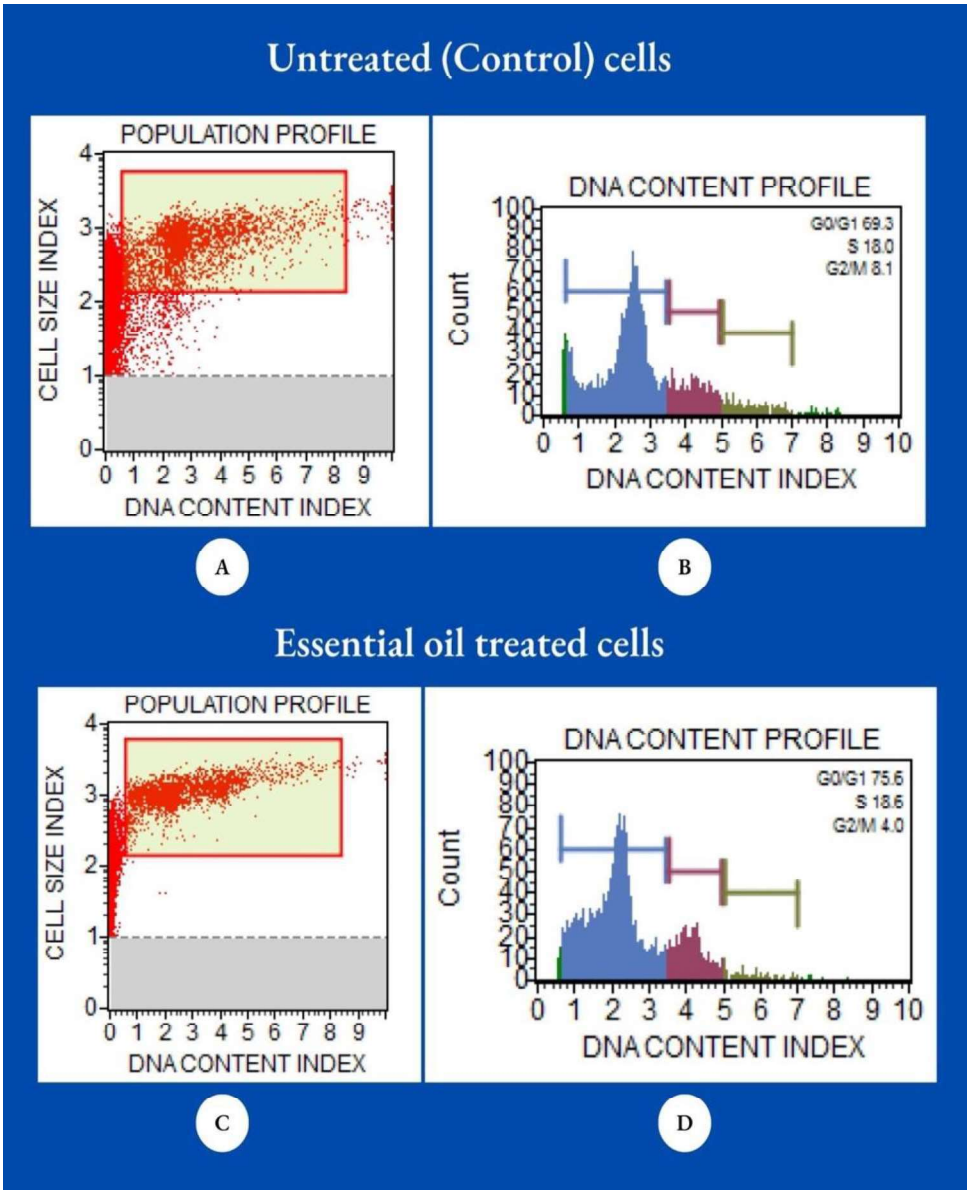


Fig. 41. Cell cycle analysis of untreated and *Syzygium travancoricum* leaf essential oil-treated B16 melanoma cells **A.** Population profile of control cells; **B.** DNA content profile of control cells; **C.** Population profile of Treated cells; **D.** DNA content profile of treated cells.

4.5.5 In vitro anti-inflammatory assay

4.5.5.1 Cycloxygenase (COX) activity

The anti-inflammatory activity of the oil of *S. travancoricum* was measured in terms of cyclooxygenase inhibition percentage compared to the untreated (control) LPS-induced RAW 264.7 cells. Protein denaturation is a well-established cause of inflammation. According to the findings of the present

study (Fig. 42), the essential oil of *S. travancoricum* effectively inhibited LPS-induced protein denaturation in the LPS-induced RAW 264.7 cell line (EC_{50} 12.33 µg/ml) compared to control. The results of the present study demonstrate the anti-inflammatory effects of *S. travancoricum* leaf essential using LPS-induced RAW 264.7 cell line. The results are displayed in Table 22.

Table 22. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil using COX assay

Concentration (µg/ml)	Average OD at 632nm	Percentage inhibition (%)	ED50 (µg/ml)
Control	0.18±0.01	96.44	
6.25	0.11±0.01	38.28	**12.33**
12.5	0.08±0.004	55.56	
25	0.06±0.001	65.23	

Values are given as mean ± SD of triplicate experiment

4.5.5.2 Lipoxygenase (LOX) activity

By inhibiting 5-lipoxygenase activity, the effects of *S. travancoricum* essential oil on the production of leukotrienes were determined, and the results are presented in Table 23. The above results suggest that *S. travancoricum* essential oil inhibits 5-lipoxygenase significantly (EC_{50} 17.44 µg/ml) compared to control.

Table 23. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil on LPS-stimulated RAW 264.7 cell line using LOX assay

Concentration (µg/ml)	Average OD at 234nm	Percentage inhibition (%)	ED50 (µg/ml)
Control	0.22±0.01	96.32	
6.25	0.16±0.02	28.68	**17.44**
12.5	0.12±0.001	46.10	
25	0.09±0.004	61.06	

Values are given as mean ± SD of triplicate experiment

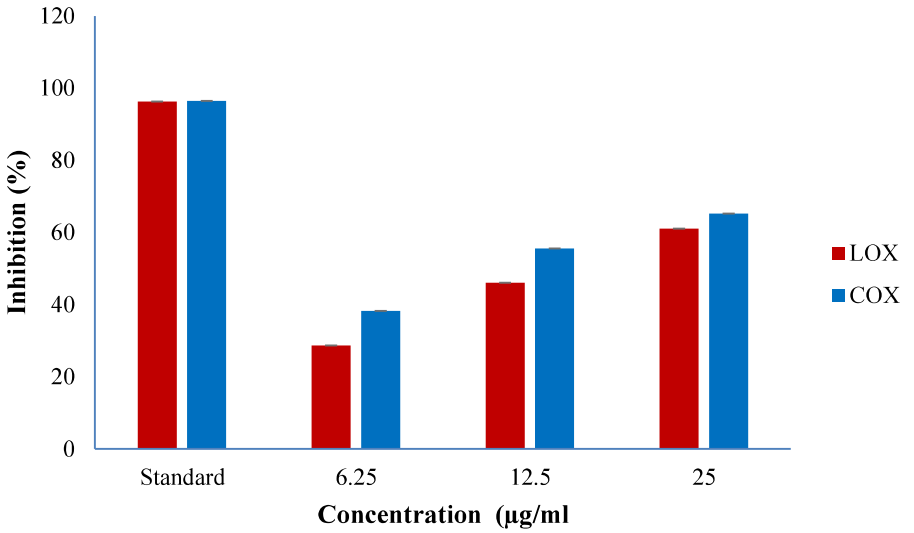


Fig. 42. Comparative anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil on LPS-stimulated RAW 264.7 cell line using COX and LOX assays

Fig. 42, illustrates a comparison of cyclooxygenase and 5-lipoxygenase inhibitory activities of *S. travancoricum* leaf essential oil. The essential oil demonstrated potent anti-inflammatory effects when tested on LPS-stimulated RAW 264.7 cells, as evidenced by the COX and LOX assays.

4.5.5.3 Myeloperoxidase (MPO) activity

The study unequivocally demonstrated the potency of *S. travancoricum* essential oil in inhibiting myeloperoxidase activity. The essential oil was found to be highly effective in inhibiting the enzyme activity and the strength of inhibition was directly proportional to the concentration of the oil.

Table 24. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil on LPS-stimulated RAW 264.7 cell line using MPO assay

Concentration (µg/ml)	ΔOD	Enzyme Activity (U/ml)
LPS	0.04	0.012
6.25	0.02	0.006
12.5	0.01	0.004
25	0.01	0.003

Values are given as mean ± SD of triplicate experiment

Significantly, the highest level of inhibition was observed with higher concentrations of the essential oil (25g/ml and 12.5g/ml) as compared to the standard drug (Table 24, Fig. 43)..

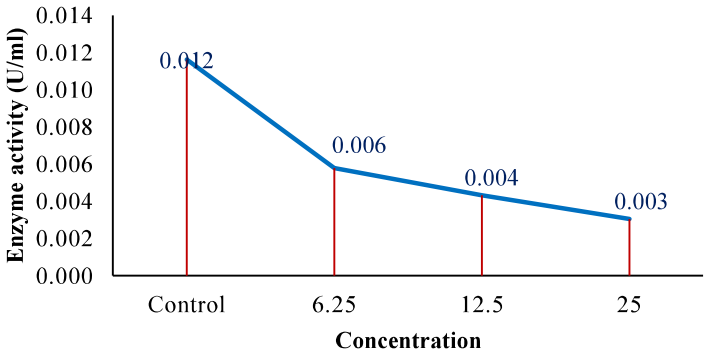


Fig. 43. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil LPS-stimulated RAW 264.7 cell line using MPO assay

4.5.5.4 Inducible Nitric Oxide Synthase and Cellular Nitrite Levels

According to the research findings, *S. travancoricum* leaf essential oil has anti-inflammatory properties. When applied to LPS-induced RAW 264.7 cell lines, the essential oil reduced the amount of nitric oxide synthase compared to control in a dose-dependent manner. nitrite concentrations (Table 25, Fig. 44). These results strongly suggest that *S. travancoricum* essential oil has the potential to be used as a natural anti-inflammatory agent.

Table 25. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil using Inducible Nitric Oxide assay

Concentration (µg/ml)	Percentage of Inhibition (%)
Control	98.24
6.25	28.29
12.5	40.24
25	60.24

Values are given as mean ± SD of triplicate experiment

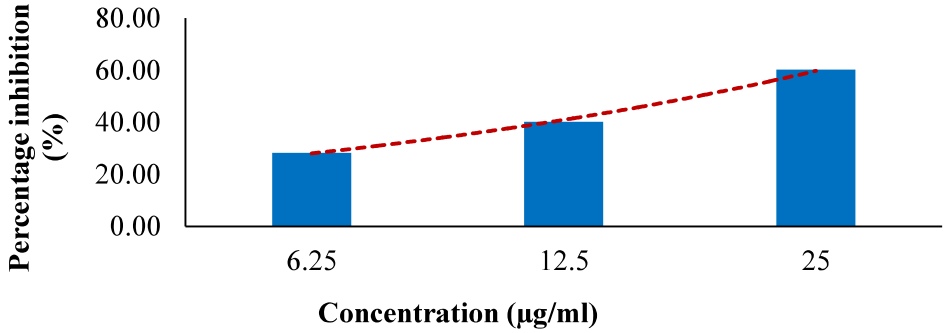


Fig. 44. In vitro anti-inflammatory activity of *Syzygium travancoricum* leaf essential oil LPS-stimulated RAW 264.7 cell line using Inducible Nitric Oxide assay

Similarly, LPS-induced RAW 264.7 cell lines with *S. travancoricum* leaf essential oil showed a reduced amount of cellular nitrite level compared to control in a dose-dependent manner. nitrite concentrations (Table 26, Fig. 45). These results also strongly suggest that *S. travancoricum* essential oil has the potential to be used as a natural anti-inflammatory agent.

Table 26. In vitro cellular nitrite levels in *Syzygium travancoricum* leaf essential oil treated cells and untreated (control) RAW 264.7 cells

Sample Concentration (µg/ml)	Average OD	Concentration of Nitrite (µg)
Control	0.10	477.18
6.25	0.07	368.28
12.5	0.05	268.29
25	0.04	181.17

Values are given as mean ± SD of triplicate experiment

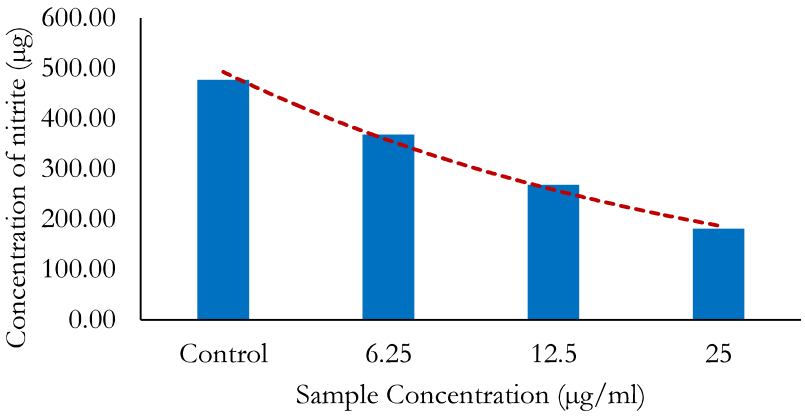


Fig. 45. Comparison of *in vitro* cellular nitrite levels in *Syzygium travancoricum* leaf essential oil treated and untreated LPS-stimulated RAW 264.7 cell line

4.6 Antidiabetic Assay

The present study evaluated the antidiabetic properties of *S. travancoricum* leaf extracts by evaluating its inhibitory effects on enzymes such as lipases, α-amylase, and α-glucosidase. The standard graph of glucose is presented in Fig. 49. The results (Fig. 46, Fig. 47, and Fig. 48) clearly revealed that the extracts significantly reduced enzyme activity in a dose-dependent manner. The water extract was the most potent among the three extracts, followed by ethanol, as indicated in Table 27, Table 28, and Table 29.

4.6.1 Lipase Inhibition Assay

Table 27. Effect of *Syzygium travancoricum* leaf extracts on lipase activity

Concentration (µg/ml)	Inhibition Percentage (%)					
	STWE	ED_{50}	STEE	ED_{50}	STEO	ED_{50}
50	21.29±0.12		12.47±0.09		10.11±0.07	
100	32.26±0.2		19.35±0.11		13.44±0.13	
150	36.45±0.11	**212.9**	32.37±0.13	**271.5**	18.39±0.12	**512.2**
200	45.16±0.2		35.91±0.21		23.44±0.08	
250	59.14±0.13		46.67±0.12		26.88±0.11	

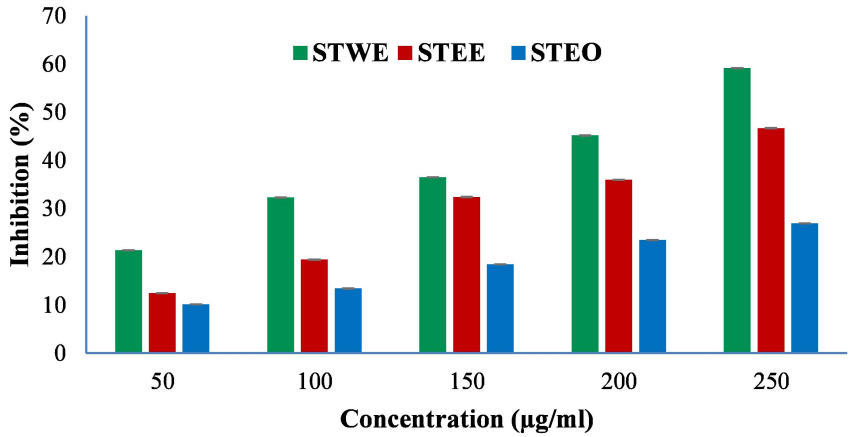


Fig. 46. Effect of *Syzygium travancoricum* leaf extracts on lipase activity

4.6.2 Alpha Amylase Inhibition Assay

Table 28. Effect of *Syzygium travancoricum* leaf extracts on α-amylase activity

Concentration (µg/ml)	Inhibition Percentage (%)					
	STWE	ED_{50}	STEE	ED_{50}	STEO	ED_{50}
50	22.17±0.1		13.96±0.13		4.93±0.05	
100	41.05±0.2		30.38±0.24		24.63±0.12	
150	61.58±0.02	**126.7**	32.02±0.3	**182.9**	38.59±0.11	**223.5**
200	76.35±0.15		55.01±0.11		49.26±0.21	
250	87.85±0.23		72.25±0.05		63.22±0.12	

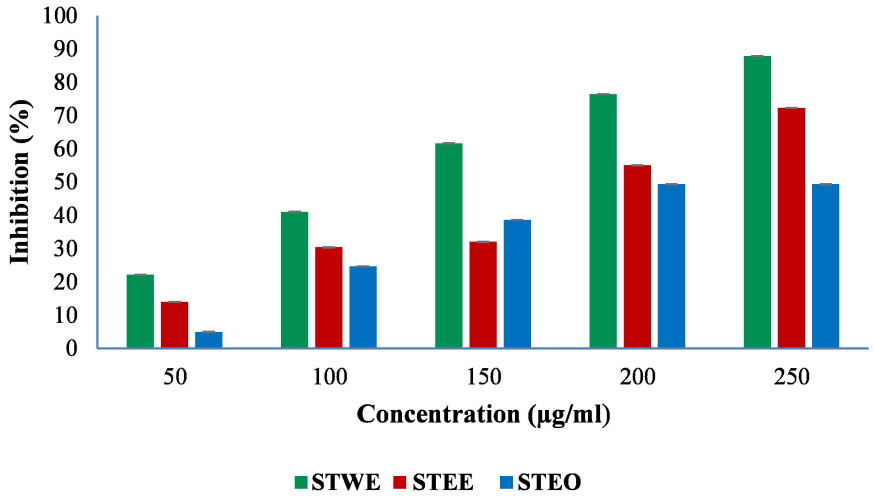


Fig. 47. Effect of *Syzygium travancoricum* leaf extracts on α-amylase activity

4.6.3 Alpha Glucosidase Inhibition Assay

Table 29. Effect of *Syzygium travancoricum* leaf extracts on α-glucosidase activity

Concentration (µg/ml)	Inhibition Percentage (%)					
	STWE	ED50	STEE	ED50	STEO	ED50
50	25.48±0.15		20.32±0.09		8.28±0.13	
100	47.10±0.09		32.04±0.14		15.91±0.12	
150	56.67±0.23	**127.7**	47.20±0.12	**165.7**	35.27±0.08	**512.2**
200	66.67±0.12		57.31±0.2		43.12±0.23	
250	84.95±0.08		72.69±0.16		53.55±0.16	

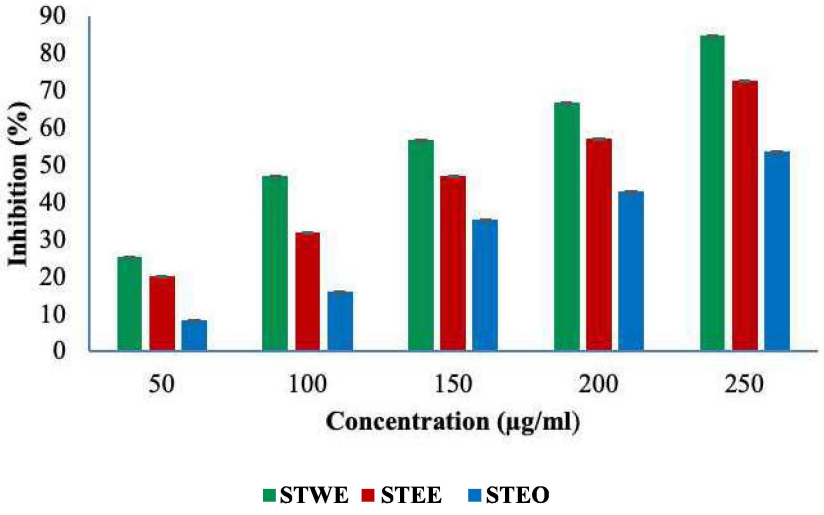


Fig. 48. Effect of *Syzygium travancoricum* leaf extracts on α-glucosidase activity

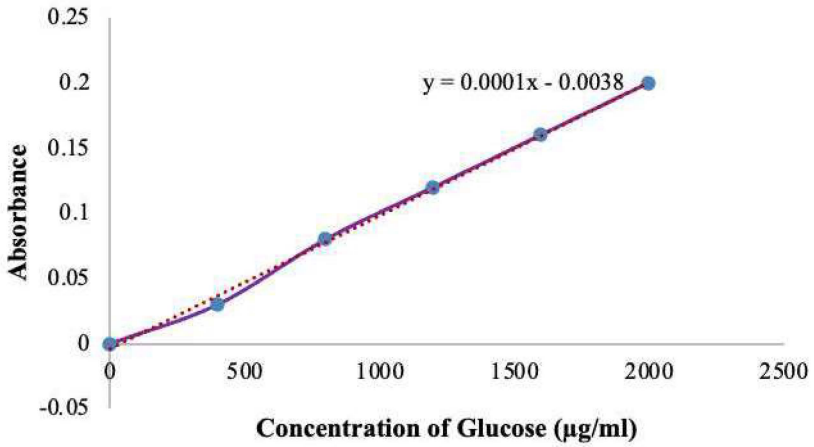


Fig. 49. Standard graph of glucose

4.7 Conservation Strategies

The conservation strategies were carried out with two key objectives; first was to pinpoint safe locations, which was accomplished through small-scale restoration efforts in currently occupied or potentially suitable unoccupied areas. The second objective was to identify appropriate propagation methods for expanding the population size, which was accomplished through seed germination, shoot cuttings and standardizing an appropriate transplanting method.

The ex situ conservation strategy was effectively implemented in five different Panchayaths located in Thrissur, Palakkad, and Pathanamthitta districts in Kerala (Fig. 50, Fig. 51, Fig. 52 & Fig. 53). The growth and establishment rate of the transplanted tree saplings were enhanced while utilising coconut husk as a transplantation medium. A total of 200 tree saplings were successfully planted utilising this ex situ conservation approach.

In the present study, it was determined that *S. travancoricum* has a low seed germination rate, which is likely due to the poor seed regeneration capacity and their susceptibility to early pest infestations. However, stem cuttings proved to be highly responsive to rooting with IBA treatment at 2000 ppm for 2-3 minutes. Moreover, a planting technique that prioritises the preservation of soil moisture by incorporating coconut husks around the saplings was identified as an effective method to substantially boost the survival rate of transplanted saplings (Fig. 54). This method effectively facilitated the establishment of saplings, thereby tackling the issue of low survival rates of seedlings caused by their sensitivity to environmental fluctuations.

Fig. 50. *Ex situ* conservation of *Syzygium travancoricum* at Elavally Grama Panchayath **A**. Planting of tree sapling and inauguration by former Minister of Agriculture, Kerala, Sri. Adv. V. S. Sunil Kumar **B, C.** Plants after successful establishment.

Fig. 51. *Ex situ* conservation of *Syzygium travancoricum* at Varavoor Grama Panchayath, Thrissur

Fig. 52. *Ex situ* conservation of *Syzygium travancoricum* **A. .** Government College, Chittur, Palakkad **B.** Enadimangalam Grama Panchayath, Pathanamthitta

Fig. 53. *Ex situ* conservation of *Syzygium travancoricum* at Panjal Grama Panchayath, Thrissur

Fig. 54. Initial phase of green space establishment using *Syzygium travancoricum* saplings at Elavally Grama Panchayath, Thrissur A, B. Saplings ready for transplantation C. Transplantation using coconut husk D. Protection of saplings using tree guard E. Growth of saplings after one year F. Growth of saplings after two years.

Chapter 5

Discussion

CHAPTER 5 DISCUSSION

5.0 Systematic analysis of *Syzygium travancoricum* Gamble

The study entitled, '*Characterization of Syzygium travancoricum Gamble (Myrtaceae), an endemic endangered species of Kerala*', aimed to gain a comprehensive understanding of this unique endemic species of the Myrtaceae family. Through various perspectives, including morphological, anatomical, histochemical, phytochemical, molecular, and pharmacological analysis, the study was able to provide a detailed characterization of this endemic and endangered species. Additionally, the study also established a conservation strategy by creating green spaces to protect *S. travancoricum* from further endangerment. The study focused on eight populations of the species located in five districts of Kerala, and the results obtained are discussed briefly.

5.1 Morphological characterization

The characters observed in the live specimens were compared with type specimens and original protologues during the study to determine their morphological characteristics. This involved close examination of various aspects of the plants, including its vegetative characteristics such as habit, bark, blaze colour, young branchlets, stem, leaves, petiole size, number of secondary nerves, intramarginal nerves, as well as their reproductive characteristics, such as peduncle size, inflorescence, sepals, petals, stamen, anther, L.S of the ovary, number of ovules, fruits, and seeds. A thorough evaluation of these features enabled a deeper understanding of the populations and their unique characteristics.

The morphological characteristics of live samples from six populations collected from JNTBGRI, Idinjaar, Kollam, Thrissur, MSSRF and

Konginichalkavu perfectly matched with the characteristics outlined in the protologue as well as type material of *S. travancoricum*. The chief characteristic features included medium to large-sized trees; bark longitudinally fissured and greyish brown, reddish brown blaze, tetragonous branchlets, chartaceous, leaf ratio wider than long, leaf shape ovate-oblong, lamina size (area) 8–10 x 5–6 cm; leaf base narrow and decurrent, leaf margin entire undulate, lateral nerves 10–5 pairs, cymose corymbose inflorescence which was terminal and axillary; long peduncle 8–10 cm long. However, in the current study, it was observed that the populations from Pookode (Wynad) and Paliyeri Mookambika Kavu (Kannur) exhibited distinct characteristics that are similar to *S. stocksii* as mentioned in the protologue. These populations were large-sized trees with greyish brown blaze colour and large-sized leaves that measured 15–20 cm in length and 8–10 cm in breadth. The shape of the leaves was elliptic to obovate, with an obtuse base and an obtusely acuminate tip without oblique ends. The petiole was shorter and thinner without a distinct groove. The number of lateral nerves ranged between 18 and 20, and the intra-marginal veins were prominent, though they faded towards the margins. The inflorescence of these populations was comparatively shorter, measuring 5–10 cm in size with a peduncle length of 2–5 cm. Consequently, it is evident from these morphological observations that these populations could be confidently categorized as *S. stocksii*.

5.2 Anatomical and Histochemical Characterization

According to this research findings, all populations shared common anatomical features. Still, certain populations exhibited significant differences in key features such as stomatal types, midrib vascular shape, distribution of midrib sclerenchyma, number of adaxial palisade layer, presence of druses and prismatic crystals, nature of pith, and cross-sectional shape of twigs.

These unique anatomical characteristics, combined with other traits, could be useful in species delineation. In the transverse section, both adaxial and abaxial epidermal cells of leaves in all populations were wider, squarish, to rectangular shaped with paracytic stomatal type except for the Pookode and Paliyeri Mookambika Kavu populations which possessed anisocytic type of stomata. This research findings corroborate with earlier findings on the leaf anatomy of *Syzygium* species (Khatijah *et al*., 1992; Noorma & Moore, 1996; Kantachot *et al*., 2007).

A single-layered adaxial palisade tissue was consistent in all the samples; however, the distribution was continuous in *S. travancoricum* but discontinuous in *S. stocksii* populations. The adaxial surface of the midrib was concave and grooved, while the abaxial surface was convex in all samples. The vascular system was found to be bicollateral. The adaxial and abaxial phloem completely surrounded the xylem, giving a characteristic 'U' shape with straight arms to the midrib vasculature in both the lamina and petiole. However, the Pookode and Paliyeri Mookambika Kavu samples showed incurved margins with discontinuous phloem branched into lateral partitions. The sclerenchyma sheath was continuous in *S. travancoricum* but was discontinuous in these populations. In lateral vein bundles, vertical bundle sheath extensions were usually absent in *S. travancoricum*, whereas they were present in these populations. The present study, however, did not reveal any observable crystal macro pattern. Nonetheless, it was noted that *S. travancoricum* had mainly druses, while *S. stocksii* had predominantly prismatic crystals.

The anatomical findings of the present study concur with previous findings of Khatijah *et al.* (1992), who also claimed that the midrib vascular type is an effective marker for species diagnosis among *Syzygium* populations.

Additionally, other anatomical characteristics, such as foliar sclereids, midrib sclerenchyma, adaxial palisade layer, and bundle sheath extensions, were discovered, which can be combined with stomatal types, midrib vascular system, and crystals to enhance species identification. However, the number and thickness of palisade layers in mesophyll did not exhibit any taxonomic significance and may be influenced by environmental factors. Oil glands were present in all samples, but any distinct patterns in their frequency or distribution were not observed.

S. travancoricum, in particular, was unambiguously observed to have oil cavities that actively secrete aromatic volatile oil in various parts of the plant. It was also identified that these secretory cavities are located abundantly in all parts of the plant, including the hypodermal regions of the stem, leaf, and petiole. This research finding definitively corroborates earlier literature on the presence of secretory cavities in other *Syzygium* species, which had previously provided indeterminate information regarding the specific plant parts where these structures are present (Khatijah *et al*., 1992; Merrill & Perry, 1939).

The histochemical characterization revealed that *S. travancoricum* populations possessed significant secondary metabolites, including tannin, lignin, starch, and essential oils, which are found in different plant parts. It is noteworthy that oil cavities were observed not only in the leaves but also in the petiole and stem. Additionally, the study has confirmed that numerous secretory substances have yielded positive test results, suggesting their accumulation within the cellular structures. These substances play a significant role in the distinct aroma of the leaves.

Syzygium is a genus with a diverse range of species, and according to Nair and Rana (2017), the taxonomy of this genus is quite complex. Therefore, there is a significant requirement for in-depth studies on the different species

within this group to determine their taxonomic classification. Conducting morphological and anatomical studies could aid in future ecological and phytosociological research and provide additional evidence to validate the taxonomic classification of the group, as well as identify new species. In the present study, it was discovered that *S. travancoricum* populations differed in terms of key anatomical characteristics such as type of stomata, midrib vasculature, midrib sclerenchyma, crystal types and frequency, distribution of adaxial palisade layer, adaxial phloem partition, pith and stem cross-section which could be useful in species delineation.

5.2.1. Comparative morphological and anatomical characterization

The morphological findings of the study recommended that *S. travancoricum* populations diverged into two species based on concrete morphological characteristics. Similarly, the anatomical analysis of eight populations based on key anatomical characteristics also provided substantial evidences for species dileneation. The comparative analysis based on UPGMA also provided a comprehensive understanding of the distinguishing features of *S. travancoricum* populations, strongly recommending their interpretation as two distinct species.

5.3 Phytochemical characterization

5.3.1 Essential Oil Profiling

The GC-MS profiling of eight populations revealed thirty three phytochemicals of significant medicinal value. Among the different classes of compounds, sesquiterpenoids comprised a major proportion of the essential oil constituents in *S. travancoricum* populations, accounting for more than 60% of the total oil content. The major sesquiterpenoids identified were β-caryophyllene and β-caryophyllene epoxide, which accounted for 80% of

sesquiterpenoids. Other sesquiterpenes discovered were α-geraniol, α-farnesene, α-selinene, and β-selinene. Monoterpenoids were the second most abundant compound, accounting for more than half of the essential oil. The major monoterpenoid compounds identified were α-pinene, β-pinene, α-ocimene, β-myrcene, β-limonene, and α-terpineol isomers. +-cembrene represented the diterpenoid compounds. Aside from that, phenol was present in moderate amounts, and the diglyceride compound 1, 3 diolein was present in trace amounts.

S. travancoricum comprised more of non-oxygenated sesquiterpenoids than oxygenated sesquiterpenoids. Similarly, acyclic monoterpenoids were the most common type of monoterpene, followed by bicyclic monoterpenoids and monocyclic monoterpenoids. The presence of some unusual chemical compounds such as viridiflorol, +-cembrene, 6 epi-shyobunol, 1, 3 diolein, and calerene epoxide was observed in Pookode leaf samples. The chemical profile of *S. travancoricum* populations revealed chemical diversity regarding major chemical compound composition and concentration.

The current study employed principal component analysis (PCA) to investigate the interrelationship between eight populations of *S. travancoricum* based on the chemical composition of their leaf essential oil. The findings unveiled significant disparities in the phytochemical composition. Concurrently, it unveiled specific common attributes within certain populations, facilitating the categorization of said populations into three primary components, namely PCA1, PCA2, and PCA3, predicated on their chemical composition (Figure 21). The populations collected from JNTBGRI, Thrissur, and MSSRF exhibited a higher degree of genetic relatedness. On the contrary, there were notable differences in the chemical constituents between the populations of Paliyeri Mookambika Kavu and Pookode. The volatile

constituents found in this study corroborate with the constituents that are commonly found in several plants of the Myrtaceae family and belonging to the *Syzygium* genus, such as *S. cumini* (Machado *et al*., 2013), *S. aromaticum* (Raina *et al*., 2001; Pino *et al*. 2001; Srivastava *et al*., 2005; Razafimamonjison *et al*., 2014), *S. calophyllifolium* (Vignesh *et al*., 2013), *S. benthamianum* (Deepika *et al*., 2013), *S. jambos* (Ghareeb *et al*., 2017; Rezende *et al*., 2013)), *S. lineatum*, *S. hancei*, *S. caryophyllatum* (Khanh & Ban, 2020), and *S. grande* (Huong *et al*., 2017).

Hence, it is evident from these research reports that each plant species has a unique phytochemical composition, which may be influenced by ecological and climatic differences, as well as the age and chemotype of the plants. These compositional patterns might have contributed to the variability in the phytoconstituents among different populations of *S. travancoricum*. The leaf samples from Pookode and Paliyeri Mookambika Kavu exhibited maximum variability concerning chemical constituents and percentage composition. These compositional patterns may contribute to the wide range of biological activities of *S. travancoricum* essential oil. β-caryophyllene is reported to have anticancer, analgesic, and anti-inflammatory properties (Zheng *et al*., 1992; Fidyt *et al*., 2016; Ames *et al*., 2018). Other notable compounds identified in most samples were α-ocimene, β-ocimene, α-pinene, and β-pinene. β-ocimene is a major compound reported in the essential oils of *Coreopsis triloba* and *Coronilla orientalis*, which have been claimed to inhibit acetylcholinesterase, butyrylcholinesterase, and α-glucosidase (Renda *et al*., 2019). The potential effects of other major compounds, such as viridiflorol, +-cembrene, 6 epi-shyobunol, 1, 3 diolein, and calerene epoxide, in this species are intriguing for future research. The antibacterial and anticancer properties of α-humulene have already been reported (Jang *et al*., 2020). Thus, the present study confirms that *S. travancoricum* may be an important source for the extraction of essential oil, as it contains a high percentage of β-caryophyllene and β-

pinene, which has antitumor, antibacterial, antifungal, and inactivation of phospholipase and esterase activities (Xu *et al*., 2018; Hou *et al*., 2019; Kovae *et al*., 2015; Silva *et al*., 2012). Hence, the essential oils from the leaves of *S. travancoricum* offer hope that this plant species could serve as the foundation for developing new biologically active substances with commercial value.

5.3.2 Phytochemical screening of extracts

The results of the phytochemical characterization provided encouraging evidence for the pharmaceutical properties of *S. travancoricum* due to an abundance of phenol and flavonoid content. Based on the analysis conducted, it was found that the leaf and bark water extracts of *S. travancoricum* had a higher concentration of phenolic compounds and flavonoids compared to ethanol extracts. Moreover, it was also observed that the plant leaves contained more of these phytochemicals than the bark. These findings suggest that the extraction method and the part of the plant used can significantly affect the concentration of these phytochemicals.

Flavonoids are a natural polyphenolic compound known for their numerous health benefits. Due to their beneficial properties such as antioxidants, anti-inflammatory effects, antidiabetic properties, and antiallergic effects, these substances are in high demand among individuals seeking to enhance their overall well-being. Moreover, the leaf and bark extracts of *S. travancoricum* exhibited a diverse range of phytochemical constituents, including tannins, terpenoids, steroids, alkaloids, carbohydrates, reducing sugars, and proteins. Fruits at various stages of maturity were discovered to possess high nutritional value, containing abundant vitamins, minerals, and dietary fibres. Multiple research studies have documented the occurrence of various chemical compounds in the genus *Syzygium*, including triterpenes,

hydrolysable tannins, anthocyanins, flavonoids, chromone derivatives, phenylpropanoids, and phloroglucinol derivatives (Ayyanar & Subash-Babu, 2012; Reynerston *et al*., 2008; Djoukeng *et al*., 2005; Kuiate *et al*., 2007; Hu *et al*., 2020; Zhang *et al*., 2020).

5.4 Molecular characterisation

Phylogenetic data

The molecular systematics of the genus *Syzygium* are poorly understood, which led to a dearth of literature in this particular research domain (Parnell *et al*., 2007). There is a lack of comprehensive studies on the *Syzygium-Eugenia* group in its primary areas of diversity despite its vast genus and global distribution (Govaerts *et al*., 2008). Understanding the phylogeny of *Syzygium* poses significant challenges. One of the main objectives of this study was to investigate the evolutionary relationships among the populations of *S. travancoricum*. The study was conducted by analysing the morphological, molecular, and combined morphological and molecular data of the specimens, including 24 *Syzygium* species retrieved from NCBI.

The study utilized both morphological and molecular data to identify unique genetic lineages within *S. travancoricum* populations. Furthermore, the phylogenetic analyses confirmed the differentiation of *S. travancoricum* from *S. stocksii* (Fig. 25 & Fig. 26). The Bayesian phylogenetic tree revealed paraphyletic relationships between these two species in relation to the concatenated dataset. The results suggest that *S. travancoricum* has undergone divergence, leading to the formation of two genetically distinct lineages. This justifies its classification as an independent species rather than a synonymous designation of *S. stocksii*. The study also highlights that integrating morphological and genetic data would be an effective and combinatorial approach for resolving species conflicts at the infra-specific level, specifically

at deeper nodes. Biffin *et al*. (2006) examined the correlation between the group *Syzygium* Gaertn. by applying parsimony and Bayesian analysis of cpDNA sequences. Vasconcelos *et al*. (2017) conducted a phylogenetic analysis of the tribe Myrteae, utilizing one nuclear (*ITS*) and seven chloroplast DNA regions. This study examined maximum likelihood and Bayesian inference methods in constructing phylogenetic trees. Craven and Biffin (2010) proposed an infrageneric classification for *Syzygium* based on its evolutionary relationship using plastid and nuclear DNA sequences, further supported by morphological evidence. Hence, this present study proposes the necessity of conducting research with Indian sampling subset to understand phytogeography and its species diversification in the Indian subcontinent.

5.5 Pharmacological characterization

The present study investigated the pharmacological properties of *S. travancoricum*, specifically its antimicrobial, antioxidant, anticancer, cytotoxic, anti-inflammatory, and antidiabetic properties. Several research studies have provided ample evidence of the therapeutic potential of *Syzygium* species and their importance in traditional, classical, and modern medicine (Ayyanar *et al*., 2013; Costa *et al*., 2013).

5.5.1 Antimicrobial property

The antimicrobial activity of essential oils derived from *S. travancoricum* leaves was evaluated against eight bacterial and three fungal strains. The essential oils exhibited notable antibacterial efficacy, specifically against *B. cereus*, *S. mutans*, and *E. coli*. The essential oil of *S. travancoricum* demonstrated significant inhibitory activity against Gram-positive and Gram-negative bacteria. Gram-positive bacteria exhibited greater sensitivity to the essential oil compared to Gram-negative bacteria. Among the three fungal strains tested, *C. albicans* exhibited significant inhibition of essential oil compared to

the positive control, Clotrimazole. Similar findings were reported by Shafi *et al*., 2002; Joji and Beena, 2011; Rahnama *et al*., 2012; Thinh *et al*., 2022, and Saroj *et al*., 2015).

The mechanism behind the antimicrobial property of *S. travancoricum* could be ascribed to the presence of some unique sesquiterpenoids in the leaf essential oil. Previous research studies have also suggested similar correlations, where α-terpineol demonstrated profound antimicrobial and antiplasmodic properties, as well as the ability to enhance the skin's permeability to lipid-soluble compounds (Williams & Barry, 1991; Lim *et al*., 2012). Similarly, α-pinene, β-pinene, trans-caryophyllene, 1,3,6-octatriene, delta-3-carene, α-caryophyllene, and limonene in *S. cumini,* were responsible for its antimicrobial activity against various pathogenic bacterial strains (Mohamed *et al*., 2013). Hence, it is evident from the given results that the antimicrobial activity of *S. travancoricum* leaf essential oil is attributed to its phytochemical compounds, such as α-pinene, β-pinene, caryophyllene, 1,3,6-octatriene, calarene, α-humulene, and α-limonene.

5.5.2 Antioxidant property

The antioxidant activity of leaf extracts from *S. travancoricum* was comparable with that of ascorbic acid, as demonstrated by DPPH and FRAP assays. The leaf and bark extracts demonstrated strong antioxidant activity, with IC_{50} values of 27.48 μg/ml and 25.30 μg/ml, respectively, compared to the standard quercetin, which had an IC_{50} value of 24.09 μg/ml. Moreover, the IC_{50} values corresponded precisely with the phenol concentration observed in the leaves and bark. The results of the present study corroborated with Uddin *et al*. (2022), who also reported antioxidant properties of *S. cymosum*, *S. paniculatum*, and *S. caryophyllatum*, ascribed to polyphenols, tannins, and flavonoids.

Several previous studies in *Syzygium* (Ruan *et al*., 2008; Mzindle, 2017; Wathsara *et al*., 2020) also support the results of the present study and suggest that leaf and bark contain high levels of antioxidants. This also confirms their potential use in various formulations for treating disorders caused by excessive oxidative stress. The antioxidant potential of essential oil was found to be moderate compared to leaf and bark extracts, possibly due to the limited presence of phenols in the essential oil.

5.5.3 Cytotoxicity

The cytotoxicity of *S. travancoricum* essential oil was assessed using the MTT assay, revealing moderate toxicity against the L929 fibroblast cell line. The observed effect could be attributed to the synergistic interaction of the active components present in the oils. Prashar *et al*. (2006) found comparable outcomes when studying the effects of *S. aromaticum* oil on human skin cancer cells. Essential oils have been shown to possess anticancer properties through multiple mechanisms, such as activating detoxifying enzymes, inducing DNA damage caused by oxidative stress, exerting antimetastatic and cytotoxic effects, inhibiting cell viability, inducing cell cycle arrest or apoptosis, reducing phosphate-Akt expression levels, inhibiting MMP-2 and protein leakage, and suppressing cell viability (Dwivedi *et al*., 2011). Batiha *et al.* (2020) identified eugenol, humulene, and caryophyllene as significant components of the essential oil in *S. aromaticum* exhibiting cytotoxic and antitumor properties.

5.5.4 Anticancer Activity

The anticancer property of *S. travancoricum* essential oil was assessed using the MTT assay, revealing effective anti-proliferative activity against the B16 melanoma cell line. The possible mechanism attributed to this anticancer activity was further assessed *via* cell apoptosis, ROS activity, mitochondrial membrane potential, cell cycle, and DNA content analyses.

5.5.4.1 Cell Apoptosis

This study aimed to determine the mechanisms underlying the anticancer activity of *S. travancoricum* essential oil. The results revealed that apoptosis was induced in the B16 melanoma cell line treated with *S. travancoricum* essential oil, causing cell death in cancer cells. Apoptosis induction can act as a mechanism that enables naturally occurring anti-cancer agents to activate cell death. Unlike cytotoxic agents, which can harm normal cells, the dys-regulatory apoptosis machinery focuses specifically on cancer cells. Therefore, these agents can be employed as an effective complementary treatment to chemotherapy. These plant-derived natural alternatives can help to reduce the chances of cancer recurrence by inducing programmed cell death. Numerous studies have been conducted on the anti-cancer properties of various plant compounds like alkaloids, terpenes, and polyphenols. D-limonene and perillyl alcohol have demonstrated their ability to induce apoptosis in cancer cell lines, inhibiting cancer growth (Klener *et al*., 2006; Chaudhry *et al*., 2022). These findings highlight the potential of plant-derived compounds for developing cancer treatments.

5.5.4.2 ROS activity

This study utilized flow cytometry to evaluate the reactive oxygen species (ROS) level in cancer cell lines treated with *S. travancoricum* leaf essential oil. The findings demonstrated a threefold rise in ROS production (indicated by green fluorescence) in the treated cell lines. The ROS production level in B16 cancer cells was higher in treated cells compared to control cells, leading to apoptosis in cancer cells. The results strongly indicate that the IC_{50} concentration of essential oil (57.26 μg/ml) can induce cell death in human B16 melanoma cells through ROS-dependent and ROS-independent mechanisms. Elevated levels of ROS and impaired mitochondrial function,

commonly observed in cancer, can lead to oxidative damage to cellular structures such as genomic and mitochondrial DNA. This damage can cause alterations in metabolic processes and signalling pathways, ultimately contributing to various human diseases, including inflammation, cancer, mitochondrial and neurodegenerative diseases, diabetes, chronic diseases, and ageing (Kudriyavktseva *et al*., 2016; Picca *et al*., 2020; van der Vliet *et al*., 2018).

5.5.4.3 Mitochondrial Membrane Potential

The mitochondrial potential of B16 melanoma cells treated with the essential oil of *S. travancoricum* was analyzed. The results demonstrated a significant decrease in mitochondrial membrane potential in the *S. travancoricum* leaf essential oil-treated B16 melanoma cells compared to the control, strongly indicating the disruption of mitochondrial metabolic activity in B16 melanoma cells. Loss of the mitochondrial inner transmembrane potential is often observed to be associated with the early stages of apoptosis. The collapse of this potential is believed to coincide with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which then triggers the downstream events in the apoptotic cascade. Mitochondrial membrane potential changes have been implicated in apoptosis, necrotic cell death, and caspase-independent cell death processes. Depolarization of the inner mitochondrial membrane potential is thus a reliable indicator of mitochondrial dysfunction and cellular health, which has become increasingly important in the study of apoptosis, drug toxicity and multiple disease states (Udensi & Tchounwou, 2014; Elkhateeb *et al*., 2018).

5.5.4.4 Cell Cycle Analysis

Apoptosis and cell cycle are crucial regulatory systems that control cell growth. The flow cytometric cell cycle distribution analysis in *S. travancoricum*

leaf essential oil-treated B16 melanoma cells confirmed the antiproliferative properties of the *S. travancoricum* leaf essential oil during mitosis. The impact of *S. travancoricum* essential oil on the cell cycle was assessed to determine the ability to inhibit B16 cell growth. The results revealed that *S. travancoricum* essential oil stopped the B16 cell cycle in the S and G2/M phases and induced apoptosis after 24 hours. The results corroborated with the previous study reported by Fernandez-Gil *et al*. (2019), who also claimed that cell cycle arrest at the G0/G1, S, and/or G2/M phases can result in apoptosis. The present study demonstrated for the first time the antiproliferative effect of *S. travancoricum* essential oil based on cell cycle disruption. The inhibitory concentration of oil specifically blocked the cell cycle at the S-phase and prevented cells from entering the proliferative phase. Additionally, the S-phase transition offers a powerful checkpoint during the cell cycle that can influence B16 cell proliferation. This suggests that the apoptotic response is influenced by cellular sensitivity during the S-phase.

The present study also demonstrated that the G2/M checkpoint in the cell cycle is a potential target for anticancer therapy. Cells with DNA damage cannot divide or repair their damaged DNA, and hence, the damaged cells may be able to enter mitosis and undergo apoptosis if the G2/M arrest checkpoint is compromised. The results of the present study demonstrated that *S. travancoricum* essential oil-induced cell cycle arrest in the S and G2/M phases can lead to apoptosis in B16 cell line which can be effectively used in cancer therapy.

5.5.5 Antiinflammatory property

The results of the present study indicated that the essential oil of *S. travancoricum* exhibited substantial anti-inflammatory properties using in vitro assays. The essential oil treated LPS-activated RAW 264.7 cells

demonstrated significant reduction in the activity of cyclooxygenase enzymes, associated with inflammatory responses, compared to untreated (control) cells. A concentration of 25 μg/ml, exhibited an inhibition rate exceeding 60%. Thus, the results indicated that *S. travancoricum* essential oil could be an effective anti-inflammatory agent.

The enzyme lipoxygenase (LOX) produces leukotrienes during the metabolism of arachidonic acid (Wisastra & Dekker, 2014). Inhibiting lipoxygenase activity is considered anti-inflammatory, and the *S. travancoricum* essential oil was found to be an effective inhibitor in this study. Myeloperoxidase produces hypochlorous acid (HOCl) and is primarily expressed in neutrophils. While myeloperoxidase and HOCl can kill pathogens, they can also damage tissue and cause cytotoxicity, leading to inflammation (Aratani, 2018). Hence, inhibiting myeloperoxidase activity is considered an effective anti-inflammatory property, and in the present study, the ability of *S. travancoricum* leaf essential oil to inhibit myeloperoxidase activity was evaluated. The results showed that the essential oil effectively inhibited myeloperoxidase activity, with enzyme activity decreasing as essential oil concentration increased. A higher essential oil concentration displayed a higher inhibitory effect than the standard drug. Inflammatory conditions increase cellular nitrite levels and the presence of the nitric oxide synthase enzyme. Therefore, the drug's anti-inflammatory properties can be assessed by quantifying inducible nitric oxide synthase and cellular nitrite levels. This study observed that the application of *S. travancoricum* leaf essential oil to the LPS-activated RAW 264.7 cell line resulted in decreased levels of nitric oxide synthase (iNOS) and cellular nitrite concentration. Increasing the essential oil concentration resulted in a progressive decrease in cellular nitrite and nitric oxide synthase levels. A significant body of literature exists on sesquiterpenes with anti-inflammatory properties obtained from

essential oils of different plant species. Caryophyllene and caryophyllene epoxide are the major sesquiterpenoids found in the leaf essential oil of *S. travancoricum*. These compounds are believed to contribute to its anti-inflammatory properties. Machado *et al*. (2013); Chanudom and Tangpong (2015); Siani *et al*. (2013), and Qamar *et al*. (2021, 2022) have reported similar findings in *S. cumini*.

5.5.6 Antidiabetic Properties

The literature suggests that the primary mechanism of action for the anti-diabetic potential of certain compounds involves inhibiting enzymes involved in carbohydrate metabolism, including α-glucosidase, maltase, and α-amylase. The results of antidiabetic activity revealed that the water and ethanol leaf extracts of *S. travancoricum* effectively suppressed the activity of lipases, α-amylase, and α-glucosidase enzymes in a dose-dependent manner. In comparison to other extracts, the leaf essential oil of *S. travancoricum* exhibited moderate inhibitory properties against lipase (26.8%), α-amylase (63.22%), and glucosidase (53.55%). The moderate antidiabetic effects of *S. travancoricum* essential oil may be attributed to the oxygenated terpenoids, specifically monoterpenes and sesquiterpenes. These terpenoids can bind to amino and sulfhydryl groups of enzymes, resulting in a conformational change and subsequent loss of enzyme activity. This mechanism suggests that the moderate antidiabetic effects observed could be due to the inhibition of enzymes by these volatile components. Consistent with prior research (Zulcafli *et al*., 2020; Rudrapal *et al*., 2022), it is anticipated that the inhibitory activity of *S. travancoricum* essential oil can be attributed to α-pinene and β-pinene.

The research findings of this study indicated that the leaf extracts of *S. travancoricum* resulted in remarkable reductions in glucose levels compared to

the untreated control group. The antidiabetic activity of the plant can be attributed to the presence of antioxidants, mainly polyphenols, as indicated by previous studies (Banda *et al*., 2018; Umeno *et al*., 2016). In diabetes, hormone-sensitive lipase exhibits enhanced ability to convert triacylglycerols into fatty acids, leading to increased release of fatty acids into the bloodstream. Rajaei *et al*. (2015) found that this process promotes the transformation of surplus fatty acids into phospholipids and cholesterol within the liver. Previous research has demonstrated that saponins and phenolic compounds derived from plants have notable anti-hyperlipidemic properties (Banda *et al*., 2018; Adeneye *et al*., 2011; Francis *et al*., 2002; Marrelli *et al*., 2016).

The present study has demonstrated that leaf extracts of *S. travancoricum* have antihyperglycemic and antihyperlipidemic properties. The study also proposes that additional research and *in vivo* application of *S. travancoricum* extracts would be advantageous for comprehending the underlying mechanism and advancing the development of antidiabetic medications. The limited effectiveness of current antidiabetic drugs is attributed to their sole focus on blood glucose levels, neglecting the treatment of hyperlipidemia. The current research findings indicate that *S. travancoricum* leaf extracts possess noteworthy anti-hyperglycemic properties, which can efficiently alleviate hyperlipidemia, comorbidity commonly linked with diabetes.

5.6 Conservation Strategies

S. travancoricum is an endemic endangered tree species confined to rain-fed environments, including sacred groves, myristica swamps, and riversides, as it has specific habitat preferences. These ecosystems are important because they serve as natural water reservoirs. *S. travancoricum* plays a vital role in ecosystem restoration due to its resilient root systems, capable of withstanding floods and replenishing groundwater resources. Unfortunately,

the populations of *S. travancoricum* are under significant threats from habitat destruction, inbreeding, low seed germination potential, and climate change. In addition, the disruptions to these delicate habitats can also lead to heightened surface water runoff, resulting in monsoon flooding, erosion, and dry stream beds throughout the year. This can cause reduced water absorption and retention, diminished soil quality, and negative impacts on agriculture (Chandran & Mesta, 2008). Therefore, it is imperative to take prompt action to safeguard this endangered plant species. Conservation efforts, mainly *via* ex situ conservation methods, were utilised to increase the population size. This study also aimed to establish green spaces in five different Grama Panchayaths across Kerala as an effective strategy for the ex situ conservation of *S. travancoricum*.

The ex situ conservation strategies were carried out by identifying secure locations and implementing small-scale restoration initiatives in those selected areas that were currently uninhabited or had the potential to be suitable for establishment. Appropriate propagation methods were also accomplished to increase the population by conducting seed germination experiments, utilising shoot cuttings, and establishing a standardised planting technique. The current study identified that *S. travancoricum* exhibits a diminished rate of seed germination, potentially attributed to its limited seed regeneration capacity and vulnerability to early pest infestations. Nevertheless, stem cuttings have demonstrated a significant level of responsiveness to rooting when treated with IBA (2000 ppm). Furthermore, it has been discovered that implementing a planting technique using coconut husk, which focuses on preserving soil moisture, can significantly enhance the viability of transplanted plants. This method aids in the successful establishment of saplings, thereby mitigating the low survival rate of seedlings due to their susceptibility to environmental fluctuations. In

contemporary conservation practices, *in situ* conservation is widely acknowledged as the most effective method for safeguarding endangered species within their native habitats. However, there is an increasingly prevalent recognition of the significance of ex situ conservation endeavours. Implementing ex situ conservation is currently recognised as a vital component for ensuring the survival of various species (Kramer *et al*., 2011; Oldfield & Newton, 2012; Pritchard *et al*., 2012).

This study highlights the significance of field gene banks as green spaces due to their various functions, such as acting as local climate change buffers and offering ornamental and aesthetic plantations. Trees can effectively mitigate the impacts of climate change by sequestering carbon dioxide from the atmosphere. Unused land areas have the potential to serve as suitable sites for the creation of green spaces. These spaces can serve various purposes, such as minimizing the impact of increasing greenhouse gas emissions and protecting against natural disasters like floods and droughts. Ex situ gardens or green spaces have multiple benefits, including soil erosion prevention, water contamination prevention, and wildlife habitat preservation. Furthermore, the presence of endangered or threatened tree species in living collections presents a valuable opportunity for experts to engage in extensive research regarding their growth patterns, reproductive processes, strategies for managing diseases, and potential reactions to climate change. Additional research is required to investigate the carbon storage potential of trees in established green spaces despite the well-established significance of forests in carbon sequestration. Planting trees on educational campuses and government organisations can effectively mitigate climate change and promote the significance of green spaces.

Chapter 6

Conclusion & Major Findings

CHAPTER 6 CONCLUSION AND MAJOR FINDINGS

Syzygium travancoricum Gamble, commonly referred to as '*Poriyal*,' '*Vathamkollimaram*,' '*Kulirmavu*,' or '*Kollignaval*,' is an endangered tree species belonging to the genus *Syzygium*. It is endemic to the southern Western Ghats region of India. According to the IUCN (2022), Red List of Threatened Species, this tree species has been categorized as "Critically Endangered" because they are at risk of extinction and can only survive in rain-fed areas due to its specific habitat requirements. Moreover, the taxonomic status of *S. travancoricum* has been debated among taxonomists due to recent confusion and synonymization with *S. stocksii*. There is an ongoing debate among taxonomists regarding the classification of this species. Identification of *Syzygium* species is challenging because of the scarcity of diagnostic morphological characteristics. Hence, accurate diagnostic characteristics are crucial for addressing the taxonomic challenges associated with these species, effective utilisation of plant resources and preserving genetic resources. The present study aimed to provide a comprehensive characterization of the species *S. travancoricum* which may provide taxonomic resolution for two species in response to an urgent need. Moreover, there is a dearth of literature regarding the morpho-anatomical, histochemical, phytochemical, molecular and pharmacological characteristics of *S. travancoricum*. Also, there is a notable absence of extensive research and proactive measures focused on identifying and mitigating the barriers encountered by endangered or rare species to promote their population growth and resolve their challenges. Hence, conducting a thorough characterization of this species before establishing and carrying out conservation strategies and engaging in bioprospecting and product development endeavours is crucial. So, the

present study entitled, 'Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), a critically endangered endemic species of Kerala' aimed to comprehensively investigate the morphology, anatomy, phytochemistry, molecular phylogeny, and pharmacological benefits of this species. Additionally, it aimed to propose ex situ conservation strategies for future bioprospecting.

Plant samples of *S. travancoricum* were collected from eight populations located at JNTBGRI, Palode; Idinjaar; Kollam; Kalassamala sacred grove in Thrissur district; Pookode; MSSRF, Wynad; Konginichalkavu, and Paliyeri Mookambika Kavu at Kannur district. The collection period spanned from March 2018 to June 2022. The morphological characteristics were compared to the protologue and type specimens retreived from the herbarium repositories of Kew Botanical Garden and the Botanical Survey of India, Coimbatore. Despite morphological similarities, Pookode (Wynad) and Paliyeri Mookambika Kavu (Kannur) had distinct morphological characteristics. These traits included a greyish-brown blaze and larger leaves measuring 15–20 cm long and 8–10 cm wide. The leaves were elliptic and obovate with obtuse bases and acuminate tips without oblique ends. These populations also had shorter, thinner petioles without grooves, 18–20 lateral nerves, and prominent intra-marginal veins that faded towards the leaf margins. The inflorescence in these populations was 5–10cm long and had a 2–5 cm peduncle. The fruit also differed in colour and size. The present study found that *S. travancoricum* has distinct morphological characteristics, including blaze colour, leaf size, shape, base, tip, petiole size, number of lateral nerves in the leaf inflorescence size and position, peduncle length, and fruit size and colour. The sample populations had no overlapping characters, so we concluded that the two species are distinct, and our recommendation is to reinstate *S. travancoricum*. The study suggests using additional morphological

traits from multiple populations to confirm the distinctiveness of *Syzygium* species and reconstruct the phylogeny.

The findings of the anatomical examination indicated that all populations exhibited common anatomical characteristics. However, key characteristics varied significantly in some populations. Stomatal types, midrib vascular shape, midrib sclerenchyma distribution, number of adaxial palisade layers, druses and prismatic crystals, pith type, and twig cross-sectional shape differed. The transverse section showed all leaf populations had wider, squarish to rectangular adaxial and abaxial epidermal cells. Except for the Pookode and Paliyeri Mookambika Kavu populations, stomata were mostly paracytic. The adaxial palisade tissue in all samples was single-layered. *S. travancoricum* and *S. stocksii* populations had distinct distribution patterns. The former had a continuous distribution, while the latter was discontinuous. The upper midrib surface was concave and grooved, while the lower surface was convex in all samples. The bicollateral vascular system was noted, with upper and lower phloem completely surrounding the xylem. This created a unique 'U' shape with straight midrib vasculature extensions in the leaf blade and petiole. However, the samples from Pookode and Paliyeri Mookambika Kavu had incurved margins with adaxial phloem partitions, which further split into lateral partitions. The sclerenchyma sheath was continuous in *S. travancoricum* but discontinuous in *S. stocksii*. Plant crystals are predominantly comprised of calcium oxalate and exhibit diverse shapes and distributions within plant tissues without discernible macroscopic crystal patterns. However, it was observed that *S. travancoricum* exhibited a predominance of druses, whereas *S. stocksii* displayed a higher prevalence of prismatic crystals. In conclusion, this investigation has successfully identified variations in significant anatomical features among the eight populations of *S. travancoricum*. These variations encompass differences in stomata type,

midrib vasculature, midrib sclerenchyma, crystal types and frequency, distribution of the adaxial palisade layer, adaxial phloem partition, pith, and stem cross-section. These characteristics have the potential to be valuable for distinguishing between different species. The potential value of unique anatomical features becomes apparent when considering their combination with other characteristics for species identification. The results of histochemical analysis revealed the presence of a variety of significant secondary metabolites in different plant vegetative parts of *S. travancoricum*. The metabolites constituted tannin, lignin, starch, and essential oil. Oil cavities have been found in various parts of plants, including the leaves, petioles, and stems. During the histochemical investigation, it was observed that most of the secretory substances exhibited positive reactions in the test. This suggests that these secondary metabolites accumulate within the cells of vegetative parts like leaves bark, and stem, contributing to this species' characteristic leaf aroma and distinctiveness. The UPGMA phylogram constructed based on comparative morphology and anatomy of eight populations with other 25 *Syzygium* species also revealed clear divergence of two populations into *S. travancoricum* and *S. stocksii* and their paraphyletic relationships.

The qualitative analysis of leaf and bark extracts of *S. travancoricum* identified various classes of secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, steroids, phenols, and terpenoids. The quantitative evaluation of leaf and bark extracts of *S. travancoricum* indicated the existence of significant amounts of secondary metabolites, including phenolic compounds and flavonoids. The concentration of chemical constituents in the water extract was higher than that of the ethanol extract. Furthermore, the leaves displayed a higher concentration of phytoconstituents compared to the bark. The results of the nutritional composition and mineral contents of

S. travancoricum fruit samples at different stages of ripening found that the fruits contain a diverse range of essential nutrients such as vitamins, minerals, phenols, anthocyanins, phenolic acids, and carbohydrates. Significant amounts of minerals, including Sodium, Potassium, Calcium, Magnesium, and Phosphorus, were detected. The samples exhibited higher concentrations of Potassium (137.55–170.39 mg/100g) and Calcium (40.75–47.99 mg/100g) in comparison to the other dietary minerals. The samples exhibited variability in the concentrations of Magnesium (10.88–11.54 mg/100g), Sodium (12.33–14.63 mg/100g), and Phosphorus (13.66–14.17 mg/100g). In addition, trace amounts of microelements, including Iron, Copper, Nickel, Chromium, Zinc, and Cobalt, were identified. The mineral content of the raw fruits was found to be the highest, followed by semi-ripe and ripened fruits.

S. travancoricum populations exhibited significant variations in the composition and concentration of major chemical compounds within their chemical profiles. The chemo-profiling results of the leaf essential oil of *S. travancoricum* populations, by GC-MS analysis, indicated a predominance of non-oxygenated sesquiterpenoids over oxygenated sesquiterpenoids in terms of concentration. The most common monoterpene observed was acyclic monoterpenoids, followed by bicyclic and monocyclic monoterpenoids. The Pookode leaf samples exhibited the presence of unique chemical compounds, including viridiflorol, +-cembrene, 6 epi-shyobunol, 1, 3 diolein, and calerene epoxide. The study affirms the potential of *S. travancoricum* as a valuable source for the development of commercially significant biologically active substances such as β-caryophyllene and β-pinene, which possess antitumor, antibacterial, antifungal and anti-inflammatory properties.

The study on molecular taxonomy provided a detailed idea regarding the similarities and dissimilarities between different taxa of *Syzygium* Gaertn. The

Bayesian phylogenetic tree indicated paraphyletic relationships between these two species in relation to the concatenated dataset. The findings indicate that *S. travancoricum* underwent divergence, resulting in two genetically distinct lineages. This encourages its categorization as a distinct species rather than being considered as a synonym of *S. stocksii*.

The antimicrobial assays revealed remarkable antimicrobial activity of the essential oils of *S. travancoricum* against specific strains of both Gram-positive and Gram-negative bacteria, as well as fungal strains. The essential oil exhibited inhibitory effects on *B. subtilis*, *B. cereus*, *E. coli*, and *S. mutans* compared to the positive and negative controls. The antioxidant assays demonstrated that the extracts of *S. travancoricum* had antioxidant properties, The results of the DPPH assay indicated that *S. travancoricum* leaf and bark extracts possessed significant amount of antioxidants with IC_{50} values of 27.48 μg/ml and 25.30 μg/ml, respectively. The FRAP assay results also suggested that the antioxidant activities were more remarkable in water extracts, followed by ethyl alcohol extracts and essential oil. The potential applications of *S. travancoricum* in various industries, such as pharmaceuticals, foods, cosmetics, dentistry, and agriculture, have been indicated by this research.

The *S. travancoricum* essential oil was tested for cytotoxicity on the L929 fibroblast cell line using MTT assay. The essential oil showed moderate cytotoxicity LC_{50} of 65.09. The anticancer property of *S. travancoricum* leaf essential oil was evaluated by exploring the underlying mechanisms, such as cell apoptosis, reactive oxygen species (ROS), mitochondrial potential, and cell cycle analysis. Treatment of B16 melanoma cell line with the essential oil at its IC_{50} concentration significantly increased late apoptotic or necrotic B16 cells, demonstrating its effectiveness in inducing cell death. Moreover, ROS

production was increased significantly in essential oil treated B16 cancer cells, ultimately leading to apoptosis. The findings demonstrate that the IC_{50} concentration of *S. travancoricum* essential oil has the potential to stimulate intracellular ROS production in cancer cells when compared to the control group. Additionally, *S. travancoricum* leaf essential oil treated B16 cells exhibited a notable decrease in mitochondrial membrane potential compared to untreated cells suggesting that the essential oil interferes with mitochondrial metabolic activity. Similarly, the research findings on cell cycle analysis suggest that the *S. travancoricum* leaf essential oil induced cell cycle arrest, specifically in the S-phase. The proportion of B16 cells in the G2/M phase decreased to 4.1%, representing a nearly 50% reduction compared to the control group, indicating significant anti-cancer properties. Hence the above results confirms that *S. travancoricum* leaf essential oil has the potential to be used as a therapeutic agent for cancer treatment.

Various assays were used to compare the anti-inflammatory activity of the leaf essential oil of *S. travancoricum* with that of the standard Diclofenac sodium. As observed through COX and LOX assays, the essential oil of *S. travancoricum* demonstrated significant anti-inflammatory effects on LPS-activated RAW 264.7 cell line. The drug diclofenac inhibited 96.44% of the target, while the essential oil of *S. travancoricum* at a concentration of 25 μg/ml inhibited more than 60%. In this study, we observed that the application of *S. travancoricum* essential oil to LPS-induced RAW 264.7 cell lines resulted in a decrease in the levels of nitric oxide synthase (iNOS) and cellular nitrite concentration. Similarly, the study also demonstrated the effectiveness of *S. travancoricum* leaf essential oil to inhibit myeloperoxidase activity, which decreased as the concentration of essential oil increased. The anti-diabetic properties of *S. travancoricum* extracts (water, ethanol, essential oil) were evaluated using lipase inhibition assay, α-amylase inhibition assay

and α-glucosidase inhibition assay. The water and ethanol leaf extracts of *S. travancoricum* were observed to inhibit lipases, α-amylase, and α-glucosidase in a dose-dependent manner. The water extract exhibited greater potency compared to the other extracts.

The distribution of *S. travancoricum* has been impeded by several factors, such as habitat loss, alterations in land use, and insufficient fruit production. In addition, the wetland habitats of this species have been greatly affected by human construction and improper garbage disposal, emphasising the urgency of implementing conservation measures. Several green spaces were established in five different grama Panchayaths in Thrissur, Palakkad and Pathanamthitta districts of Kerala to safeguard this endemic and endangered tree species. In these locations, we could potentially conserve about 200 trees. The conserved genetic material can effectively modify the existing environmental conditions of these areas and could also contribute to carbon sequestration. Establishing a green space of this nature will play a crucial role in safeguarding the biodiversity of the endangered plants in our state. This initiative will not only increase awareness among the local population but also offer a dependable source of high-quality planting materials for interested cultivators, thereby promoting the growth of many potential plant species.

MAJOR FINDINGS OF THE STUDY

The primary goal of the present research titled, "**Characterization of *Syzygium travancoricum* Gamble (Myrtaceae), an endemic endangered species of Kerala**" was to acquire a thorough comprehension of this distinctive endemic endangered species belonging to the Myrtaceae family. By employing a range of analytical approaches, such as morphological, anatomical, histochemical, phytochemical, molecular, and pharmacological analyses of eight populations of *S. travancoricum* situated within five districts of Kerala, the study successfully conducted a comprehensive characterization of this species. Furthermore, the investigation also formulated a conservation strategy by creating green spaces to safeguard *S. travancoricum* from further endangerment. The major findings of the study are summarized below.

- The morphological characteristics of live samples from six populations, namely JNTBGRI, Idinjaar, Kollam, Thrissur, MSSRF, and Konginichalkavu, exhibited a precise correspondence with the characteristics described in the protologue, as well as the type material of *S. travancoricum*.
- Pookode (Wynad) and Paliyeri Mookambika Kavu (Kannur) populations exhibited distinct characteristics that resemble those described in the protologue and type material for *S. stocksii*.
- Pookode (Wynad) and Paliyeri Mookambika kavu (Kannur) displayed discernible traits that closely resembled those of *S. stocksii*, including the presence of large elliptic to obovate-shaped leaves measuring approximately 15–20 cm in length and 8–10 cm in breadth, featuring an obtuse leaf base with an obtusely acuminate tip, without any oblique ends, short petiole without any prominent groove, the lateral nerves ranged from 18-20, with noticeable intra-marginal veins, albeit diminishing

towards the margins, short inflorescence measuring 5–10 cm in size, accompanied by a short peduncle ranging from 2–5 cm.

- The populations of *S. travancoricum* exhibited variations in important anatomical features, including stomatal type, midrib vasculature, midrib sclerenchyma, crystal types and frequency, distribution of the adaxial palisade layer, adaxial phloem partition, pith, and stem cross-section.
- A comprehensive histochemical analysis of *S. travancoricum* populations revealed that major phytochemical constituents, including tannin, lignin, starch, and essential oils, were consistently present in all populations.
- UPGMA phylogram of *S. travancoricum* based on morphological and anatomical characters exhibited clade divergence for Pookode and Paliyeri Mookambika Kavu, showing close relatedness to *S. stocksii*.
- Qualitative phytochemical analysis of leaf and bark extracts of *S. travancoricum* exhibited a diverse range of phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids, phenols, and terpenoids.
- The water extracts of the leaves of *S. travancoricum* exhibited the highest concentration of phenolic compounds (240.2 mg GAE/g) than ethanol extracts.
- The water extracts of both leaf and bark demonstrated the highest flavonoid content, with 78 mg QUE/g and 40 mg QUE/g, respectively, compared to ethanol extracts.
- The nutrient analysis of *S. travancoricum* fruit samples at various stages of ripening demonstrated that these fruits possess a copious reservoir of vital nutrients, encompassing vitamins, minerals, phenols, anthocyanins, and carbohydrates.

- Potassium and Calcium were identified as the major minerals, ranging from 137.55–170.39 mg/100g and 40.75–47.99 mg/100g, respectively.
- The leaf essential oil of *S. travancoricum* comprised a complex mixture of monoterpenoids, diterpenoids, and sesquiterpenoids
- The GC-MS profile of *S. travancoricum* populations showed variations in the composition and concentration of major chemical compounds.
- The sesquiterpenoid compounds, β-caryophyllene and caryophyllene epoxide, β-geraniol, α-farnesene, and α-selinene were the primary constituents, comprising more than 60% of the total oil content, followed by monoterpenoids that included α-pinene, β-pinene, ocimene isomers, myrcene, β-limonene, and α-terpineol. Diterpenoids were present in trace quantities.
- Phylogenetic tree constructed based on DNA barcodes (*matK*, *ITS* and *rbcL*) using Bayesian inference exhibited clade divergence for Pookode and Paliyeri Mookambika Kavu, showing close relatedness to *S. stocksii* with 73% branch support.
- Phylogenetic tree constructed based on combined morphological and molecular data exhibited distinct clade divergence for Pookode and Paliyeri Mookambika Kavu, showing close relatedness to *S. stocksii* with 100% branch support confirming reinstatement of *S. travancoricum* from *S. stocksii*
- The antimicrobial study showed that the leaf essential oil *S. travancoricum* had inhibitory effects on selected bacterial strains such as *B. subtilis*, *B. cereus*, *E. coli*, and *S. mutans* compared to the positive and negative control groups. However, the essential oil demonstrated moderate activity

against few bacterial strains, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*.

- The leaf and the bark extracts of *S. travancoricum* exhibited significant antioxidant potential, with IC_{50} values of 27.48 μg/ml and 25.30 μg/ml, respectively.
- *S. travancoricum* leaf essential oil had moderate cytotoxicity with an LC_{50} value of 65.09 μg /ml against the L929 fibroblast cell line.
- *S. travancoricum* leaf essential oil exhibited significant antiproliferative effects against the B16 melanoma cell line with an IC_{50} value of 57.26 μg/ml.
- Cell apoptosis analysis demonstrated that *S. travancoricum* leaf essential oil has the potential to induce cellular apoptosis, effectively inhibiting cancer cell proliferation in the B16 melanoma cell line.
- A significant increase in reactive oxygen species (ROS) production, as evidenced by the green fluorescence in the *S. travancoricum* leaf essential oil-treated B16 melanoma cells compared to the control, led to apoptosis in the cancer cells.
- The administration of *S. travancoricum* leaf essential oil to B16 melanoma cells resulted in a substantial decrease in mitochondrial membrane potentials compared to the control group.
- The analysis of cell cycle distribution indicated a reduction in cell accumulation during the G0/G1 and G2/M phases after a 24-hour treatment with the essential oil, indicating induced cell cycle arrest, specifically in the S-phase.

- The anti-inflammatory effects of the leaf essential oil of *S. travancoricum* were statistically significant in the LPS-activated RAW 264.7 cell line, as evidenced by the results of COX and LOX assays.
- The application of *S. travancoricum* leaf essential oil on LPS-induced RAW 264.7 cell lines reduced the levels of nitric oxide synthase (iNOS) and cellular nitrite concentration.
- Applying *S. travancoricum* leaf essential oil on LPS-induced RAW 264.7 cell lines inhibited the myeloperoxidase activity, confirming the anti-inflammatory activity.
- The water extract of *S. travancoricum* leaves demonstrated higher inhibitory effects on lipases, α-amylase, and α-glucosidase than other extracts.
- Approximately 200 trees were conserved by establishing green spaces in five distinct Grama Panchayaths in the districts of Thrissur, Palakkad, and Pathanamthitta in Kerala.

Chapter 7

Future Recommendations

CHAPTER 7 FUTURE RECOMMENDATIONS

The primary objective of our study was to gain a comprehensive understanding of the endemic and endangered species, *S. travancoricum* Gamble, belonging to the *Syzygium* genus. To accomplish this task, eight populations from five districts in Kerala were meticulously analyzed using various techniques such as morphological, anatomical, histochemical, phytochemical, molecular, and pharmacological analyses. Our research findings have conclusively resolved existing uncertainties surrounding the morphology and correct identification of two *Syzygium* species. However, we propose several additional research areas that could be prioritized in future research endeavours.

Based on a comparative analysis with prior research, our findings have substantiated the utility of *ITS, matK*, and *rbcL* as DNA barcodes for the identification of *Syzygium* species, as well as for the construction of reasonably dependable molecular phylogenies within the genus. However, utilization of contemporary phylogenetic methodologies can be incorporated to understand the taxonomy of *S. travancoricum* comprehensively. Moreover, incorporating additional *Syzygium* species in forthcoming investigations will be imperative to delineate the taxonomic ambiguities among closely related taxa.

Further preclinical and clinical research is necessary to confirm the overall pharmacological efficacy of *S. travancoricum* leaf essential oil. Extensive research is necessary to optimise individualised and targeted cancer care by exploring the therapeutic potential of *S. travancoricum* leaf essential oil in combination with cancer medications, given the promising outcomes observed thus far.

References

Adeneye, A. A., Agbaje, E. O., & Olagunju, J. A. (2011). Metformin: An effective attenuator of risperidone-induced insulin resistance hyperglycemia and dyslipidemia in rats. *Indian Journal of Experimental Biology*, 49(5), 332–338.

Ahmed, R., Tariq, M., Hussain, M., Andleeb, A., Masoud, M. S., Ali, I., Mraiche, F., & Hasan, A. (2019). Phenolic contents-based assessment of therapeutic potential of *Syzygium cumini* leaves extract. *PLOS ONE*, *14*(8), e0221318. https://doi.org/10.1371/journal.pone.0221318

Alfikri, F. N., Pujiarti, R., Wibisono, M. G., & Hardiyanto, E. B. (2020). Yield, quality, and antioxidant activity of clove (*Syzygium aromaticum* L.) bud oil at the different phenological stages in young and mature trees. *Scientifica*, *2020*, 1–8. https://doi.org/10.1155/2020/9701701

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amaral, A. C. F., Kuster, R. M., Bessa, W. S., Barnes, R. A., Kaplan, M. A. C., & Wessjohann, L. A. (2001). Flavonoids and other phenolics from leaves of two Marlierea species (Myrtaceae). *Biochemical Systematics and Ecology*, *29*(6), 653–654. https://doi.org/10.1016/s0305-1978(00)00089-2

Ames-Sibin, A. P., Barizão, C. L., Castro-Ghizoni, C. V., Silva, F. M. S., Sá-Nakanishi, A. B., Bracht, L., Bersani-Amado, C. A., Marçal-Natali, M. R., Bracht, A., & Comar, J. F. (2018). β-caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats. *Journal of Cellular Biochemistry*, *119*(12), 10262–10277. https://doi.org/10.1002/jcb.27369

Amir Rawa, M. S., Mazlan, M. K. N., Ahmad, R., Nogawa, T., & Wahab, H. A. (2022). Roles of *Syzygium* in Anti-Cholinesterase, Anti-Diabetic, anti-inflammatory, and Antioxidant: From Alzheimer's Perspective. *Plants*, *11*(11), 1476. https://doi.org/10.3390/plants11111476

Apaza Ticona, L., Souto Pérez, B., Martín Alejano, V., & Slowing, K. (2021). Anti-inflammatory and anti-arthritic activities of glycosylated flavonoids from *Syzygium jambos* in edematogenic agent-induced paw edema in mice. *Revista Brasileira de Farmacognosia*, *31*(4), 429–441. https://doi.org/10.1007/s43450-021-00167-0

Applied Biosystems. (2021). *ExoSAP-IT™ PCR product cleanup quick reference* https://assets.thermofisher.com/TFSAssets/LSG/manuals/78200b.pdf.

Aratani, Y. (2018). Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Archives of Biochemistry and Biophysics*, *640*, 47–52. https://doi.org/10.1016/j.abb.2018.01.004

Arumugam, B., Palanisamy, U. D., Chua, K. H., & Kuppusamy, U. R. (2016). Potential antihyperglycaemic Effect of myricetin Derivatives from *Syzygium malaccense*. *Journal of Functional Foods*, *22*, 325–336. https://doi.org/10.1016/j.jff.2016.01.038

Axelrod, B., Cheesbrough, T.M., & Laakso, S. (1981). [53] Lipoxygenase from soybeans: EC 1.13.11.12 Linoleate:oxygen oxidoreductase. *Methods in Enzymology, 71*, 441-451.

Ayyanar, M., & Subash-Babu, P. (2012). *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), 240–246. https://doi.org/10.1016/S2221-1691(12)60050-1

Ayyanar, M., Subash-Babu, P., & Ignacimuthu, S. (2013). *Syzygium cumini* (L.) Skeels., a novel therapeutic agent for diabetes: Folk medicinal and pharmacological evidences. *Complementary Therapies in Medicine*, *21*(3), 232–243. https://doi.org/10.1016/j.ctim.2013.03.004

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, *27*(4), 1326. https://doi.org/10.3390/molecules27041326

Banda, M., Nyirenda, J., Muzandu, K., Sijumbila, G., & Mudenda, S. (2018). Antihyperglycemic and antihyperlipidemic Effects of Aqueous Extracts of *Lannea edulis* in alloxan-Induced Diabetic Rats. *Frontiers in Pharmacology*, *9*(9), 1099. https://doi.org/10.3389/fphar.2018.01099, PubMed: 30323764, PubMed Central: PMC6172360

Banadka, A., Wudali, N. S., Al-Khayri, J. M., & Nagella, P. (2022). The role of Syzygium samarangense in nutrition and economy: An overview. *South African Journal of Botany*, 145, 481–492. https://doi.org/10.1016/j.sajb.2022.03.014

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2),202. https://doi.org/10.3390/biom10020202

Beddome, R. H. (1872). *The flora sylvatica for Southern India (parts 15–24)* (pp. 169–288). Gantz Brothers.

Beddome, R. H. (1874). Icones plantarum Indiae orientalis: Or plates and descriptions of new and rare plants, from southern India and Ceylon, *1*. *Gantz brothers*.

Bentham, G., & Mueller, F. (1867). Ordo LXI. Rubiaceae. *Flora Australiensis: A Description of the Plants of the Australian Territory*, *3*, 399–447.

Biffin, E., Craven, L. A., Crisp, M. D., & Gadek, P. A. (2006). Molecular systematics of *Syzygium* and allied genera (Myrtaceae): Evidence from the chloroplast genome. *Taxon*, *55*(1), 79–94. https://doi.org/10.2307/25065530

Biffin, E., Lucas, E. J., Craven, L. A., Ribeiro da Costa, I., Harrington, M. G., & Crisp, M. D. (2010). Evolution of exceptional species richness among lineages of fleshy-fruited Myrtaceae. *Annals of Botany*, *106*(1), 79–93. https://doi.org/10.1093/aob/mcq088

BigDye Terminator v3.1 Cycle sequencing Kit – User Manual, Applied Biosystems

Bourdillon, F. W. (1894). Memoriam: WHP. In H. Hart (Ed.), *printer to the University*.

Bridson, D., & Forman, L. (1992). *The herbarium handbook* (3rd ed), Royal Botanical Garden, Kew, UK, *10*, 1900347431.

Byng, J., Wilson, P., & Snow, N. (2015). Typifications and nomenclatural notes on Indian Myrtaceae. *Phytotaxa*, *217*(2), 101–116. **h**ttps://doi.org/10.11646/phytotaxa.217.2.1

Cardoso, C. M. V., Proença, S. L., & Sajo, M. G. (2009). Foliar anatomy of the subfamily Myrtoideae (Myrtaceae). *Australian Journal of Botany*, *57*(2), 148–116. https://doi.org/10.1071/BT07176

Chandran, S., Mesta, D. K., Ali, S., Gururaja, K. V., Rao, G. R., & Ramachandra, T. V. (2008). Discovery of two critically endangered tree species and issues related to relic forests of the Western Ghats. *Open Conservation Biology Journal*, *2*(1).

Chandran, M. D. S., & Sivan, V. V. (1999). Project lifescape, 2. Flowering plants. *Resonance Journal of Science Education*, *4*, 69–77.

Chandran, M. S., Rao, G. R., Gururaja, K. V., & Ramachandra, T. V. (2010). Ecology of the swampy relic forests of Kathalekan from Central Western Ghats, India. *Bioremediation, Biodiversity and Bioavailability*, *4*(1), 54–68.

Chanudom, L., & Tangpong, J. (2015). Anti-inflammation property of Syzygium cumini (L.) Skeels on indomethacin-induced acute gastric ulceration. *Gastroenterology Research and Practice*, *2015*, 343642. https://doi.org/10.1155/2015/343642

Chatatikun, M., & Chiabchalard, A. (2013). Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (Daucus carota Linn.) root crude extracts. *Journal of Chemical and Pharmaceutical Research*, *5*(4), 97–102.

Chaudhry, G. E., Md Akim, A., Sung, Y. Y., & Sifzizul, T. M. T. (2022) Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. *Frontiers in Pharmacology*, *13*, 842376. https://doi.org/10.3389/fphar.2022.842376

Cheong, M. S., & Ranghoo-Sanmukhiya, V. M. (2013). Phylogeny of Syzygium species using morphological, RAPD and ISSR markers. *International Journal of Agriculture and Biology*, *15*, 511–516.

Chithra, V., Henry, A. N., Kumari, R., & Chithra, V. (1987). Syzygium flora of Tamil Nadu Series I. *Analysis*, *2*, 152–158.

Cock, I. E, and Cheesman, M. A. T. T. H. E. W. (2018). *Plants of the genus Syzygium (Myrtaceae): A review on ethnobotany, medicinal properties and phytochemistry*. *Bioactive compounds of medicinal plants: Properties and potential for human health*, 35–84.

Consortium of Bar Code of Life (CBOL). https://www.barcodeoflife.org

Craven, L. A., & Biffin, E. (2010). An infrageneric classification of *Syzygium* (Myrtaceae). *Blumea-biodiversity, evolution and biogeography of* Plants, *55*, *1*, 94–99.

Craven, L. A. (2001). Unravelling knots or plaiting rope: What are the major taxonomic strands in *Syzygium* sens. lat. (Myrtaceae) and what should be done with them? In: Saw, L. G., Chua, L. S. L., & Khoo, K. C. Taxonomy: The cornerstone of biodiversity In *Proceedings of the Fourth Flora Males. Symp.*:. Inst. Pen. Perhutanan Malaysia (pp. 75–85). Kuala Lumpur.

Cutler, D. F., Botha, C. E. J., & Stevenson, D. W. (2008). *Plant anatomy: An applied approach*. Blackwell Publishing.

Deepika, N., Eganathan, P., Sujanapal, P., & Parida, A. (2013). Chemical composition of *Syzygium benthamianum* (wt. ex Duthie) Gamble essential oil-An endemic and Vulnerable Tree species. *Journal of Essential Oil Bearing Plants*, *16*(2), 289–293. https://doi.org/10.1080/0972060X.2013.793976

Djoukeng, J. D., Abou-Mansour, E., Tabacchi, R., Tapondjou, A. L., Bouda, H., & Lontsi, D. (2005). Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). *Journal of Ethnopharmacology*, *101*(1–3), 283–286. https://doi.org/10.1016/j.jep.2005.05.008

Duthie, J. F. (1878–9). Myrtaceae. In J. D. Hooker (Ed.), *Flora of British India*, *2* (pp. 462–506). Reeve, and Co.

Dwivedi, V., Shrivastava, R., Hussain, S., Ganguly, C., & Bharadwaj, M. (2011). Comparative anticancer potential of clove (*Syzygium aromaticum*)-an Indian spice-against cancer cell lines of various anatomical origin. *Asian Pacific Journal of Cancer Prevention: APJCP*, *12*(8), 1989–1993. PubMed: 22292639

Ecker, A., Araujo Vieira, F., de Souza Prestes, A., Mulling Dos Santos, M., Ramos, A., Dias Ferreira, R., Teixeira de Macedo, G., Vargas Klimaczewski, C., Lopes Seeger, R., Teixeira da Rocha, J. B., & de Vargas Barbosa, N. B. (2015). Effect of *Syzygium cumini* and *Bauhinia forficata* aqueous-leaf extracts on oxidative and mitochondrial parameters in vitro. *Excli Journal*, *14*, 1219–1231. https://doi.org/10.17179/excli2015-576

Elkhateeb, W. A., Zaghlol, G. M., El-Garawani, I. M., Ahmed, E. F., Rateb, M. E., & Abdel Moneim, A. E. A. (2018). Ganoderma applanatum secondary metabolites induced apoptosis through different pathways: In vivo and in vitro anticancer studies. *Biomedicine and Pharmacotherapy*, *101*, 264–277. https://doi.org/10.1016/j.biopha.2018.02.058

Eshwarappa, R. S. B., Iyer, R. S., Subbaramaiah, S. R., Richard, S. A., & Dhananjaya, B. L. (2014). Antioxidant activity of Syzygium cumini leaf gall extracts. *BioImpacts, BI*, *4*(2), 101–107. https:/doi.org/10.5681/bi.2014.018

Fernandez-Gil, B. I., Guerra-Librero, A., Shen, Y. Q., Florido, J., Martínez-Ruiz, L., García-López, S., Adan, C., Rodríguez-Santana, C., Acuña-Castroviejo, D., Quiñones-Hinojosa, A., Fernández-Martínez, J., Abdel Moneim, A. E., López, L. C., Rodríguez Ferrer, J. M., & Escames, G. (2019). Melatonin enhances cisplatin and radiation cytotoxicity in head and neck squamous cell carcinoma by stimulating mitochondrial ROS Generation, apoptosis, and autophagy. *Oxidative Medicine and Cellular Longevity*, 2019, 7187128. https://doi.org/10.1155/2019/7187128

Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Medicine*, *5*(10), 3007–3017. https://doi.org/10.1002/cam4.816

Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition*, *88*(6), 587–605. https://doi.org/10.1079/BJN2002725

Frodin, D. G. (2004). History and concepts of big plant genera. *Taxon*, *53*(3), 753–776. https://doi.org/10.2307/4135449

Gaertner, J. (1788). *De fructibus et seminibus plantarum*, *1*. Typis Academie Carolinae.

Gamble, J. S. (1919). Myrtaceae. In *Flora of the Presidency of Madras*. Adlard & Son Ltd, *1*(3), 391–577.

Ghareeb, M. A., Hamed, M. M., Abdel-Aleem, A. A. H., Saad, A. M., Abdel-Aziz, M. S., & Hadad, A. H. (2017). Extraction, isolation, and characterization ofbioactive compounds and essential oil from Syzygium jambos. *Extraction*, *10*(8).

Govaerts, R., Sobral, M., Ashton, P., Barrie, F., Holst, B. K., Landrum, L. L., Matsumoto, K., Mazine, F. F., Nic Lughadha, E., Proenca, C., Soares-Silva, L. H., Wilson, P. G., & Lucas, E. (2008). *World checklist of Myrtaceae* p. 455. Kew Publishing. Board of Trustees of the Royal Botanic Gardens.

Gupta, M., Sharma, S., & Bhadauria, R. (2015). Fungitoxic activity of fruit extracts of *Syzygium cumini* (L.) Skeels against plant pathogenic fungi *Alternaria alternata* and *Fusarium oxysporum*. *Archives of Phytopathology and Plant Protection*, *48*(4), 354–364. https://doi.org/10.1080/03235408.2014.888875

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41* (pp. 95–98).

Harborne, J. B. (1984). Methods of plant analysis. In *Phytochemical methods: A guide to modern techniques of plant analysis* (pp. 1–36). Springer Netherlands.

Hassan, E. A., & El-Awadi, M. E. (2013). Brief review on the application of histochemical methods in different aspects of plant research. *Natural Science*, *11*(12), 54–67.

Hooker, J. D. (1879). *The flora of British India*, *2*. Reeve, and Co.

Convent Garden, London.

Hossain, H., Rahman, S. E., Akbar, P. N., Khan, T. A., Rahman, M. M., & Jahan, I. A. (2016). HPLC profiling, antioxidant and *in vivo* anti-inflammatory activity of the ethanol extract of *Syzygium jambos* available in Bangladesh. *BMC Research Notes*, *9*, 191. https://doi.org/10.1186/s13104-016-2000-z

Hou, J., Zhang, Y., Zhu, Y., Zhou, B., Ren, C., Liang, S., & Guo, Y. (2019). α-Pinene Induces apoptotic Cell Death via Caspase Activation in Human Ovarian Cancer Cells. *Medical Science Monitor*,25, 6631–6638 https://doi.org/10.12659/MSM.916419

Huelsenbeck, J. P., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Indian Virtual Herbarium. (2023). https://ivh.bsi.gov.in Retrieved September 01, 2022

Hu, Y. K., Wang, L., Zhao, Y., Liu, J., Wang, J., & Zhao, Y. (2020). Two New oleanane triterpenoids from *Syzygium samarangense*. *Chemistry of Natural Compounds*, *56*(4), 692–695. https://doi.org/10.1007/s10600-020-03121-z

Huang, D. W., Chang, W. C., Wu, J. S. B., Shih, R. W., & Shen, S. C. (2016). Vescalagin from pink wax apple [Syzygium samarangense (Blume) Merrill and Perry] alleviates hepatic insulin resistance and ameliorates glycemic metabolism abnormality in rats fed a high-fructose diet. *Journal of Agricultural and Food Chemistry*, *64*(5), 1122–1129. https://doi.org/10.1021/acs.jafc.5b05558

Huong, L. T., Hung, N. V., Chac, L. D., Dai, D. N., & Ogunwande, I. A. (2017). Essential oils from *Syzygium grande* (wight) walp. and *Syzygium sterrophyllum* merr. et perry. *Journal of Essential Oil Bearing Plants*, *20*(6), 1620–1626. https://doi.org/10.1080/0972060X.2017.1409658

Hussin, K. H., Cutler, D. F., & Moore, D. M. (1992). Leaf anatomical studies of Eugenia L. (Myrtaceae) species from the Malay Peninsula. *Botanical Journal of the Linnean Society*, *110*(2), 137–156. https://doi.org/10.1111/j.1095-8339.1992.tb00287.x

Ismail, A., Mohamed, M., Sulaiman, S. A., & Wan Ahmad, W. A. N. (2013). Autonomic nervous system mediates the hypotensive effects of aqueous and residual methanolic extracts of *Syzygium polyanthum* (Wight) Walp. var. *polyanthum* leaves in anaesthetized rats. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2013, 716532. https://doi.org/10.1155/2013/716532

Itam, A., Wati, M. S., Agustin, V., Sabri, N., Jumanah, R. A., & Efdi, M. (2021). Comparative Study of Phytochemical, Antioxidant, and cytotoxic Activities and phenolic Content of *Syzygium aqueum* (Burm. f. Alston f.) Extracts Growing in West Sumatera Indonesia. *TheScientificWorldJournal*, 2021, 5537597. https://doi.org/10.1155/2021/5537597

International Union for Conservation of Nature and Natural Resources. (2022). The IUCN red list of threatened species. *Version 2022–2*. https://www.iucnredlist.org. Retrieved [July 20, 2022]

Jang, H. I., Rhee, K. J., & Eom, Y. B. (2020). Antibacterial and antibiofilm effects of α-humulene against *Bacteroides fragilis*. *Canadian Journal of Microbiology*, *66*(6), 389–399. https://doi.org/10.1139/cjm-2020-0004

Javaid, A., & Samad, S. (2012). Screening of allelopathic trees for their antifungal potential against Alternaria alternata strains isolated from dying-back *Eucalyptus* spp. *Natural Product Research*, *26*(18), 1697–1702. https://doi.org/10.1080/14786419.2011.602638

Jayachandra, K., & Devi, V. S. (2012). Development of herbal drugs in the treatment of jaundice-An overview. *Int J Ayurvedic Herb Med*, *2*, 636–645.

Joji, R., & Beena, J. (2011). Chemical composition and antibacterial activity of the volatile oil from the leaf of *Syzygium samarangense* (Blume) Merr. and LM Perry. *Asian J. Biochem. Pharm. Res*, *3*(1), 263–269.

Joyce, J., Ramachandran, K. K., & Nair, P. V. (2007). Animal diversity of the Myristica swamp forests of Southern Kerala with special reference to herpetofauna. In *The Proceedings of the 19th Kerala Science Congress* (pp. 724–726).

Kadri, Y., Nciri, R., Bardaa, S., Brahmi, N., Saber, S., Harrath, A. H., Aldahmash, W., Alwasel, S., Mohany, M., El Feki, A., & Salah Allagui, M. (2019). *Syzygium aromaticum* alleviates cerium chloride-induced neurotoxic effect in the adult mice. *Toxicology Mechanisms and Methods*, *29*(1), 26–34. https://doi.org/10.1080/15376516.2018.1506849

Kantachot, C., Chantaranothai, P., & Thammathaworn, A. (2007). Contributions to the leaf anatomy and taxonomy of Thai Myrtaceae. *Natural History Journal of Chulalongkorn University*, *7*(1), 35–45.

Karuppusamy, S., & Ravichandran, V. (2016). Diversity Assessment of Woody Plants of Megamalai Wildlife Sanctuary. Theni District, Tamil Nadu. *Biology Bulletin*, *2*(1), 74–89.

Khanh, T. H., & Ban, P. H. (2020). Analysis of essential oils from leaf of *Syzygium hancei* Merr. & Perry, *Syzygium caryophyllatum* (L.) Alston and *Syzygium lineatum* (DC.) Merr. and Perry from Vietnam. *Journal of Essential Oil Bearing Plants*, 23(3), 548–558. https://doi.org/10.1080/0972060X.2020.1790429

Khatijah, I., Chia, J. S., & Lim, B. T. (1992). *Nutrient composition of Malaysian traditional cakes*, *159*, Mardi [Report].

Klener, P., Andera, L., Klener, P., Necas, E., & Zivný, J. (2006). Cell death signalling pathways in the pathogenesis and therapy of haematologic malignancies: Overview of therapeutic approaches. *Folia Biologica*, *52*(4), 119–136.

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (1996) Nirali Prakashan. *Pune*. Textbook of pharmacognosy, *4*, 510–511.

Konda, P. Y., Chennupati, V., Dasari, S., Sharma, N., Muthulingam, M., Ramakrishnan, R., Sade, A., Jagadheeshkumar, V., Natesan, V., & Jaiswal, K. K. (2021). Ethno-pharmacological insulin signaling induction of aqueous extract of *Syzygium paniculatum* fruits in a high-fat diet induced hepatic insulin resistance. *Journal of Ethnopharmacology*, *268*, 113576. https://doi.org/10.1016/j.jep.2020.113576

Kovaè, J., Šimunovic, K., Wu, Z., Klanènik, A., Bucar, F., Zhang, Q., Mo•ina, S.S. (2015). Antibiotic resistance modulation and modes of action of (-)-α-pinene in *Campylobacter jejuni*. PLoS One. 10(4). e0122871.

Kramer, A., Hird, A., Shaw, K., Dosmann, M., & Mims, R. (2011). *Conserving North America's threatened plants: Progress report on Target 8 of the global strategy for plant conservation. Botanic gardens conservation international U.S.* Google Scholar.

Krishnamoorthy, K. (1960). *Myristica* swamps in the evergreen forests of Travancore. In Tropical Moist Evergreen Forest Symposium. Forest Research Institute.

Kudryavtseva, A. V., Krasnov, G. S., Dmitriev, A. A., Alekseev, B. Y., Kardymon, O. L., Sadritdinova, A. F., Fedorova, M. S., Pokrovsky, A. V., Melnikova, N. V., Kaprin, A. D., Moskalev, A. A., & Snezhkina, A. V. (2016). Mitochondrial dysfunction and oxidative stress in aging and cancer. *Oncotarget*, *7*(29), 44879–44905. https://doi.org/10.18632/oncotarget.9821

Kuiate, J. R., Mouokeu, S., Wabo, H. K., & Tane, P. (2007). Antidermatophytic triterpenoids from Syzygium jambos (L.) Alston (Myrtaceae). *Phytotherapy Research*, *21*(2), 149–152. https://doi.org/10.1002/ptr.2039

Kumar, S., & Singh, B. (2021). *Syzygium cumini* (jamun) its medicinal uses. *International Journal of Pharmacognosy*, *8*, 361–372.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Kuo, Y. C., Yang, L. M., & Lin, L. C. (2004). Isolation and immunomodulatory effect of flavonoids from Syzygium samarangense. *Planta Medica*, *70*(12), 1237–1239. https://doi.org/10.1055/s-2004-835859

Latief, N., Anand, S., Lingaraju, M. C., Balaganur, V., Pathak, N. N., Kalra, J., Kumar, D., Bhadoria, B. K., & Tandan, S. K. (2015). Effect of trimeric myricetin rhamnoside (TMR) in carrageenan-induced inflammation and caecal ligation and puncture-induced lung oxidative stress in mice. *Phytotherapy Research: PTR*, *29*(11), 1798–1805. https://doi.org/10.1002/ptr.5446

Laya, A., Benoît, B. K., & Pradeep, S. N. (2023). Antidiabetic (α-amylase and α-glucosidase) and anti-obesity (lipase) inhibitory activities of edible cassava (*Manihot esculenta* Crantz) as measured by in vitro gastrointestinal digestion: Effects of phenolics and harvested time. *International Journal of Food Properties*, *25*(1), 492–508. https://doi.org/10.1080/10942912.2022.2050256

Lepoivre, M., Chenais, B., Yapo, A., Lemaire, G., Thelander, L., & Tenu, J. P. (1990). Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. *Journal of Biological Chemistry*, *265*(24), 14143–14149. https://doi.org/10.1016/s0021-9258(18)77279-7, PubMed: 2117605

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Lucas, E. J., Belsham, S. R., Lughadha, E. M. N., Orlovich, D. A., Sakuragui, C. M., Chase, M. W., & Wilson, P. G. (2005). Phylogenetic patterns in the fleshy-fruited Myrtaceae- preliminary molecular evidence. *Plant Systematics and Evolution*, *251*(1), 35–51. https://doi.org/10.1007/s00606-004-0164-9

Machado, R. R. P., Jardim, D. F., Souza, A. R., Scio, E., Fabri, R. L., Carpanez, A. G., Grazul, R. M., de Mendonça, J. P. R. F., Lesche, B., & Aarestrup, F. M. (2013). The effect of essential oil of Syzygium cumini on the development of granulomatous inflammation in mice. *Revista Brasileira de Farmacognosia*, *23*(3), 488–496. https://doi.org/10.1590/S0102-695X2013005000030

Mahmoud, M. F., Abdelaal, S., Mohammed, H. O., El-Shazly, A. M., Daoud, R., El Raey, M. A., & Sobeh, M. (2021). Syzygium jambos extract mitigates pancreatic oxidative stress, inflammation and apoptosis and modulates hepatic IRS-2/AKT/GLUT4 signaling pathway in streptozotocin-induced diabetic rats. *Biomedicine and Pharmacotherapy*, *142*, 112085. https://doi.org/10.1016/j.biopha.2021.112085

Marrelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules*, *21*(10), 1404. https://doi.org/10.3390/molecules21101404

Mazine, F. F., Souza, V. C., Sobral, M., Forest, F., & Lucas, E. J. (2014). A preliminary phylogenetic analysis of Eugenia (Myrtaceae: Myrteae), with a focus on Neotropical species. *Kew Bulletin*, *69*(2), 1–14. https://doi.org/10.1007/s12225-014-9497-x

Merrill, E. D., & Perry, L. M. (1939). The myrtaceous genus Syzygium Gaertner in Borneo. *Memoirs of the American Academy of Arts and Sciences*, *18*(3), 135–202. https://doi.org/10.2307/25058505

Metcalfe, C. R., & Chalk, L. (1979). *Anatomy of the dicotyledons: Systematic anatomy of the leaf and stem, with a brief history of the subject* p. 288. Clarendon Press.

Miyazawa, M., & Hisama, M. (2003). Antimutagenic activity of phenylpropanoids from clove (*Syzygium aromaticum*). *Journal of Agricultural and Food Chemistry*, *51*(22), 6413–6422. https://doi.org/10.1021/jf030247q

Mohamed, A. A., Ali, S. I., & El-Baz, F. K. (2013). Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. *PLOS ONE*, *8*(4), e60269. https://doi.org/10.1371/journal.pone.0060269

Mohanan, N. (1996). Rediscovery of *Syzygium bourdillonii* (Gamble) Rathkr. and N. C. Nair (Myrtaceae), an endemic and little-known species of Western Ghats. *Journal of Economic and Taxonomic Botany*, *20*(3), 729–731.

Murugan, C., & Manickam, V. S. (2004). Two additions to Myrtaceae of India. *Journal of Economic and Taxonomic Botany*, *28*(3), 523–526.

Nair, N. C., & Henry, A. N. (1983). *Flora of* Tamil Nadu, *India*, *I*, *Analysis*. *Vol. 1*. Botanical Survey of India, pp. 150.

Nair, K. K. N., & Nayar, M. P. (1986). *Flora of Courtallum*, *vol.1 Botanical survey of India*. Calcutta, pp. 134.

Nair, P. V., Ramachandran, K. K., Swarupanandan, K., & Thomas, T. P. (2007). *Mapping biodiversity of the Myristica Swamps in Southern Kerala*.

Nair, K. N., & Rana, T. (2017). Phylogeny of *Syzygium*. In *The genus Syzygium: Syzygium cumini and other underutilized species* (1st ed) (pp. 7–14). CRC Press. https://doi.org/10.1201/9781315118772

Nayar, T. S., Rasiya Beegam, A., Mohanan, N., & Rajkumar, G. (2006). *Flowering plants of Kerala* (pp. 445–453). Tropical Botanic Garden and Research Institute.

National Institute of Standards and Technology—National Institute of Standards and Technology. (2018). *Mass spectral library (NIST/EPA/NIH)*. National Institute of Standards and Technology. https://doi.org/10.18434/T4D303

Haron, N. W., & Moore, D. M. (1996). The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (Myrtaceae). *Botanical Journal*

of the Linnean Society, *120*(3), 265–277. https://doi.org/10.1111/j.1095-8339.1996.tb00776.x

Oldfield, S., & Newton, A. (2012). *Integrated conservation of tree species by botanic gardens: A reference manual. Botanic gardens Conservation International, Richmond, UK*.

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. *Japan Journal of Nutrition*, *44*, 307–315. http://doi.org/10.5264/eiyogakuzashi.44.307

Parnell, J. A. N., Craven, L., & Biffin, E. (2006). Matters of scale: Dealing with one of the largest genera of Angiosperms. In T. Hodkinson, J. Parnell (Eds.), *Towards the tree of life: Systematics of species-rich groups. Systematics association special series*, *72* (pp. 251–273). London. https://doi.org/10.1201/9781420009538.ch16

Pascal, J. P. (1988). Wet Evergreen forests of the Western Ghats of India. Inst. Fr. De Pondichery, Trav. sect i. *Science and Technology*, 337.

Picca, A., Calvani, R., Coelho-Junior, H. J., Landi, F., Bernabei, R., & Marzetti, E. (2020). Mitochondrial dysfunction, oxidative stress, and neuroinflammation: Intertwined roads to neurodegeneration. *Antioxidants*, *9*(8), 647. https://doi.org/10.3390/antiox9080647

Pino, J. A., Marbot, R., Agüero, J., & Fuentes, V. (2001). Essential oil from buds and leaves of clove (Syzygium aromaticum (L.) Merr. et Perry) Grown in Cuba [*Syzygium aromaticum* (L.) Merr. et Perry]. *Journal of Essential Oil Research*, *13*(4), 278–279. https://doi.org/10.1080/10412905.2001.9699693

POWO. (2023). *Plants of the world online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet*.

http://www.plantsoftheworldonline.org/Retrieved September 01, 2022

Prabhugaonkar, A., Mesta, D. K., & Janarthanam, M. K. (2014). First report of three red-listed tree species from swampy relics of Goa State, India. *Journal of Threatened Taxa*, *6*(2), 5503–5506. https://doi.org/10.11609/JoTT.o3376.5503-6

Prashar, A., Locke, I. C., & Evans, C. S. (2006). Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. *Cell Proliferation*, *39*(4), 241–248. https://doi.org/10.1111/j.1365-2184.2006.00384.x

Pritchard, D. J., Fa, J. E., Oldfield, S., & Harrop, S. R. (2012). Bring the captive closer to the wild: Redefining the role of ex situ conservation. *Oryx*, *46*(1), 18–23. https://doi.org/10.1017/S0030605310001766

Qamar, M., Akhtar, S., Ismail, T., Yuan, Y., Ahmad, N., Tawab, A., Ismail, A., Barnard, R. T., Cooper, M. A., Blaskovich, M. A. T., & Ziora, Z. M. (2021). *Syzygium cumini*(L.), Skeels fruit extracts: In vitro and in vivo anti-inflammatory properties. *Journal of Ethnopharmacology*, *271*, 113805. https://doi.org/10.1016/j.jep.2021.113805

Qamar, M., Akhtar, S., Ismail, T., Wahid, M., Abbas, M. W., Mubarak, M. S., Yuan, Y., Barnard, R. T., Ziora, Z. M., & Esatbeyoglu, T. (2022). Phytochemical profile, biological properties, and food applications of the medicinal plant *Syzygium cumini*. *Foods*, *11*(3), 378. https://doi.org/10.3390/foods11030378

Rahnama, P., Montazeri, A., Huseini, H. F., Kianbakht, S., & Naseri, M. (2012). Effect of Zingiber officinale R. rhizomes (ginger) on pain relief in primary dysmenorrhea: A placebo randomized trial. *BMC Complementary and Alternative Medicine*, *12*, 92. https://doi.org/10.1186/1472-6882-12-92

Raina, V. K., Srivastava, S. K., Aggarwal, K. K., Syamasundar, K. V., & Kumar, S. (2001). Essential oil composition of *Syzygium aromaticum* leaf from Little Andaman, India. *Flavour and Fragrance Journal*, *16*(5), 334–336. https://doi.org/10.1002/ffj.1005

Rajaei, E., Mowla, K., Ghorbani, A., Bahadoram, S., Bahadoram, M., & Dargahi-Malamir, M. (2015). The effect of omega-3 fatty acids in patients with active rheumatoid arthritis receiving DMARDs therapy: Double-blind randomized controlled Trial. *Global Journal of Health Science*, *8*(7), 18–25. https://doi.org/10.5539/gjhs.v8n7p18

Ramana, M. V., Chorghe, A., & Venu, P. (2014). Two new species of *Syzygium* (Myrtaceae) from Saddle Peak National Park, Andaman and Nicobar Islands, India. *Blumea – Biodiversity, Evolution and Biogeography of Plants*, *59*(1), 42–48. https://doi.org/10.3767/000651914X683593

Ramasubbu, R., Anjana, S., Divya, C., Sasi Kala, N., & Manikandan, G. (2018). A new species of *Syzygium* (Myrtaceae) from Southern Western Ghats of Tamil Nadu, India. *Phytotaxa*, *374*(3), 263–267.

Ramesh, B. R., & Pascal, J. P. (1997). *Atlas of endemics of the Western Ghats (India). Distribution of tree species in the evergreen and semievergreen forest*. French Institute of Pondicherry.

Rameshkumar, K. B., Anu Aravind, A. P., & Vinod Kumar, T. G. (2015). Leaf essential oil composition of six *Syzygium* species from the Western Ghats, South India. *Records of Natural Products*, *9*(4), 592–596.

Ratheesh Narayanan, M. K., Shareef, S. M., Shaju, T., Sivu, A. R., Sujana, K. A., Nandakumar, M. K., & Satheesh, K. T. (2014). A new species of *Syzygium* (Myrtaceae) from the Southern Western Ghats of Kerala, India. *International Journal of Advanced Research*, *2*(3), 1055–1058.

Ravikumar, K. (1999). Novelties from high wavy Mountains, Southern Western Ghats, Theni District, Tamil Nadu, India. *Rheedea*, *9*, 55–75.

Ray, R. (2011). *Developing Strategies for Conservation of ThreatenedEndemic biodiversity of the Sacred Groves of central Western GhatsProject report* p. 146. Centre for Ecological Sciences, Indian Institute of Science.

Razafimamonjison, G., Michel Jahiel, M., Duclos, T., Ramanoelina, P., Fawbush, F., & Danthu, P. (2014). Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. *International Journal of Basic and Applied Sciences*, *3*, 224–233.

Renda, G., Özel, A., Barut, B., Korkmaz, B., & Yayli, N. (2019). The Volatile Chemical Compositions of the Essential Oil/SPME and Enzyme Inhibitory and radical Scavenging Activities of Solvent Extracts and the Essential oils from *Coronilla orientalis* Miller and *C. Varia* L. grows in Turkey. *Iranian Journal of Pharmaceutical Research*, *18*(4), 1831–1842. https://doi.org/10.22037/ijpr.2019.1100802

Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry*, *109*(4), 883–890. https://doi.org/10.1016/j.foodchem.2008.01.021

Rezende, W. P., Borges, L. L., Alves, N. M., Ferri, P. H., & Paula, J. R. (2013). Chemical variability in the essential oils from leaves of *Syzygium jambos*. *Revista Brasileira de Farmacognosia*, *23*(3), 433–440. https://doi.org/10.1590/S0102-695X2013005000035

Risterucci, A. M., Duval, M. F., Rohde, W., & Billotte, N. (2005). Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Molecular*

Ecology Notes, *5*(4), 745–748. https://doi.org/10.1111/j.1471-8286.2005.01050.x

Robi, T. J. (2009). Floristic structure and diversity of Myristica swamps at Kulathupuzha in a GIS perspective. *Dehradun University*, *VIII*, 148.

Ruan, Z. P., Zhang, L. L., & Lin, Y. M. (2008). Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules*, *13*(10), 2545–2556. https://doi.org/10.3390/molecules13102545

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. *Frontiers in Pharmacology*, *13*, 806470. https://doi.org/10.3389/fphar.2022.806470

Sadasivam, S., & Manickam, A. (1992). *Biochemical methods for agricultural sciences*. Wiley Eastern Ltd.

Sahana, D. A., Shivaprakash, G., Baliga, R., Prabha, A., & Ganesh, J. P. M. R. (2010). Effect of *Eugenia jambolana* on plasma glucose, insulin Sensitivity and HDL-C Levels: Preliminary Results of a Randomized Clinical Trial. *Journal of Pharmacy Research*, *3*(6), 1268–1270.

Sajwan, S., Sajwan, K., & Agarwal, U. (2014). Comparative studies and quality evaluation of some important Una ni herbal drugs. *Indian Journal of Drugs*, *2*(3), 104–113.

Salter, M., Duffy, C., Garthwaite, J., & Strijbos, P. J. (1996). Ex vivo measurement of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity in vivo, *J.Neurochem*, *66*, 1683–1690.

Sanches, J. R., França, L. M., Chagas, V. T., Gaspar, R. S., Dos Santos, K. A., Gonçalves, L. M., Sloboda, D. M., Holloway, A. C., Dutra, R. P., Carneiro, E. M., Cappelli, A. P., & Paes, A. M. (2016). Polyphenol – Rich extract of *Syzygium cumini* Leaf dually improves peripheral insulin sensitivity and pancreatic islet function in monosodium L – Glutamate-induced obese rats. *Frontiers in Pharmacology*, *7*, 48.

Saroj, A., & Pragadheesh, V. S. Palanivelu, Yadav, *A.K.* Singh, Samad, A., Negi, A.S., & Chanotiya, C. (2015). Anti-phytopathogenic activity of *Syzygium cumini* essential oil, hydrocarbon fractions and its novel constituents. *Industrial Crops and Products, 74*, 327-335.

Sasisdharan, N., & Jomy, A. (1999). A new species of Syzygium Gaertn. (Myrtaceae) from southernWestern Ghats, India. *Rheedea*, *9*, 155–158.

Sasidharan, N. (2004). Biodiversity Documentation for Kerala, part 6: Flowering plants. *Kerala Forest Research Institute, Peechi*, 173–180.

Schmid, R. (1972). A resolution of the *Eugenia-Syzygium* controversy (Myrtaceae). *American Journal of Botany*, 59(4), 423–436. https://doi.org/10.1002/j.1537-2197.1972.tb10113.x

Sekhon-Loodu, S., & Rupasinghe, H. P. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. *Frontiers in Nutrition*, *6*, 53. https://doi.org/10.3389/fnut.2019.00053

Shafi, P. M., Rosamma, M. K., Jamil, K., & Reddy, P. S. (2002). Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils. *Fitoterapia*, *73*(5), 414–416. https://doi.org/10.1016/s0367-326x(02)00131-4

Shareef, S. M., Geetha Kumary, M. P., Santhosh Kumar, E. S., & Shaju, T. (2010). *Syzygium claviflorum* (Myrtaceae) – A new record for South India. *Rheedea*, *20*(1), 53–55.

Shareef, S. M., Santhosh Kumar, E. S., & Shaju, T. (2012a). A new species of *Syzygium* (Myrtaceae) from the Southern Western Ghats of Kerala, India. *Phytotaxa*, *71*, 28–33.

Shareef, S. M., Kumar, S., & Roy, P. E. (2013). A new species of *Syzygium* (Myrtaceae) from Kerala, India. *Phytotaxa*, *129*(1), 34–38. https://doi.org/10.11646/phytotaxa.129.1.2

Shareef, S. M., Roy, P. E., & Krishnaraj, M. V. (2014). *Syzygium munnarensis* sp. nov. (Myrtaceae): An overlooked endemic species from southern Western Ghats of Kerala, India. *Webbia*, *69*(1), 53–57. https://doi.org/10.1080/00837792.2014.899727

Shareef, S. M., & Rasiya, B. A. (2015). Lectotypification and status of *Syzygium myhendrae* (Bedd. ex Brandis) Gamble (Myrtaceae)- an endemic myrtle of southern Western Ghats, India. *Taiwania*, *60*(1), 59–62.

Shareef, S. M., Beegam, A. R., & Byng, J. W. (2020). Lectotypification and taxonomic reinstatement of *Syzygium benthamianum* (Myrtaceae). *Phytotaxa*, *455*(1), 53–59. https://doi.org/10.11646/phytotaxa.455.1.7

Shareef, S. M., & Santhosh Kumar, E. S. (2021). Census of *Syzygium* Gaertn. in India. Abrahamia, *6*(2), 90–107.

Sharma, S. B., Rajpoot, R., Nasir, A., Prabhu, K. M., & Murthy, P. S. (2011). Ameliorative Effect of Active Principle Isolated from Seeds of *Eugenia jambolana* on carbohydrate Metabolism in Experimental Diabetes. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2011, 789871. https://doi.org/10.1093/ecam/nep233

Sharma, R., Kishore, N., Hussein, A., & Lall, N. (2013). Antibacterial and anti-inflammatory effects of *Syzygium jambos* L. (Alston) and isolated compounds on acne vulgaris. *BMC Complementary and Alternative Medicine*, *13*, 292. https://doi.org/10.1186/1472-6882-13-292

Sheeba Irwin, J., Narasimhan, D., & Ganeshan, R. (2003). Status of *Syzygium gambleanum* Rathakr. & Chithra (Myrtaceae) from southern Western Ghats. *Bulletin of the Botanical Survey of India*, *45*, 111–120.

Shen, S. C., Chang, W. C., & Chang, C. L. (2012). Fraction from wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] fruit extract ameliorates insulin resistance via modulating insulin signaling and inflammation pathway in tumor necrosis factor α-treated FL83B mouse hepatocytes. *International Journal of Molecular Sciences*, *13*(7), 8562–8577. https://doi.org/10.3390/ijms13078562

Shrikanta, A., Kumar, A., & Govindaswamy, V. (2015). Resveratrol content and antioxidant properties of underutilized fruits. *Journal of Food Science and Technology*, l52, 52(1), 383–390. https://doi.org/10.1007/s13197-013-0993-z

Siani, A. C., Souza, M. C., Henriques, M. G., & Ramos, M. F. (2013). Anti-inflammatory activity of essential oils from *Syzygium cumini* and *Psidium guajava*. *Pharmaceutical Biology*, *51*(7), 881–887. https://doi.org/10.3109/13880209.2013.768675

Siddique, S., Parveen, Z., Firdaus-e-Bareen, C. MN, Chaudhary, M. N., Mazhar, S., & Nawaz, S. (2017). The essential oil of *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. leaves from Pakistan: A potential source of eugenol methyl ether. *Industrial Crops and Products*, *109*, 912–917. https://doi.org/10.1016/j.indcrop.2017.09.048

Rivas da Silva, A. C., Lopes, P. M., Barros de Azevedo, M. M., Costa, D. C., Alviano, C. S., & Alviano, D. S. (2012). Biological activities of α-pinene and β-pinene enantiomers. *Molecules*, *17*(6), 6305–6316. https://doi.org/10.3390/molecules17066305

Singh, J. P., Kaur, A., Singh, N., Nim, L., Shevkani, K., Kaur, H., & Arora, D. S. (2016). In vitro antioxidant and antimicrobial properties of jambolan (*Syzygium cumini*) fruit polyphenols. *LWT – Food Science and Technology*, *65*, 1025–1030. https://doi.org/10.1016/j.lwt.2015.09.038

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sreekala, A. K., Pillai, D. S., Akhil, R., Surendran, A., & Ramasubbu, R. (2019). A new species of *Syzygium* (Myrtaceae) from the Agasthyamalai Biosphere Reserve, Kerala, India. *Phytotaxa*, *403*(1), 066–070. https://doi.org/10.11646/phytotaxa.403.1.6

Sreekumar, V. B., Sreejith, K. A., Sanil, M. S., Harinarayanan, M. K., Prejith, M. P., & Varma, R. V. (2020). Distribution of *Syzygium travancoricum* Gamble (Myrtaceae), a Critically Endangered tree species from Kerala part of Western Ghats, India. *Journal of Threatened Taxa*, *12*(17), 17340–17346. https://doi.org/10.11609/jott.5231.12.17.17340-17346

Srivastava, A. K., Srivastava, S. K., & Syamsundar, K. V. (2005). Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. *Flavour and Fragrance Journal*, *20*(1), 51–53. https://doi.org/10.1002/ffj.1364

Srivastava, J., Chandra, H., Nautiyal, A. R., & Kalra, S. J. (2014). Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDA$_m$s) as an alternative drug line to control infections. *3 Biotech*, *4*(5), 451–460. https://doi.org/10.1007/s13205-013-0180-y

Sujanapal, P., Robi, A. J., Udayan, P. S., & Dantus, K. J. (2013). *Syzygium sasidharanii sp. nov.* (*Myrtaceae*) – A new species with edible fruits from Agasthyamala Hills of Western Ghats, India. *International Journal of Advanced Research*, *1*(5), 44–48.

Jose, R. A., Puthiyapurayil, S., & Nanu, S. (2014). *Syzygium sahyadricum* (Myrtaceae), a new tree species from India, and notes on the distribution of *S. spathulatum* Thwaites. *Phytotaxa*, *174*(5), 285–290. https://doi.org/10.11646/phytotaxa.174.5.5

Sujanapal, P., & Kunhikannan, C. (2017). The genus *Syzygium* in Western Ghats. In *The genus Syzygium* (pp. 15–56). CRC Press.

Swofford, D. L., Waddell, P. J., Huelsenbeck, J. P., Foster, P. G., Lewis, P. O., & Rogers, J. S. (2001). Bias in phylogenetic estimation and its relevance to

the choice between parsimony and likelihood methods. *Systematic Biology*, *50*(4), 525–539. https://doi.org/10.1080/106351501750435086

Swofford, D. L. (2002). *PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10*. Sinauer Associates, Inc. Publishers. Sunderland.

Talarico, L. B., Zibetti, R. G., Faria, P. C., Scolaro, L. A., Duarte, M. E., Noseda, M. D., Pujol, C. A., & Damonte, E. B. (2004). Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*. *International Journal of Biological Macromolecules*, *34*(1–2), 63–71. https://doi.org/10.1016/j.ijbiomac.2004.03.002

Tamiello, C. S., do Nascimento, G. E., Iacomini, M., & Cordeiro, L. M. C. (2018). Arabinogalactan from edible jambo fruit induces different responses on cytokine secretion by THP-1 macrophages in the absence and presence of proinflammatory stimulus. *International Journal of Biological Macromolecules*, *107*(A), 35–41. https://doi.org/10.1016/j.ijbiomac.2017.08.148

Tanaka, T., Orii, Y., Nonaka, G., & Nishioka, I. (1993). Tannins and related compounds. CXXIII. Chromone, acetophenone and phenylpropanoid glycosides and their galloyl and/or hexahydroxydiphenoyl esters from the leaves of *Syzygium aromaticum* Merr. et Perry. *Chemical and Pharmaceutical Bulletin*, *41*(7), 1232–1237. https://doi.org/10.1248/cpb.41.1232

Thermo Fisher Scientific. (2021). RapidHIT ID system v1.3.1 user guide. MAN0018938:A. https://www.thermofisher.com/documentconnect/documentconnect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFSAssets%2FLSG%2Fmanuals%2FMAN0018938_RapidHIT_ID_System_v1_3_1_UG.pdf&title=VXNlciBHdWlkZTogUmFwaWRISVQgSUQgU3lzdGVtIHYxLjMuMQ== Retrieved July 20, 2021

Thornhill, A. H., Ho, S. Y. W., Külheim, C., & Crisp, M. D. (2015). Interpreting the modern distribution of Myrtaceae using a dated molecular phylogeny. *Molecular Phylogenetics and Evolution*, *93*, 29–43. https://doi.org/10.1016/j.ympev.2015.07.007

Uddin, A. B. M. N., Hossain, F., Reza, A. S. M. A., Nasrin, M. S., & Alam, A. H. M. K. (2022). Traditional uses, pharmacological activities, and phytochemical constituents of the genus *Syzygium*: A review. *Food Science and Nutrition*, *10*(6), 1789–1819. https://doi.org/10.1002/fsn3.2797

Udensi, U. K., & Tchounwou, P. B. (2014). Dual effect of oxidative stress on leukemia cancer induction and treatment. *Journal of Experimental and Clinical Cancer Research: CR*, *33*, 106. https://doi.org/10.1186/s13046-014-0106-5

Udhayavani, C., Ramachandran, V. S., & John, H. A. (2013). Ecology and population study on *Syzygium travancoricum* Gamble (Myrtaceae) – A critically endangered species from Nilgiris. *NeBIO*, *4*, 68–71.

Umeno, A., Horie, M., Murotomi, K., Nakajima, Y., & Yoshida, Y. (2016). Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. *Molecules*, *21*(6), 708. https://doi.org/10.3390/molecules21060708

Vaishnava, M. M., Tripathy, A. K., & Gupta, K. R. (1992). *Fitoterapia*, *63*, 259.

Vasconcelos, T. N. C., Proença, C. E. B., Ahmad, B., Aguilar, D. S., Aguilar, R., Amorim, B. S., Campbell, K., Costa, I. R., De-Carvalho, P. S., Faria, J. E. Q., Giaretta, A., Kooij, P. W., Lima, D. F., Mazine, F. F., Peguero, B., Prenner, G., Santos, M. F., Soewarto, J., Wingler, A., & Lucas, E. J. (2017). Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae. *Molecular Phylogenetics and Evolution*, *109*, 113–137. https://doi.org/10.1016/j.ympev.2017.01.002

Venkat Ramana, M., Chorghe, A., & Venu, P. (2014). Two new species of *Syzygium* (*Myrtaceae*) from Saddle Peak National Park, Andaman and Nicobar Islands, India. *Blumea*, *59*, 42–48.

Verma, R. K., Verma, R. S., Chauhan, A., & Bisht, A. (2015). Evaluation of essential oil yield and chemical composition of eight lemongrass (*Cymbopogon* spp.) cultivars under Himalayan region. *Journal of Essential Oil Research*, *27*(3), 197–203. https://doi.org/10.1080/10412905.2015.1014936

Vignesh, R., Puhazhselvan, P., Sangeethkumar, M., Saranya, J., Eganathan, P., & Sujanapal, P. (2013). GC-MS analysis, antimicrobial, scavenging ability and cytotoxic activity of leaves of Syzygium calophyllifolium Walp. *Journal of Biologically Active Products from Nature*, *3*(2), 121–129. https://doi.org/10.1080/22311866.2013.817741

Viswanathan, M. B. G., & Manikandan, U. (2008). Rediscovery of *Syzygium courtallense* (Gamble) Alston (Myrtaceae) and *Dioscorea spicata* Roth (Dioscoreaceae) from the Kalakkad- Mundanthurai tiger Reserve in India. *Bulletin of the Botanical Survey of India*, *50*, 157–160.

van der Vliet, A., Janssen-Heininger, Y. M. W., & Anathy, V. (2018). Oxidative stress in chronic lung disease: From mitochondrial dysfunction to dysregulated redox signaling. *Molecular Aspects of Medicine*. Walker Publishing, *63*, 59–69. https://doi.org/10.1016/j.mam.2018.08.001,

Walker, M. C., & Gierse, J. K. (2010). In vitro assays for cyclooxygenase activity and inhibitor characterization. Methods in Molecular Biology, 644, 131–144. https://doi.org/10.1007/978-1-59745-364-6_11

Wathsara, H. P. T., Weeratunge, H. D., Mubarak, M. N. A., Godakumbura, P. I., & Ranasinghe, P. (2020). In vitro antioxidant and antidiabetic potentials of Syzygium caryophyllatum L. Alston. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2020*, 9529042. https://doi.org/10.1155/2020/9529042

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR protocols: A guide to methods andApplications* (pp. 315–322). Academic Press, Inc.

Wight, R. (1842). X.— On the Separation of the Pomegranate as a distinct Natural Order from Myrtaceæ. *Annals and Magazine of Natural History*, *9*(56), 86–92. https://doi.org/10.1080/03745484209442794

Wight, R. (1846). *Icones plantarum Indiae orientalis*, *3*. Frank, & Co.

Williams, A. C., & Barry, B. W. (1991). Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. *Pharmaceutical Research*, *8*(1), 17–24. https://doi.org/10.1023/a:1015813803205

Wilson, P. G., O'Brien, M. M., Gadek, P. A., & Quinn, C. J. (2001). Myrtaceae revisited: A reassessment of infrafamilial groups. *American Journal of Botany*, *88*(11), 2013–2025. https://doi.org/10.2307/3558428

Wisastra, R., & Dekker, F. J. (2014). Inflammation, cancer and oxidative lipoxygenase activity are intimately linked. *Cancers*, *6*(3), 1500–521. https://doi.org/10.3390/cancers6031500

Wong, M. L., Liew, J. W. K., Wong, W. K., Pramasivan, S., Mohamed Hassan, N., Wan Sulaiman, W. Y., Jeyaprakasam, N. K., Leong, C. S., Low, V. L., & Vythilingam, I. (2020). Natural Wolbachia infection in field-collected Anopheles and other mosquito species from Malaysia. *Parasites and Vectors*, *13*(1), 414. https://doi.org/10.1186/s13071-020-04277-x

Xu, Q., Li, M., Yang, M., Yang, J., Xie, J., Lu, X., Wang, F., & Chen, W. (2018). α-pinene regulates *miR-221* and induces G_2/M phase cell cycle arrest in

human hepatocellular carcinoma cells. *Bioscience Reports*, *38*(6). https://doi.org/10.1042/BSR20180980

Yao, N., Yao, W., Lei, J., & Wang, K. (2013). Lignans from Syzygium grijsii and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, *46*, 79–82. https://doi.org/10.1016/j.bse.2012.09.009

Zhang, D., Hu, Y., Wang, L., Huang, S., Zhao, Y., Li, M., Li, F., & Zhao, Y. (2020). Diterpenoids and sesquiterpenoids from *Syzygium fluviatile*. *Records of Natural Products*, *14*(3), 190–195. https://doi.org/10.25135/rnp.158.19.08.1393

Zheng, G. Q., Kenney, P. M., & Lam, L. K. (1992). Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. *Journal of Natural Products*, *55*(7), 999–1003. https://doi.org/10.1021/np50085a029

Zou, J., Mi, Y., Chen, W., Liu, Q., Wang, J., Lou, L., & Zhao, W. (2006). Alkyl phloroglucinol derivatives from Syzygium levinei and their differentiation-inducing activity. *Planta Medica*, *72*(6), 533–538. https://doi.org/10.1055/s-2006-931559

Zulcafli, A. S., Lim, C., Ling, A. P., Chye, S., & Koh, R. (2020). Focus: Plant-Based Medicine and Pharmacology: Antidiabetic Potential of *Syzygium* sp.: An Overview. *Yale Journal of Biology and Medicine*, *93*(2), 307–325.

Appendices

APPENDIX 1 - MORPHOLOGICAL CHARACTERS ANALYSED IN THE STUDY

Sl. No	Characters
1	Habit
1	Blaze colour
2	Leaf ratio
3	Leaf shape
4	Lamina size (area)
5	Leaf base
6	Leaf margin
7	Leaf apex
8	Petiole size
9	Petiole length
10	Petiole shape
11	No. of lateral nerves
12	Inflorescence position
13	Peduncle length
14	Petal shape
15	Flower bud shape
16	Sepal shape
17	Anther size
18	Fruit size
19	Fruit colour
20	Fruit shape

APPENDIX 2 - ANATOMICAL CHARACTERS ANALYSED IN THE STUDY

Sl. No.	Characters
1.	Lateral vein orientation
2.	Lateral vein loop
3.	Stomata
4.	Epidermis abaxial anticlinal cell wall
5.	Lamina cuticular ornamentation
6.	Leaf crystals
7.	Midrib adaxial surface cross-section
8.	Midrib abaxial surface cs
9.	Midrib adaxial phloem partition
10.	Midrib vascular shape
11.	Midrib sclerenchyma
12.	Lamina adaxial cell shape (cs)
13.	Lamina abaxial cell shape (cs)
14.	Adaxial palisade layer
15.	Abaxial palisade layer
16.	Lateral vein bundle sheath extension
17.	Foliar sclereid
18.	Twig cross-section (mature)
19.	Pith

APPENDIX 3 - MORPHOLOGICAL AND ANATOMICAL DATA WITH CHARACTER CODING

Characters	Character Codes
Habit	0, Large tree; 1, Medium tree; 2, Small tree
Blaze colour	0, Reddish brown; 1, Greyish brown; 2, Creamy brown
Leaf ratio	0, Wider than long; 1, Two times longer than wide; 2, Longer than wide
Leaf shape	0, Ovate oblong; 1, Elliptic to obovate; 2, Obovate
Lamina size (area)	0, 5-10 cm x 0-4 cm; 1, 10-15 cm x 4-8 cm; 2, 15-20 cm x 8-12 cm; 3, 20-25 cm x12-16 cm
Leaf base	0, Narrow decurrent; 1, Obtuse; 2, Attenuate; 3, Cuneate
Leaf margin	0, Entire undulate; 1, Entire
Leaf apex	0, Obtuse folded; 1, Obtuse to acuminate; 2, Obtuse to round
Petiole size	0, Stout; 1, Thin
Petiole length	0, 0-1 cm; 1, 1-2 cm; 2, 2-3 cm
Petiole shape	0, presence of groove on upper side; 1, absence of groove on upper side
No. of secondary lateral nerves	0, 10-15 pairs; 1, 18-20 pairs
Inflorescence position	0, Terminal and axillary; 1, Axillary; 2, Terminal
Peduncle length	0, 0-4 cm; 1, 4-8 cm; 2, 8-12 cm
Petal shape	1, five-lobed calyptrate; 2, five-lobed round or acute; 3, five-lobed campanulate
Flower bud shape	0, Dome; 1, Pyriform
Sepal shape	0, Triangular, green coloured with purple-coloured tips; 1, Triangular, green coloured, rounded tips
Anther size	0, 0-5 mm x 0-3 mm; 1, 5-10 mm x 3-6 mm
Fruit size	0, 2-3 mm; 1, 3-4 mm; 2, 5-6 mm; 3, 6-12 mm
Fruit shape	0, Globose; 1, Sub-globose; 2, Oval
Fruit colour	0, Pinkish purple; 1, Purplish Black; 2, Black
Lateral vein orientation	0, Parallel; 1, Irregular; 2, Reticulate
Lateral vein loop	0, Present; 1, Absent
Stomata	0, Paracytic; 1, Anisocytic; 2, Anomocytic
Epidermis abaxial anticlinal cell wall	0, Straight; 1, Straight curved; 2, Curved
Lamina cuticular ornamentation	0, Curved undulate; 1, Smooth; 2, Striate

Leaf crystals	0, druses predominant; prismatic crystals absent; 1, prismatic crystals predominant; druses absent; 2, Both druses and prismatic crystals present
Midrib adaxial surface cross-section	0, Canaliculate; 1, Flat
Midrib abaxial surface cross-section	0, Convex; 1, Flat
Midrib adaxial phloem partition	0, Absent; 1, Present
Midrib vascular shape	0, Arc with straight margins; 1, Arc with incurved margins; 2, Arc with interrupted bundles
Midrib sclerenchyma	0, Discontinuous; 1, Continuous
Lamina adaxial cell shape (CS)	0, equal width and height; 1, wider than high
Lamina abaxial cell shape (CS)	0, equal width and height; 1, wider than high
Adaxial palisade layer	0, Single layered; 1, Multilayered
Adaxial palisade layer	0, Continuous; 1, Discontinuous
Lateral vein bundle sheath extension	0, Absent; 1, Present
Foliar sclereid	0, Absent; 1, Present
Twig cross-section (mature)	0, Terete; 1, Angular and winged; 2, Sub-terete
Pith	0, Angular, 1, rectangular, 2, circular

APPENDIX 4 - DATA MATRIX OF PHYLOGENETIC ANALYSIS USING MORPHOLOGICAL AND ANATOMICAL CHARACTERS

Taxon/Characters12345678910111213141516171819202122232425262728293031323334353637383 9

Species 1 S. acuminatissimum 0223031110102122030200 01{2}001001100000111

Species 2 S. aquem 2121211100010222233300 01{2}012000000000101

Species 3 S. aromaticum 2111 1 1 3 0 1 1 2
1 2 2 0 0 1 0 4 3 3 0
0 1{2} 0 0 2 0 0 0 0 1 0
0 0 0 1 0 1

Species 4 S. caryophyllatum 2 0 0 0 0 2 1
2 0 0 0 0 2 2 0 0 1 0
4 0 2 0 0 1{2} 0 0 2 0 1
0 0 0 0 0 0 0 0 0 1

Species 5 S. claviflorum 0 1 1 1 2 2 1 1
1 0 1 1 2 0 2 0 2 1 4
3 4 0 0 1{2} 2 1 2 0 0 1
1 0 1 0 0 0 0 0 1

Species 6 S. cumini 0 1 2 1 1 3 1 1
1 0 1 0 0 1 1 1 1 1 4
2 4 0 0 2 0 1 2 0 0 0
0 0 0 0 0 0 0 0 1

Species 7 S. cymosum 0 1 1 1 3 3 0 3
1 2 1 0 0 1 2 2 1 4 3
0 0 0 0 2 0 1 2 0 0 0
0 0 0 0 0 0 0 0 1

Species 8 S. gardneri 0 0 1 3 0 1 0 3
1 1 1 0 0 2 2 1 1 1 2
2 0 0 0 2 1 1 2 0 0 0
0 0 1 1 0 0 0 0 1

Species 9 S. grande 0 1 1 1 3 2 0 1
1 2 1 1 2 2 2 0 1 1 4
2 5 0 0 2 0 0 2 0 0 0
0 0 0 0 1 0 0 0 1

Species 10 S. hemisphericum 1 1 0 1 1 1 0
1 0 2 0 0 0 1 2 2 3 1
4 2 6 0 0 2 0 2 2 0 1
0 0 0 0 0 0 0 0 0 1

Species 11 S. jambos 2 1 2 1 2 1 1 1
0 0 0 1 0 2 2 2 2 3 3

3	0	0	0	2	0	0	2	0	0	0
0	1	0	0	0	0	0	0	1		
Species 12	S. lanceolatum		2	1	2	1	1	3	1	1
1	0	1	0	0	1	1	1	1	1	4
2	4	0	0	2	2	1	2	0	0	0
0	0	1	1	0	0	0	0	1		
Species 13	S. luehmannii		1	0	2	1	0	3	1	3
1	1	1	0	0	2	0	1	2	1	4
3	4	0	0	2	2	0	1	0	1	1
0	0	0	0	0	0	0	1	1		
Species 14	S. malaccense		0	1	2	1	2	1	1	1
0	0	0	1	0	2	2	2	2	3	3
3	3	0	0	2	2	2	2	0	0	0
0	0	0	0	1	0	0	0	0		
Species 15	S. nervosum		1	0	1	1	2	2	0	1
1	0	1	0	1	1	2	2	1	0	3
0	0	0	0	2	0	0	2	0	0	0
2	0	1	0	0	0	1	1	0		
Species 16	S. oblatum		0	1	1	1	2	3	0	2
0	2	0	2	2	1	0	1	1	1	4
0	5	0	0	2	1	0	2	0	0	0
0{2}	0	0	0	1	0	1	1	0		
Species 17	S. palghatense		1	1	2	1	0	3	1	1
0	0	1	0	2	0	0	2	1	0	4
0	3	0	0	2	1	0	2	0	0	0
0	0	0	0	0	0	1	1	1		
Species 18	S. polyanthum		1	1	1	1	3	1	0	1
1	1	1	1	1	1	0	1	1	1	3
1	1	0	0	2	1	2	0	0	0	0
3	0	0	0	1	0	1	1	0		
Species 19	S. rubicundum		0	0	2	1	1	0	0	1
1	0	1	0	0	1	0	2	1	0	2
0	2	0	0	2	1	0	2	0	0	0
0	0	0	0	0	0	1	1	0		
Species 20	S. samarengense		2	1	2	1	2	1	1	1
0	0	0	1	0	2	2	2	2	3	3
3	0	0	0	2	0	0	2	0	0	0
0	1	0	0	0	0	1	1	0		
Species 21	S. stocksii		0	1	1	1	3	1	0	1
1	1	1	1	1	1	0	1	1	1	3
1	1	0	0	1	0	0	2	0	0	0
1{2}	1	0	0	0	1	1	0	1		
Species 22	S. tamilnadensis		1	2	2	1	1	3	1	2
1	0	1	0	2	2	0	0	1	0	3

0 6 0 0 1 0 0 2 0 0 0
0 0 0 0 0 0 0 1 0

Species 23 S. tetragonum 1 1 0 1 1 3 1 1
1 0 1 0 2 2 0 0 1 0 3
0 0 0 0 1 0 0 1 0 0 0
3 "0,1" 0 0 0 0 1 1 0

Species 24 S. travancoricum 1 0 0 0 1 0 0 0
0 2 0 0 0 2 0 0 0 1 2
1 0 0 0 0{1} 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0

Species 25 S. zeylanicum 2 1 2 1 3 1 1 4
1 0 1 0 0 0 0 1 2 1 3
2 6 0 0 0{1} 2 0 2 0 0 0
0 0 0 0 0 0 0 1 0

Species 26 JNTBGRI 1 0 0 0 1 1 0 0
0 2 0 0 0 2 0 0 0 1 2
1 0 0 0 0{1} 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0

Species 27 IDINJAAR 1 0 0 0 1 1 0 0
0 2 0 0 0 2 0 0 0 1 2
1 0 0 0 0{1} 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0

Species 28 KOLLAM 1 0 0 0 1 1 0 0
0 2 0 0 0 2 0 0 0 1 2
1 0 0 0 0{1} 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0

Species 29 THRISSUR 1 0 0 0 1 1 0 0
0 2 0 0 0 2 0 0 0 1 2
1 0 0 0 0{1} 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0

Species 30 KONGINICHALKAVU 1 0 0 0 1 1 0
0 0 2 0 0 0 2 0 0 0 1
2 1 0 0 0 0{1} 0 0 0 0 0
0 0 0 0 0 0 0 0 1 0

Species 31 PALIYERIKAVU 0 1 1 1 3 1 0 1
1 1 1 1 1 1 0 1 1 1 3
1 1 0 0 1 0 0 1 0 0 0
1{2} 1 0 0 0 1 0 0 1

Species 32 POOKODE 0 1 1 1 3 1 0 1
1 1 1 1 1 1 0 1 1 1 3
1 1 0 0 1 0 0 1 0 0 0
1{2} 1 0 0 0 1 0 0 1

Species 33 MSSRF 1000110002000200012 1 0 0 0 0{1} 0
0 0 0 0 0 0 0 0 0 0 0
0 1 0

APPENDIX 5 - DNA SEQUENCES DEPOSITED AT GENBANK

Loci	Samples	GenBank Accession No.
ITS	IDINJAAR	MN064801
	KOLLAM	MN064802
	JNTBGRI	MN064803
	WYANAD	MN064804
	PALIYERIKAVU	OL375229
	POOKODE	OL375228
	KONGINICHALKAVU	ON597486
	THRISSUR	ON597488
matK	IDINJAAR	MN072849
	KOLLAM	MN072850
	JNTBGRI	MN072851
	THRISSUR	MN072852
	WYNAD	MN072853
	POOKODE	OL473988
	PALIYERIKAVU	OL473989
	KONGINICHALKAVU	OL473990
rbcL	IDINJAAR	MN072854
	KOLLAM	MN072855
	JNTBGRI	MN072856
	WYANAD	MN072857
	PALIYERIKAVU	OL473984
	POOKODE	OL473985
	KONGINICHALKAVU	OL473986
	THRISSUR	OL473987

ANNEXURE 6 - GENBANK REFERENCES OF THE SELECTED *SYZYGIUM* SPECIES INCLUDED IN PHYLOGENETIC TREE CONSTRUCTION

Loci	Species	GenBank Accession
	Syzygium_acuminatissimum	MW854216
	Syzygium_aquem	JF682809
	Syzygium_aromaticum	KX079334
	Syzygium_caryophyllatum	OR055972
	Syzygium_claviflorum	MH109177
	Syzygium_cumini	JX856510
	Syzygium_cymosum	OM218687
	Syzygium_gardneri	KT970719
	Syzygium_grande	KT982669
	Syzygium_hemisphericum	JX856511
	Syzygium_jambos	KC815991
	Syzygium lanceolatum	KT982670
ITS	*Syzygium_luehmannii*	AY187197
	Syzygium_malaccense	MN104141
	Syzygium nervosum	MH884765
	Syzygium_oblatum	MW854197
	Syzygium palaghatense	KT982674
	Syzygium_polyanthum	MW854218
	Syzygium_rubicundum	KT982675
	Syzygium_samarengense	OP897497
	Syzygium_tamilnadensis	KT982672
	Syzygium_tetragonum	KR532644
	Syzygium_travan_GENBK	KY607797
	Syzygium_zeylanicum	EF026650
	Syzygium_acuminatissimum	MW854112
	Syzygium_aquem	DQ088559
	Syzygium_aromaticum	MH101982
	Syzygium_caryophyllatum	KU301785
matK	*Syzygium_claviflorum*	MW854091
	Syzygium_cumini	DQ088575
	Syzygium_cymosum	MT864815
	Syzygium_gardneri	KT907477
	Syzygium_grande	AB925279

	Syzygium_hemisphericum	AB925274
	Syzygium_jambos	GUI35056
	Syzygium lanceolatum	KT936452
	Syzygium_luehmannii	JN564161
	Syzygium_malaccense	KT936453
	Syzygium nervosum	DQ088595
	Syzygium_oblatum	AB924759
	Syzygium palaghatense	KT936457
	Syzygium_polyanthum	MW854105
	Syzygium_rubicundum	KT936458
	Syzygium_samarengense	AY525141
	Syzygium_tamilnadensis	KY018931
	Syzygium_tetragonum	KR531550
	Syzygium_travan_GENBK	KT936460
	Syzygium_zeylanicum	DQ088619
	Syzygium_acuminatissimum	MW854145
	Syzygium_aquem	OK052789
	Syzygium_aromaticum	MH101978
	Syzygium_caryophyllatum	KU301786
	Syzygium_claviflorum	KF496648
	Syzygium_cumini	GU135224
	Syzygium_cymosum	KM896098
	Syzygium_grande	AB925485
	Syzygium_jambos	GU135219
rbcL	*Syzygium_luehmannii*	KM896171
	Syzygium_malaccense	LC381850
	Syzygium nervosum	DQ088595
	Syzygium_oblatum	MH113353
	Syzygium_polyanthum	MW854138
	Syzygium_samarengense	AB925321
	Syzygium_tetragonum	KR530117
	Syzygium_travan_GENBK	MW427216
	Syzygium_zeylanicum	OK052796

APPENDIX 7 - COMBINED MOLECULAR DATA

#NEXUS
[TITLE]
BEGIN DATA;
DIMENSIONS NTAX=32 NCHAR=1543;
FORMAT MISSING=? GAP=- datatype=dna interleave=yes;
MATRIX
[ITS=464]
Syzygium_acuminatissimum GGCGGGCCTCGCCCAATGTCCCC-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGC?TTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTAAACAAGAGAGCG
ATGCTCCCGTCACCCCAGACATGGTGCGTGCGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCATTGGTCGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACTTGGGTGCGTATGATGGCCTCCCGAGACAACCTTGTCCCGGTTGG
CCCAAAATCGAGCGTTGGAGCGA
Syzygium_aquem GGTGGGCCTCGCCCAACGTCTCC-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGCTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCTGCCGCCCCAGACATGGTGCGCGCGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCCTCGGCTTAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCCTAACCCCTCGCCTTGAATTGGGCGG
GCGGGACTTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCCCGGTTGGCCCAAAATCGAGCGTTGGAGCGA
Syzygium_aromaticum GGTGGGCCTCGCCCAACGTCTCT-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGCTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGCCGTCCCGGACATGGTGCGCGTGCGGGATGCCATGCAATCTCCCA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCCTAACTCCTCGCCTTGAATTGGGCGG
GCGGGACTTGGGTGCGTACGTTGGCCTCCCGAGATGACCTTATCCCGGTTGGCCCAAAATCGAGCGTTGGAGCGA
Syzygium_caryophyllatum GGTGGGCCTCGCCCAACGTCTCT-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGTTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGTCGCCCCAGACATGGTGTGTGTGCGGGATGCCATGCAATCTCCTT-
CTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACCTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCTCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_claviflorum GGTGGGCCTCGCCCAACGTCTCC-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGCTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGTCGCCCCAGACATGGTGCGTGTGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACCTGGGTGCGTACGTTGGCCTCCCGAGATGACCTTATCCCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_cumini GGTGGGCCTCGCCCAACGTCTCT-AGACGCTTGGATGGCACGGGTGCCCAC-
GGGCGCTCGGGCTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGTCGCCCCAGACATGGTGCGCGTGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAACTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACCTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCCCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_cymosum GGTGGGCCTCGCCCAACGTCTCT-AGACGCTTGGATGGCACGGGTGCCTAC-
GGGCGCTCGGGTTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGTCGCCCCAGACATGGTGCGTGTGCGGGATGCCATGCAATCTCCTT-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACCTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCCCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_gardneri GGTGGGCCTCGCCCAACGTCTCT-AAACGCTTGGATGGCACGGGTGCCTGC-
GGGCGCTCGGGTTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG
ATGCTCCCGTCGCCCCAGACATGGTGCGTGTGCGGGATGCCATGCAATCTCCTT-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGCGGGACCTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCCCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_grande GGTGGGCCTCGCCCAACGTCTCT-AGACGCTTGGATGGCACGGGCGCCTAC-
GGGCGCTCGGGCTTTTTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCG

ATGCTCCCGTCGCCCCAGACATGGTGCGCGTGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCG-
AAGCTTCGGTTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCC-
AACCCCTCGCCTTGAATTGGGCGGGTGGGACCTGGGTGCGTATGTTGGCCTCCCGAGATGACCTTATCCCGGTTGG
CCTAAAATCGAGCGTCGGAGCGA
Syzygium_hemisphericum GGCGGGCCTCACCCAACGTCTCC-AGACGCTCGGATGGCCCGGGTGCCTGC-
GGGCGCTCGGGC-
TTCTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCGATGCTCCCGCCGC
CCCAGACATGGTGCGTGCGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAAGCTTCGGTTGAGGGCACGT
TTGCCTGGGTGTCACACGCGGCGTTGCCCCTAACCCCTCGCCTTGAATTGGGCGGGCGGGACTTGGGTGCGTACGT
TGGCCTCCCGAGACGAACTTATCCCGGTTGGCCCAAAATCGAGCGTTGGAGCGA
Syzygium_jambos GGCGGGC-TCGCCCAACGTCTCC-AGACGCTTGGATGGCACGGGTGCCCAC-
GGGCGCTCGGGC-
TTCTCTCGGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCGATGCTCCCGCCGC
CCCAGACATGGTGCGTGCGCGGGATGCCATGCAATCTCCTA-
TTATTCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATACTTGG
TGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAAGCTTCGGTTGAGGGCACGT
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Syzygium_luehmannii

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Syzygium_malaccense

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Syzygium_nervosum

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Syzygium_oblatum

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Syzygium_palghatense

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Syzygium_polyanthum

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Syzygium_grande
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Syzygium_luehmannii
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Syzygium_oblatum
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Syzygium_polyanthum
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Syzygium_samarengense ----------

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END;

APPENDIX 8 – COMBINED MORPHOLOGICAL AND MOLECULAR DATA

#NEXUS
[TITLE]
BEGIN DATA;
DIMENSIONS NTAX=32 NCHAR=1582;
FORMAT MISSING=? GAP=- datatype=mixed(DNA:1-1543, STANDARD:1544-1582) interleave=yes;
MATRIX
[ITS=464]
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Syzygium_grande

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LIST OF PUBLICATIONS

Sl. No	Title	Authors	Journal/Books/ Book Chapter	National/ International	ISBN/ISSN	Scopus/Web of Science	doi	Impact factor
1	The Genus *Syzygium* Gaertn- A review of its Systematics and Endemism	P.S Smitha & P.V Anto	Book Chapter	National				
2	Chemical composition and antibacterial activity of the leaf essential oil from Syzygium travancoricum Gamble	P.S Smitha & P.V Anto	Book Chapter	National Frontiers in Life Science Volume V	ISBN: 978- 978-93-91768-54-6			
3	*Syzygium travancoricum* Gamble, an endangered endemic species of Southern Western Ghats	P.S Smitha & P.V Anto	Book Chapter	National Proceedings of National Seminar Species the Passion 7	ISBN-978-93-91691-01-1			
4	Endemic Syzygium species of Western Ghats	P.S Smitha & P.V Anto	Book Chapter	National Proceedings of National Seminar Species the Passion 8	ISBN-978-93-91691-01-1			
5	Phytochemical Evaluation of *Syzygium travancoricum* Gamble (Myrtaceae) from Southern Western Ghats	P.S Smitha, P.V Anto & U. Senthilkumar	Book Chapter	National Recent Advances in Healthcare Research	ISBN 978-81-957251-0-6			
6	Anatomical and histochemical characterization of *Syzygium travancoricum* Gamble (Myrtaceae)	P.S Smitha & P.V Anto	Journal of Microscopy Research & Technique Vol 86, Issue 3	International	ISSN:1097-0029	Scopus and Web of Science	https://doi.org/10.1002/jemt.24321	**2.893**

LIST OF PRESENTATIONS

Sl. No	Author	Title	Conference name & place	Year
1	P.S Smitha and P. V Anto	"Nutritional and mineral profiling of *Syzygium travancoricum* fruits"	International Seminar on Phytochemistry, Phytochemistry & Pharmacology Division, JNTBGRI, Palode	26 th-27 th March, 2018
2	P.S Smitha and P. V Anto	"Phytochemical screening of *Syzygium travancoricum* (Gamble)	International Conference on Phytomedicine ICPM 2018, Bharathiar University, Coimbatore	28 th-30 th August, 2018
3	P.S Smitha and P. V Anto	*Syzygium travancoricum* Gamble, a critically endangered endemic tree species of Western Ghats – Bioprospecting and Conservation'	International Endangered Species Day - Poster Presentation Competition organized by the Centre for Biodiversity, University of Kerala, Thiruvananthapuram	May 21, 2021
4	P.S Smitha and P. V Anto	*Syzygium travancoricum* Gamble, an endangered endemic species of Southern Western Ghats	National Seminar; Species the Passion 7	February, 2022
5	P.S Smitha and P. V Anto	Endemic *Syzygium* species of Southern Western Ghats	National Seminar: Species the Passion 8	February, 2023

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RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Anatomical and histochemical characterization of *Syzygium travancoricum* Gamble (Myrtaceae)

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Review Editor: Paolo Bianchini

Abstract

Syzygium travancoricum Gamble popularly known as "*Kulavettimaram*" or "*Kulir-maavu*" is a least explored endemic endangered taxa of Southern Western Ghats, Kerala. The species is often misidentified due to its close resemblance with allied species and no other studies have been reported on the anatomical and histochemical characters of this species. This article aims to evaluate the anatomical and histochemical characteristics of various vegetative parts of *S. travancoricum*. Anatomical and histochemical characters of bark, stem, and leaf were analyzed using standard microscopic and histochemical procedures. *S. travancoricum* possessed distinct anatomical characters such as, paracytic stomata, arc shaped midrib vasculature, continuous sclerenchymatous sheath around the midrib vascular region, single layer of adaxial palisade layer, presence of druses, and quadrangular cross section contour of stem which could be combined with additional morphological and phytochemical characteristics, relevant for species identification. The bark showed the presence of lignified cells, isolated groups of fibers and sclereids, starch depositions and druses. Stem has quadrangular outline with well-defined periderm. The petiole and the leaf blade have abundance of oil glands and druses with paracytic stomata. The anatomical and histochemical characterization are potential tools for the delineation of confusing taxa and provide substantial evidence to their quality control.

KEYWORDS
druses, oil glands, starch, *Syzygium travancoricum*, tannin

Research Highlights

- *S. travancoricum* had distinct anatomical and histochemical characteristics that could aid in species identification and overcoming species adulteration.
- Lignified cells, isolated groups of fibers and sclereids, starch depositions, and druses were found in the bark.
- The stem has a quadrangular outline with a well-defined periderm. The petiole and leaf blade are densely packed with oil glands and druses with paracytic stomata.
- Anatomical and histochemical characterization are potential tools for taxonomic classification.

1 | INTRODUCTION

Syzygium Gaertn. is a major genus of Myrtaceae, with approximately 1200 species dispersed throughout the tropical and subtropical parts of the world (Govaerts et al., 2008; Parnell et al., 2007). The genus is economically significant for its aromatic properties, tensile wood, fruit, and a chief resource of pharmaceutical drugs. The systematic studies of *Syzygium* species are quite perplexing and complex due to the

morphological homogeneity. Many authors have thoroughly reviewed and discussed the historical foundation and classification issues in the genus Syzygium (Craven, 2001; Schmid, 1972).

Syzygium travancoricum Gamble is an aromatic evergreen medium to large-sized tree specifically confined to wet, rainfed areas of tropical forests and sacred groves in the Southern Western Ghats. The bark is characterized by deep longitudinal fissures and appears grayish-brown in color. Leaves are simple, large-sized 8–10 cm in length and 5–6 cm in breadth, glabrous, petiolate with oblique tips. Flowers are small, developed as clusters in cymose inflorescence, bisexual, bracteate and white colored. Fruits are small, single seeded berry with deep purple color (Gamble, 1915). *S. travancoricum* is often misidentified due to its close resemblance with allied species. While the *Syzygium* genus has been extensively studied, no research on the anatomical and histochemical characterization of *S. travancoricum* have been published to date.

Comparative anatomical and histochemical studies to resolve taxonomic confusions are gaining momentum after the taxonomic disposition of New-World genus *Eugenia* from Old-World genus *Syzygium* using comparative anatomy and reproductive botany (Schmid, 1972). A few research studies focused on wood anatomy (Chattaway, 1959; Dadswell & Ingle, 1947; Ingle & Dadswell, 1953) and palynology (Pike, 1956), strongly supported the distinction between the species of *Eugenia* and *Syzygium*. Hence it seemed reasonable to expect that the anatomical and histochemical characters of a species could be of systematic value and might draw substantial evidences for species delineation. Moreover, accurate species identification is critical for reproducibility in research and therefore, standardization of anatomical and histochemical markers are the few parameters required for quality control of herbal raw materials or drugs. Hence, this research study was aimed to characterize the anatomical and histochemical characteristics of the bark, stem, and leaf of *S. travancoricum* that may help in species identification. This study also intended to examine the correlate the cell types responsible for producing the characteristic odor with the secretory materials within various plant parts of *S. travancoricum* histochemically.

2 | MATERIALS AND METHODS

2.1 | Reagents used

Safranin, Ferric chloride, HCl, Iodine solution, Phloroglucinol S and Sudan red.

2.2 | Plant collection

About five fresh plant samples of stem, bark and petiole of *S. travancoricum* distributed all across Kerala were collected during the period April 2019–June 2019. The voucher specimens of the sample were prepared (VCTBH0050) and deposited at the Herbaria maintained by the Department of Botany, Vimala College (Autonomous) Thrissur, Kerala, India.

2.3 | Anatomical characterization

Standard anatomical procedures were employed to prepare semipermanent slides and were used to examine the anatomical findings (Cutler et al., 2008). Thin tissue sections taken using hand or microtome were stained with diluted aqueous safranin, followed by rinsing in water and mounting in 40% glycerine. The analysis of the semipermanent histological slides prepared was conducted using a trinocular compound microscope (Leica DM 3000) with an eyepiece magnification of 10x attached to a Leica DFC 295 digital camera connected to the computer and Leica Application Suite software was used for the observation and capture of microscopic images at magnification 4×, 10×, 20×, and 40× with numerical apertures of 0.10, 0.25, 0.40, and 0.65, respectively. Images were observed and the anatomical traits were compared.

2.4 | Histochemical characterization

The bark, stem, and leaf was cross-sectioned using a sliding plant microtome, Automatic MT3 and were treated with the following histochemical reagents: Ferric chloride reagent for the detection of tannin compounds, Phloroglucinol S for the detection of lignin, Iodine solution for the detection of starch and aleurone grains, and Sudan Red for the detection of oil compounds and total lipids (Machado et al., 2006). To determine the presence of starch, tissue sections were stained with iodine solution, appearance of deep blue color indicated the deposition of starch grains. The presence of tannin was detected using ferric chloride solution where a bluish-black or grayish black color indicated the presence of tannins. For examining the lignified cells, the section was first moistened with phloroglucinol solution, allowed to dry and then treated with one drop of concentrated hydrochloric acid. The appearance of pink color indicated the presence of lignin. To examine the presence of lipophilic materials, the specimen was stained with Sudan red. The appearance of deep orange-pink color inside the cells confirmed the presence of lipophilic materials.

3 | RESULTS

3.1 | Anatomical characterization

3.1.1 | Bark

Cross-sections of the bark revealed periderm with phellem, phellogen, phelloderm, and phloem (Figure 1a). The phelloderm region was composed of approximately seven to eight layers of parenchymatous cells (Figure 1a), with abundant stone cells and druses which represented a conspicuous feature as shown in Figure 1b. The periderm appeared curved with continuous layers of radially flattened phellem. The discontinuous nature of periderm was due to the presence of irregular patches of sclereids and druses. The secondary phloem encircled secondary xylem and consisted of sclerenchymatous fibers. The extra

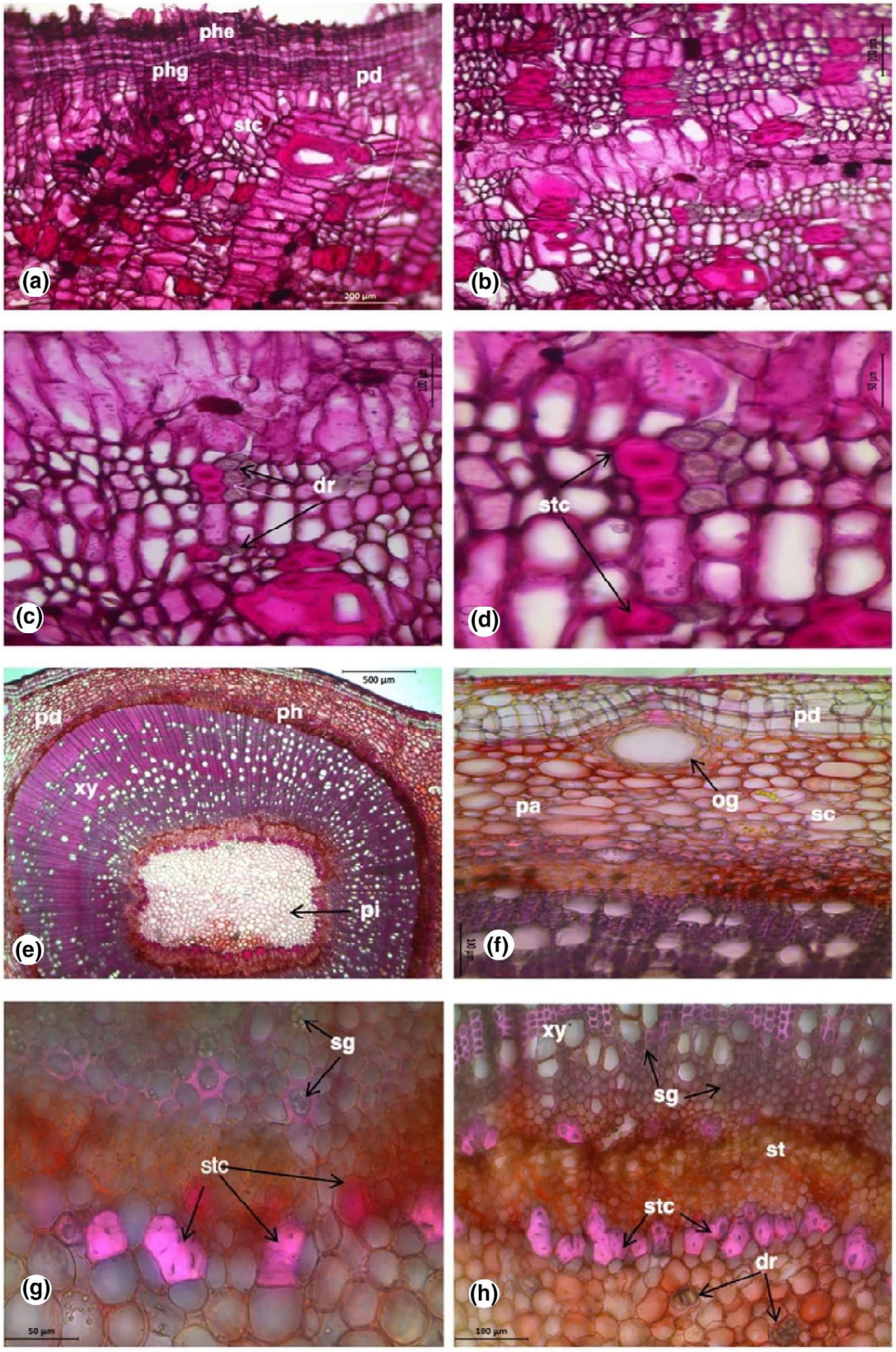

FIGURE 1 Cross-sections of bark and stem of *S. travancoricum* Gamble. Bark: (a) general view; (b) periderm layer; (c) distribution of druses; (d) distribution of stone cells Stem: (e) general view; (f) periderm; (g) distribution of sclereids; (h) distribution of druses and starch. dr, druses; og, oil glands; pa, parenchyma; pd, periderm; ph, phloem; phe, phellem; phg, phellogen; pi, pith; sc, secondary cortex; sg, starch grains; stc, stone cells; xy, xylem. Bars: (a, b): 200 µm, (c, f, h): 100 µm, (e): 500 µm, (d, g): 50 µm

stelar region including secondary phloem exhibited the presence of druses (Figure 1c, d).

3.2 | Stem

In cross-section, the secondary stem exhibited a quadrangular outline with a well-defined periderm. The periderm is comprised of continuous layers of radially flattened phellem, narrow band of phellogen and a parenchymatous secondary cortex. The secondary cortical region was made up of isodiametric parenchyma cells with lignified cells, stone cells, druses, and starch depositions (Figure 1e, f). The druses were found scattered around the vascular system, phloem and pith region. Druses were found to be more common than prismatic crystals. The vasculature of the stem was of open type. The phloem was located both externally and internally to the xylem, forming a continuous ring. The internal phloem contained groups of isolated stone cells and druses. Central pith region comprised of parenchyma, lignified cells, stone cells, druses, and starch grains (Figure 1g, h).

3.3 | Leaf

The cross-section of the leaf revealed a concave to convex midrib and two narrow side wings. The adaxial surface of epidermal cells was straight with an arc-shaped abaxial surface. The upper and lower epidermis consisted of single-layered radially flattened parenchymatous cells protected by a thin cuticle (Figure 2a). However, the adaxial epidermis was found to be broader than the abaxial epidermis. Stomata were observed to be of paracytic type. The mesophyll layer was composed of single-layered palisade parenchyma at the adaxial region and four to five layers of spongy parenchyma at the abaxial region. The leaf anatomy revealed a closed type of vascular system. The vascular system appeared "U" shaped at the midrib region and was composed of a larger bicollateral vascular bundle extending from the abaxial face to the adaxial face with outer and inner patches of phloem (Figure 2b). There was a continuous sheath of sclerenchyma around the vascular bundle. The phloem region showed abundance of sclereids and druses. Druses were also present in the midrib parenchymatous and mesophyll layers. Oil glands were found dispersed in the mesophyll layer. The oil glands seemed to be bigger than the surrounding cells and were bordered with small parenchyma cells. Oil glands and druses were dispersed between the palisade and spongy mesophyll tissue (Figure 2c, d).

3.4 | Petiole

The petiole was cylindrical in transverse section. The adaxial surface appeared U-shaped extending outwards, whereas the abaxial surface appeared V-shaped (Figure 2e). There was abundance of essential oil cavities in the cortical zone specifically in the abaxial hypodermal region (Figure 2f). The vasculature of the petiole appeared to be of open type and crescent-shaped with intra and extraxylary phloem (Figure 2e). The parenchyma cells in the cortical region comprised of more than eight layers. Druses, sclereids, and tannin depositions were predominantly present in the cortical region (Figure 2g, h).

3.5 | Histochemical analysis

The histochemical analysis revealed the deposition of several valuable secondary metabolites such as; tannin, lignin, starch and essential oil depositions in various plant parts of *S. travancoricum*. It was identified that the oil cavities were not only distributed in the leaves but also in other parts such as the leaf and stem. In this histochemical study, most secretory substances showed positive reaction to the test, indicating that these substances accumulate within the cells and attribute to the characteristic leaf aroma and distinctiveness of this species.

Lignin deposition was predominantly seen all over the mechanical tissues and xylem walls which appeared as pinkish purple on treating with Phloroglucinol S reagent. This is obvious in the cross-sections of the bark, stem, and leaf of *S. travancoricum* where the lignified cells appeared in pinkish-purple color (Figures 3d, h, 4d, h). Also, the treatment with Sudan Red gave an orange-pink colouration to the oil glands found dispersed in the cortical and hypodermal region of the leaf, petiole and stem (Figures 3g, 4c, g). The appearance of grayish-black colouration on the application of ferric chloride indicated the presence of tannin in various plant parts such as phellem and secondary cortex of bark, hypodermal, cortical and pith regions of stem and petiole; hypodermal and midrib region of leaves (Figures 3b, f, 4b, f). This shows that tannin and lignin can also accumulate within the ground tissue and not necessarily in the specific secretory structures alone. Starch depositions were observed as the abundant reserve food material in almost every part of the plant part investigated especially in the mesophyll cells of leaves, cortex and pith region of stem and petiole which appeared deep blue on the application of Iodine solution (Figures 3a, e, 4a, e). We found the presence of druses as a characteristic feature of the species and were observed in every plant part examined.

4 | DISCUSSION

Even though Myrtaceae species are abundant in essential oils and other chemical compounds, little is known about the anatomy and histochemistry of their vegetative parts. This investigation demonstrates that *S. travancoricum* shares a number of anatomical characteristics with other members of its family. In the present study, it was discovered that *S. travancoricum* possesses distinct anatomical characters such as stomatal type, midrib vascular form, midrib sclerenchyma pattern, number of adaxial palisade layer, presence of druses, and the cross-section of twig which could be combined with additional morphological and phytochemical characteristics, relevant for species identification. Although several studies have pointed out the relevance of several anatomical traits in resolving taxonomic confusions

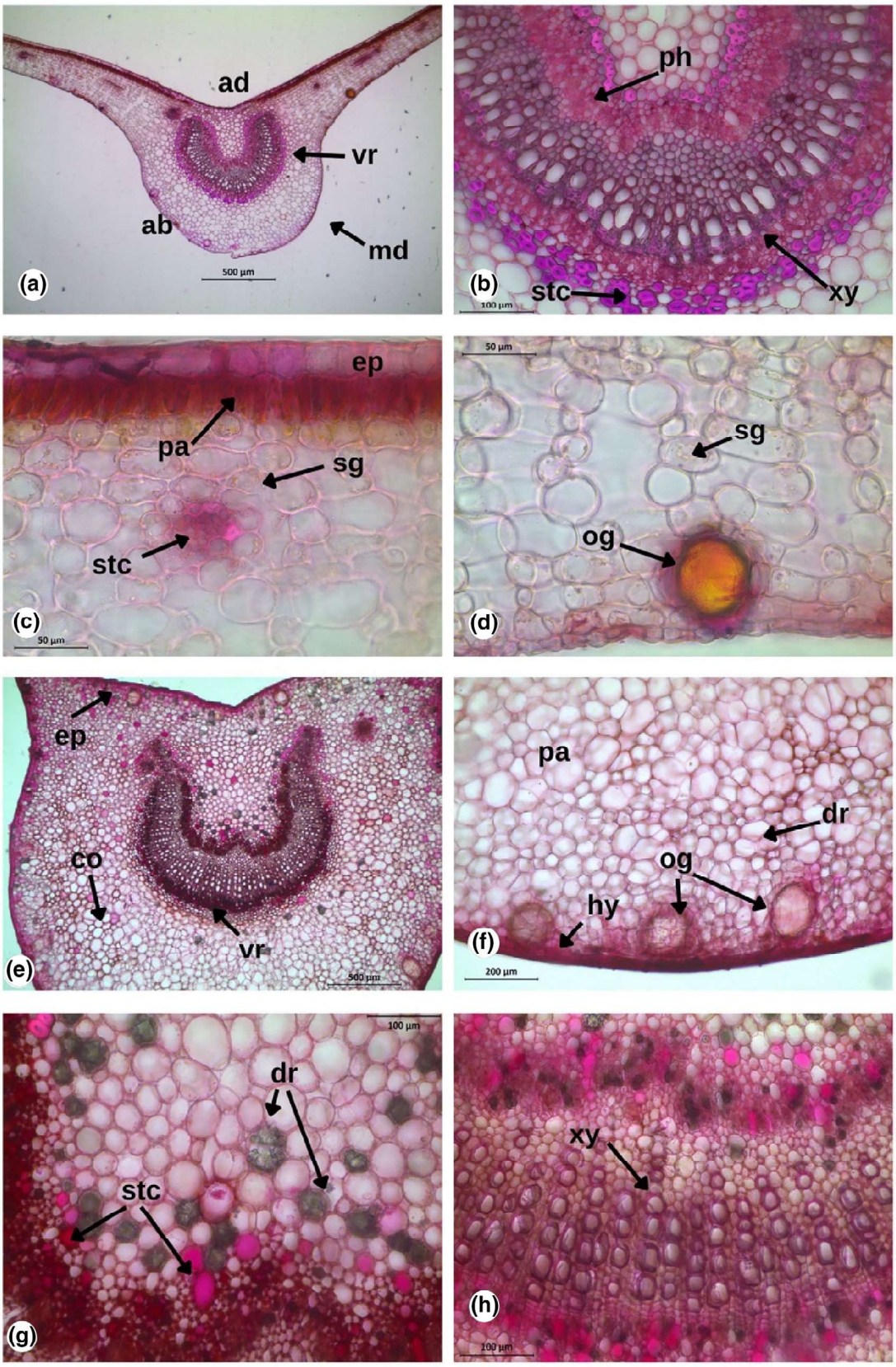

FIGURE 2 Cross-sections of lamina and petiole of *S. travancoricum* Gamble. Leaf (a) general view; (b) Midrib; (c) Starch grains, sclereids and druses; (d) Oil glands Petiole; (e) general view; (f) oil glands; (g) stone cells and druses; (h) vascular system. co, cortex; dr, druses; hy, hypodermis; og, oil glands; pa, parenchyma; ph, phloem; stc, stone cells; xy, xylem. Bars: (a, e) 500 µm; (b, g, h): 100 µm; (f): 200 µm; (c, d): 50 µm

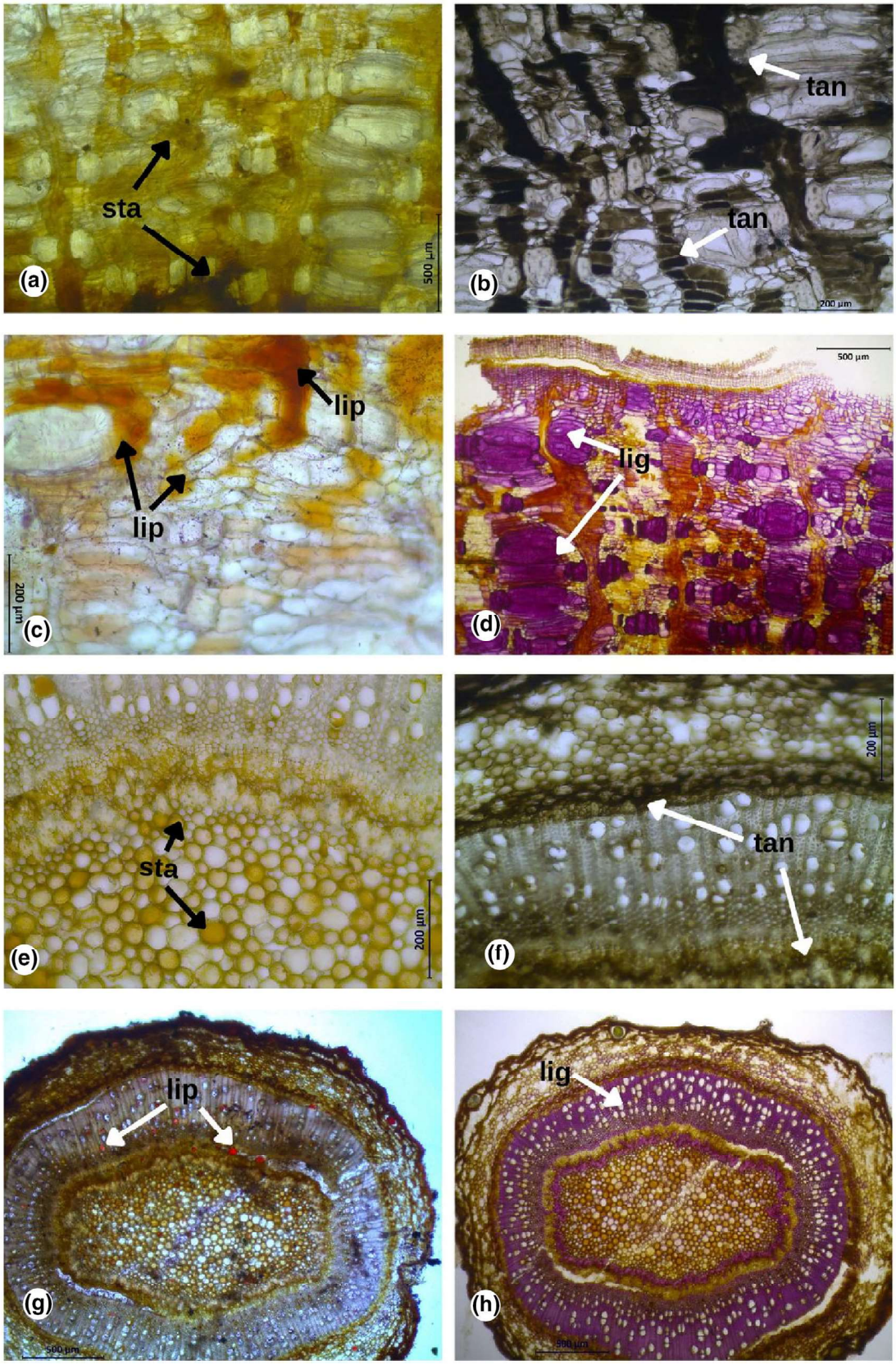

FIGURE 3 Histochemical analysis of the bark and stem of *S. travancoricum* Gamble. (a) detection of starch by Iodine solution; (b) detection of tannin by ferric chloride reagent; (c) detection of lipid by Sudan red reagent; (d) detection of lignin by Phloroglucinol S reagent. lip, lipid; lig, lignin; sta, starch; tan, tannin. Bars: (a, d, g, h): 500 µm; (b, c, e, f): 200 µm

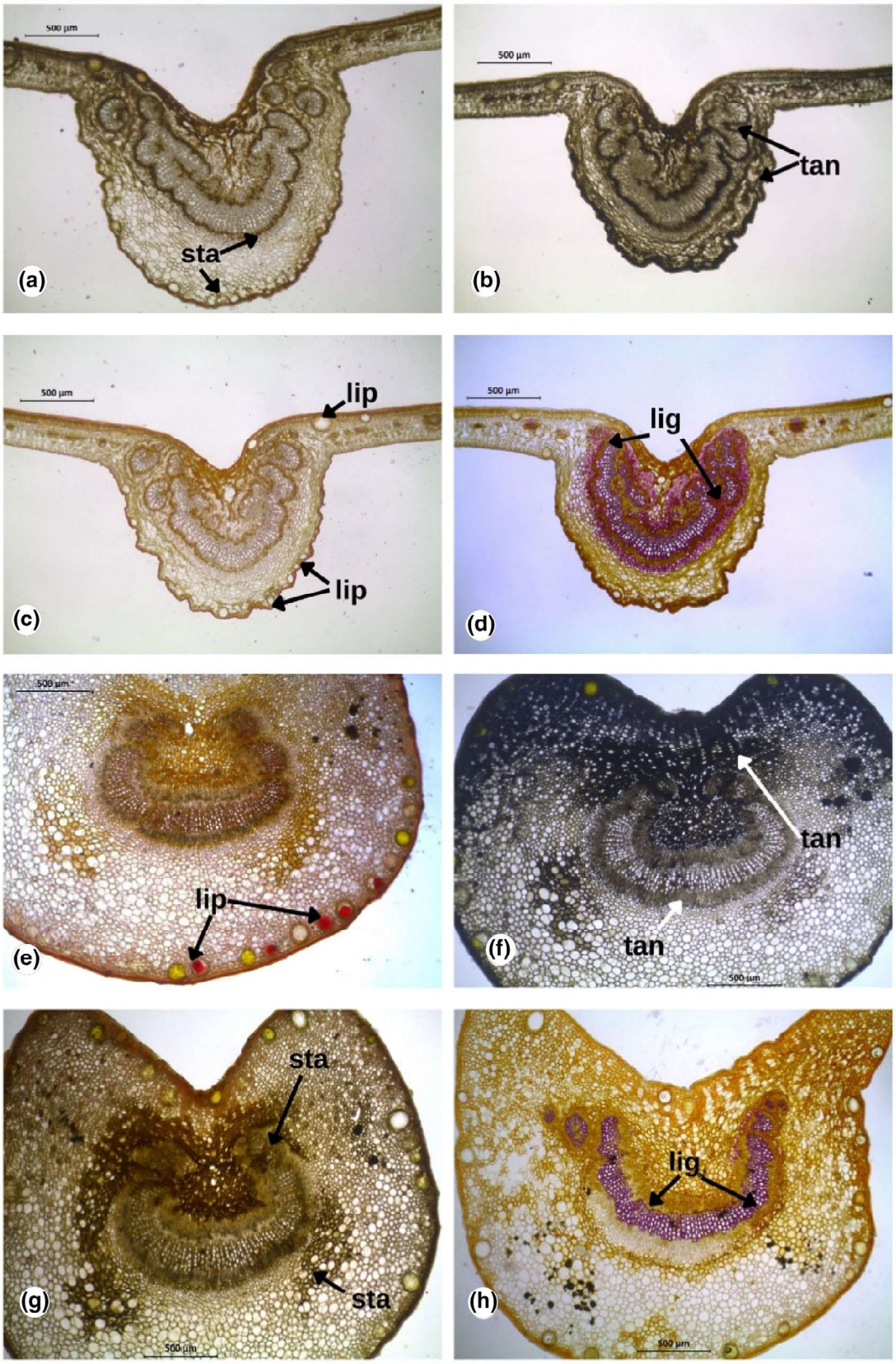

FIGURE 4 Histochemical analysis of lamina and petiole of *S. travancoricum* Gamble (a, g). Detection of starch by Iodine solution; (b, f). Detection of tannin by ferric chloride reagent; (c, e). Detection of lipid by Sudan red reagent; (d, h). Detection of lignin by Phloroglucinol S reagent. lip, lipid; lig, lignin; sta, starch; tan, tannin. Bars: (a–h): 500 µm.

across the plant kingdom no studies are found in the literature on the anatomical and histochemical investigation of this particular species.

In plants, the crystals present in the tissues are largely formed of calcium oxalate, and they can be found in a variety of shapes and places throughout the plant tissues (Franceschi & Horner, 1980). Crystals have been shown to be taxonomically useful when compared to other families or higher taxonomic ranks in the past (Dickison, 2000; Metcalfe & Chalk, 1950). Studies have shown that the vegetative and reproductive structures of many species of Myrtaceae contain druses. Alves et al. (2008) and Donato and Morretes (2007, 2009) reported the presence of calcium oxalate druses in the South American species of *Eugenia*. There have been reports of polyhedral crystals, including druses, in *Psidium*, *Eugenia*, *Gomidesia*, and *Myrcia* (Cardoso & Sajo, 2009). These structures might provide defense against herbivores and pathogens (Franceschi & Nakata, 2005; Korth et al., 2006).

However, Soh and Parnell (2011) proposed that the leaf anatomical features of *Syzygium* being homoplastic may not resolve the taxonomic problems but a combination of non-unique leaf anatomical characters such as stomatal type, crystal types and pattern, the midrib vasculature might be diagnostic for subgeneric groups. In our study we did not notice any obvious crystal macropattern among the samples; however, the primary form of crystal was druses in *S. travancoricum*.

Wilkinson (1979) proposed that the stomatal traits may be utilized for diagnostic resolution or for usage within certain taxonomic groups to distinguish between species. Similarly, Baranova (1992) pointed out in her comprehensive survey of the field of stomatology, it is critical to understand the extent to which variability and proportion of stomatal types exist so that they can be used in conjunction with other features to determine taxonomic placement. There have been some earlier leaf anatomical studies reported in *Syzygium* that have documented three different stomatal kinds, including anisocytic, anomocytic, and paracytic stomata (Haron & Moore, 1996; Kantachot et al., 2007; Khatijah et al., 1992).

Metcalfe and Chalk (1950) identified the existence of secretory structures as one of the characteristic features of the family Myrtaceae. It was observed that *S. travancoricum* possessed oil cavities that secrete aromatic volatile oil in various parts considered in our study. This finding corroborated with previous literature on the secretory cavities present in other *Syzygium* species (Al-Edany & Al-Saadi, 2012; Arruda et al., 2015; Chatri et al., 2020; Fahn, 1988; Khatijah & Mohamad Ruzi, 2006). Although earlier studies had similar findings, they revealed vague information about the distribution of the secretory cavities and the specific plant part in which these structures are present. In the present study, the secretory cavities were found located abundantly in all parts of the plant specifically, the hypodermal regions of the stem, leaf, and petiole.

The midrib vascular system observed in *S. travancoricum* had an arc with straight borders, which was characteristic of the species. The midrib region also contained phloem, either as continuous tissue or as strands on the adaxial side of the midrib. This trait is considered a typical anatomical trait of the order Myrtales (Cronquist, 1981; Takhtajan, 1980) and is consistent in Myrtaceae (Cardoso & Sajo, 2009; Schmid, 1980). The development of the adaxial phloem, the confluence of the adaxial and abaxial phloem, and the continuity of fibers around the midrib are considered suitable characters for identifying Myrtaceae species (Cardoso & Sajo, 2009; Soh & Parnell, 2011).

We considered other anatomical characters such as the distribution pattern of sclereids, midrib vasculature, number of adaxial palisade layer, stomatal type and distribution of druses as valuable anatomical markers for species diagnosis when combined with other characters because these anatomical characters are rare and consistent in their appearance. Cardoso and Sajo (2009) found that the percentage of palisade in the mesophyll is a distinguishing feature of neotropical plants in Myrtaceae. In all plant samples investigated, oil glands were present, were numerous and had a discernible pattern in the frequency of oil glands and their distribution in various parts. Merril and Perry (1939) observed, however, that the size of the oil glands and the density of punctations are frequently relevant for species identification. Oil glands were revealed to be the primary source of characteristic aroma in *S. travancoricum*.

The principal ergastic substances accumulated in various plant parts were identified using histochemical analysis. Starch was identified as the principal reserve food material stored in almost every plant part studied. Mercader et al. (2018) stated that the morphometric variations of starch grains may be utilized for taxonomic and pharmacognostic investigations. Although starch deposition occurs throughout the plant body, in the present study, starch grains were abundantly present in the mesophyll layers, cortical and pith cells of the leaf and stem. Several works have reported the use of histochemical characters within the plant cells such as terpenoids, lipids, carbohydrates and proteins in plant identification (Bosabalidis, 2014; Dubey & Trivedi, 2012; Gersbach et al., 2001; Hassan & El-Awadi, 2013). Greathead (2003) reported that the essential oil itself is a complex mixture of secondary metabolites comprising low-boiling point and molecular weight of phenylpropenes and terpenes. Several studies on aromatic plants affirmed the presence of essential oil as the contributing factor for the characteristic aroma of these plants (Chamorro et al., 2012; Figueiredo et al., 2008; Joy et al., 2001).

Essential oil, responsible for the aroma of *S. travancoricum* was found in the oil cavities distributed in their leaves and stem. Faridah et al. (2018) reported the occurrence of oil globules in the cavities of hypanthium in *S. aromaticum* when tested with Sudan III. Tannin represents a phenol derivative which occurs abundantly in various plant parts. However, in the present study, tannin depositions were found in the bark cells, mesophyll and cortical parenchymatous cells associated with vascular tissues suggesting a specific distribution pattern. In summary, the histochemical analysis helped to identify the region of biosynthesis and storage of metabolites at the tissue level.

5 | CONCLUSION

S. travancoricum Gamble (Myrtaceae) is an endangered endemic taxon of the Southern Western Ghats, India. The anatomical and

histochemical properties of the bark, stem, and leaf of *S. travancoricum* have been reported for the first time. *S. travancoricum* possessed distinct anatomical characteristics such as paracytic stomata, arc-shaped midrib vasculature, the continuous sclerenchymatous sheath around the midrib vascular region, single-layered adaxial palisade layer, presence of druses, and quadrangular cross-section contour of the stem, which could be coupled with additional morphological, phytochemical and molecular characteristics pertinent for species identification. The anatomical and histochemical characteristics are potential tools for the delineation of ambiguous taxa and provide substantial proof of their quality control. The results of the present study are useful for future anatomical research on other members of *Syzygium*. Moreover, this investigation can be further intensified by extensive phytochemical investigations which may be beneficial for modern drug discovery.

AUTHOR CONTRIBUTIONS

P. S. Smitha: Investigation; writing – original draft; methodology; conceptualization. **P. V. Anto:** Supervision; data curation; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Al-Edany, T., & Al-Saadi, S. (2012). Taxonomic significance of anatomical characters in some species of the family *Myrtaceae*. *American Journal of Plant Sciences*, *3*, 572–581. https://doi.org/10.4236/ajps.2012.35069

Alves, E., Tresmondi, F., & Longui, E. (2008). Análise estrutural de folhas de *Eugenia uniflora* L. (Myrtaceae) coletadas em ambientes rural e urbano, SP, Brasil. *Acta Botanica Brasilica*, *22*(1), 241–248. https://doi.org/10.1590/S0102-33062008000100023

Arruda, R., do Carmo de, O., & Victório, C. P. (2015). Leaf secretory structure and volatile compounds of Eugenia copacabanensis Kiaersk. (*Myrtaceae*). *Journal of Essential Oil Research*, *23*, 1–10. https://doi.org/10.1080/10412905.2011.9700472

Baranova, M. (1992). Principles of comparative stomatographic studies of flowering plants. *The Botanical Review*, *58*, 49–99. https://doi.org/10.1007/BF02858543

Bosabalidis, A. (2014). Idioblastic mucilage cells in *Teucrium polium* leaf, anatomy and histochemistry. *Modern Phytomorphology*, *5*, 49–52. https://doi.org/10.5281/zenodo.161003

Cardoso, P. S. L., & Sajo, M. G. (2009). Foliar anatomy of the subfamily Myrtoideae (*Myrtaceae*). *Australian Journal of Botany*, *57*(2), 148–161. https://doi.org/10.1071/BT07176

Chamorro, E., Zambón, S., Morales, W., Sequeira, A., & Velasco, G. (2012). Study of the chemical composition of essential oils by gas chromatography. In B. Salih (Ed.), *Gas chromatography in plant science, wine technology, toxicology and some specific applications* (pp. 307–324). InTech. https://doi.org/10.5772/33201

Chatri, M., Mella, C. E., & Des, M. (2020). Characteristics of leaves anatomy of some *Syzygium* (*Myrtaceae*). *Advances in Biological Sciences Research*, *10*, 19–22. https://doi.org/10.2991/absr.k.200807.005

Chattaway, M. M. (1959). The anatomy of bark. VII. Species of *Eugenia* (Sens. Lat.). *Tropical Woods*, *111*, 1–4.

Craven, L. A. (2001). Unravelling knots or plaiting rope: What are the major taxonomic strands in *Syzygium sens*. lat. (Myrtaceae) and what should be done with them? In L. G. Saw, L. S. L. Chua, & K. C. Khoo (Eds.), *Taxonomy: The cornerstone of biodiversity; proceedings IV international flora malesiana symposium* (pp. 75–85). Forest Research Institute Malaysia (FRIM). http://hdl.handle.net/102.100.100/202210?index=1

Cronquist, A. (1981). *An integrated system of classification of flowering plants*. Columbia University Press.

Cutler, D. F., Botha, T., & Stevenson, D. W. (2008). *Plant anatomy: An applied approach* (p. 312). Blackwell Publishing.

Dadswell, H. E., & Ingle, H. D. (1947). The wood anatomy of the *Myrtaceae* I. *Tropical Woods*, *90*, 1–7.

Dickison, W. C. (2000). *Integrative plant anatomy* (pp. 1–532). Academic Press.

Donato, A. M., & Morretes, B. L. (2007). Anatomia foliar de *Eugenia brasiliensis* Lam. (Myrtaceae) proveniente de áreas de restinga e de floresta. *Revista Brasileira de Farmacognosia*, *17*, 426–443. https://doi.org/10.1590/s0102-695x2009000500019

Donato, A. M., & Morretes, B. L. (2009). Anatomia foliar de *Eugenia florida* DC. (Myrtaceae). *Revista Brasileira de Farmacognosia*, *19*, 759–770. https://doi.org/10.1590/S0102-695X2009000500019

Dubey, W., & Trivedi, P. (2012). Histochemical localization of lipids, secondary metabolites and lignin in healthy and Meloidogyne incognita, infected okra (*Abelmoschus esculentus* [L.] Moench). *Indian Journal of Plant Sciences*, *1*(1), 91–100.

Fahn, A. (1988). Secretory tissues in vascular plants. *New Phytologist*, *108*, 229–257. https://doi.org/10.1111/j.1469-8137.1988.tb04159.x

Faridah, Q. Z., Al-Hakimi, A. S., Shamsul, K., Ruhaizin, F. F., & Zaidi, S. M. (2018). Anatomy and histochemistry of structures producing aroma in leaves of *Syzygium aromaticum* (L.) Merr. And *Clausena excavata* Burm. f. *Pertanika Journal of Tropical Agricultural Science*, *41*(1), 225–240.

Figueiredo, A. C., Barroso, J. G., Pedro, L. G., & Scheffer, J. J. C. (2008). Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour and Fragrance Journal*, *23*(4), 213–226.

Franceschi, V., & Nakata, P. (2005). Calcium oxalate in plants: Formation and function. *Annual Review of Plant Biology*, *56*, 41–71. https://doi.org/10.1146/annurev.arplant.56.032604.144106

Franceschi, V. R., & Horner, H. T. (1980). Calcium oxalate crystals in plants. *The Botanical Review*, *46*, 361–427. https://doi.org/10.1007/BF0286053

Gamble, J. S. (1915). *Flora of the presidency of Madras*. West, Newman and Adlard.

Gersbach, P. V., Wyllie, S., & Sarafis, V. A. (2001). New histochemical method for localization of the site of monoterpene phenol accumulation in plant secretory structures. *Annals of Botany*, *88*, 521–525. https://doi.org/10.1006/anbo.2001.1480

Govaerts, R., Sobral, M., Ashton, P., Barrie, F., Holst, B. K., Landrum, L. L., et al. (2008). *World checklist of Myrtaceae*. Royal Botanic Gardens.

Greathead, H. (2003). Plants and plant extracts for improving animal productivity. *Proceedings of the Nutrition Society*, *62*, 279–290. https://doi.org/10.1079/pns2002197

Haron, N. W., & Moore, D. M. (1996). The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (*Myrtaceae*). *Botanical Journal of the Linnean Society*, *120*, 265–277. https://doi.org/10.1111/j.1095-8339.1996.tb00776.x

Hassan, E., & El-Awadi, M. (2013). Brief review on the application of histochemical methods in different aspects of plant research. *Nature and Science*, *11*(12), 54–67.

Ingle, H. D., & Dadswell, H. E. (1953). The anatomy of the timbers of the south-West Pacific area. III. *Myrtaceae*. *Australian Journal of Botany*, *1*, 353–401. https://doi.org/10.1071/BT9530353

Joy, P. P., Thomas, J., Mathew, S., & Skaria, B. P. (2001). Medicinal Plants. In T. K. Bose, J. Kabir, P. Das, & P. P. Joy (Eds.), *Tropical Horticulture* (pp. 449–632). Naya Prokash.

Kantachot, C., Chantaranothai, P., & Thammathaworn, A. (2007). Contribution to the leaf anatomy and taxonomy of Thai *Myrtaceae*. *The Natural History Journal of Chulalongkorn University*, *7*(1), 35–45. https://archive.org/details/tropical-natural-history-102917-82461/page/42/mode/2up

Khatijah, H., Mohamad Ruzi, A. R. (2006). *Anatomical atlas of Malaysian medicinal plants Universiti Kebangsaan Malaysia Bangi*. Penerbit Universiti Kebangsaan Malaysia.

Khatijah, H. S., Cutler, D. F., & Moore, D. M. (1992). Leaf anatomical studies of *Eugenia* L. (*Myrtaceae*) species from the Malay peninsula. *Botanical Journal of the Linnean Society*, *110*, *2*(11), 137–156. https://doi.org/10.1111/j.1095-8339.1992.tb00287.x

Korth, K., Doege, S., Park, S., Goggin, F., Wang, Q., Gomez, S., Liu, G., Jia, L., & Nakata, P. (2006). *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. *Plant Physiology*, *141*, 188–195. https://doi.org/10.1104/pp.106.076737

Machado, S., Gregorio, E., & Guimaraes, E. (2006). Ovary peltate trichomes of *Zeyheria Montana* (*Bignoniaceae*): Developmental ultrastructure and secretion in relation to function. *Annals of Botany*, *97*(3), 357–369. https://doi.org/10.1093/aob/mcj042

Mercader, J., Abtosway, M., Bird, R., Bundala, M., Clarke, S., Favreau, J., et al. (2018). Morphometrics of starch granules from sub-Saharan plants and the identification of ancient starch. Federated Research Data. doi:10.20383/101.017

Merril, E. D., & Perry, L. M. (1939). The Myrtaceous genus *Syzygium* Gaertner in Borneo. *Memoirs of the American Academy of Arts and Sciences*, *18*(3), 135–202. https://www.biodiversitylibrary.org/page/8369700#page/102/mode/1up

Metcalfe, C. R., & Chalk, L. (1950). *Anatomy of the dicotyledons* (Vol. 1 & 2). Clarendon Press. https://archive.org/details/anatomyofthedico033552mbp/page/n13/mode/2up

Parnell, J. A. N., Craven, L. A., & Biffin, E. D. (2007). Matters of scale: Dealing with one of the largest genera of angiosperms.

Pike, K. M. (1956). Pollen morphology of *Myrtaceae* from the south-West Pacific area. *Australian Journal of Botany*, *4*, 13–53. https://doi.org/10.1071/BT9560013

Schmid, R. (1972). A resolution of the *Eugenia-Syzygium* controversy (*Myrtaceae*). *American Journal of Botany*, *59*, 423–436. https://doi.org/10.1002/j.1537-2197.1972.tb10113

Schmid, R. (1980). Comparative anatomy and morphology of Psiloxylon and Heteropyxis, and the subfamilial and tribal classification of Myrtaceae. *Taxon*, *29*, 559–595. https://doi.org/10.2307/1220329

Soh, W. K., & Parnell, J. (2011). Comparative leaf anatomy and phylogeny of *Syzygium* Gaertn. *Plant Systematics and Evolution*, *297*, 1–32. https://doi.org/10.1007/s00606-011-0495-2

Takhtajan, A. L. (1980). Outline of the classification of flowering plants (Magnoliophyta). *The Botanical Review*, *46*(3), 225–359. https://doi.org/10.1007/BF02861558

Wilkinson, H. P. (1979). The plant surface (mainly leaf). In C. R. Metcalfe & L. Chalk (Eds.), *Anatomy of the dicotyledons* (2nd ed., pp. 97–167). Clarendon Press.

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