DOCUMENTATION, BIOCHEMICAL ANALYSIS, BIOPROSPECTING AND MOLECULAR PHYLOGENY OF *GARCINIA* SPECIES FROM THE WESTERN GHATS OF INDIA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN BOTANY, SCHOOL OF LIFE SCIENCES
GOA UNIVERSITY

By

Ms. Rutuja Satish Palkar
Department of Botany
Goa University
Goa

NOVEMBER 2021

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DECLARATION

I, **Ms. Rutuja Satish Palkar** hereby declare that this thesis represents work which has been carried out by me and that it has not been submitted, either part or full, to any other University or Institute for the award of any research degree.

Place: **Taleigao Plateau, Goa.** **Rutuja Satish Palkar**

Date: 19-11-2021 Name of the candidate

CERTIFICATE

I hereby certify that the above Declaration of the candidate, **Ms. Rutuja Satish Palkar** is true and the work was carried out under our supervision.

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Dedicated to

Naturalists
&
My Family

ACKNOWLEDGEMENTS

This Ph.D. work is an important and memorable moment of my life journey that helped me to develop my theoretical, experimental, scientific, intellectual approaches and to transform me as an independent researcher. It would not have been possible without the support and guidance that I received over-time from many people including the experts, well-wishers, colleagues and various institutions. I am extremely privileged to express my thanks to all those who contributed in many ways to complete this study and made it a memorable experience for me.

First and foremost I am earnestly grateful to my research supervisor and mentor Prof. S. Krishnan, Head, Department of Botany, for all the valuable guidance, endless support, encouragement, patience and enthusiasm that inspired me to accomplish entire work with perseverance and confidence.

I am warmly thankful to Prof. M. K. Janarthanam (Co-guide, Former Head & Dean) for his valuable suggestions, guidance and support during the study.

I would like to gratefully acknowledge the Vice-Chancellor for providing necessary infrastructure to carry out my research work. I am sincerely thankful to VC's nominee, Prof. Sanjeev Ghadi (Head, Dept. of Biotechnology) and Prof. B. F. Rodrigues (Former Head, Dept. of Botany & Present Dean) for time to time evaluation of my work and their expert opinions and perspectives which were helpful to improve the work.

I am grateful to the faculty members of Department of Botany, Goa University Prof. D. J. Bhat (Rtd.) (Former Head & Dean), Prof. P. K. Sharma (Former Head & Dean), Prof. Vijaya Kerkar (Former Head), Dr. Nandkumar Kamat (Rtd.) (Assistant Professor) and Dr. Rupali Bhandari (Assistant Professor) for their guidance and support during course of my research work.

My thanks to all the non-teaching staff members of the Department Mr. Vithal Naik, Mrs. Nutan Chari, Mr. V. Gaonkar, Mr. Gajanan Tari, Mr. Dilip Agapurkar,

Ms. Teja, Mr. Samrat Gaonkar, Ms. Baby Sahara Abdul and all administrative staff of Goa University for their continuous help and co-operation throughout the study.

I am thankful to the authorities and staff of various herbaria and libraries such as Auroville herbarium, Villupuram (AURO); Western Circle Herbarium, Pune (BSI); Calicut University, Calicut (CALI); Foundation of Revitalization of Local Health Traditions, Bengaluru (FRLH); Goa University, Goa; Southern Circle Herbarium, Coimbatore (MH); Shivaji University, Kolhapur (SUK) and Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Thiruvananthapuram (TBGT) for providing permission to consult the herbarium specimens and literature available in various herbaria. I extend my thanks to authorities of BSI and Royal Botanic Garden, Edinburgh (E) for permission to reproduce type images for the publication.

I would like to express my sincere thanks to authorities and staff of the various institutions for their help during the research work namely District Agricultural Farm, Thaliparamba, Kannur; ICAR-Indian Institute of Spices Research, Calicut (IISR); Regional Fruit Research Station, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Vengurla; Krishi Vigyan Kendra, Lanja, Ratnagiri; Zonal Agriculture Office-Tiswadi, Margao and Canacona; ICAR- NBPGR Regional station, Thrissur; Lalbagh Botanical Garden, Bengaluru; Ramnarain Ruia College, Mumbai; Anti-cancer Drug Screening Facility, Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai; Regional Facility for DNA Fingerprinting, Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram.

My sincere gratitude to Dr. A. G. Pandurangan (Rtd.) (Former Director), Dr. R. PrakashKumar (Director), Dr. N. Mohanan (Rtd.) (Senior Principal Scientist), Dr. T. Sabu (Technical Officer), Dr. P. S. Shameer, Ms. Angala Mathew (Research Scholar), Mr. R. Ananthakrishnan (Research Scholar), Mr. Vinu Krishnan (Library staff) from Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Thiruvananthapuram (JNTBGRI), Thiruvananthapuram; Prof. M. Sabu (Rtd.) (CSIR-Emeritus Scientist), Dr. A. K. Pradeep (Assistant Professor), Dr. P. E. Shreejith from Calicut University, Calicut; Dr. N. Parthasarathy (Professor) and his team from Pondicherry University, Puducherry; Dr. Palaniswamy (Principal Scientist), Dr. K. A.

Sujana (Scientist D), Dr. R. Manikandan (Scientist E), Smt. R. Mehala Devi (Botanical Assistant), Mr. Prasad from MH, Coimbatore; Dr. D. Narasimhan (Rtd.) (Associate Professor & Former Head) from Madras Christian College, Tambaram, Chennai; Dr. K. Ravikumar (Rtd.) (Professor) from FRLHT, Bengaluru; Mr. Muhammed Nissar (Scientist), Mr. Ramesh (Librarian) from IISR, Calicut; Mr. Jivaraj (Superitendent), Mr. Narayanan Nambiar (Technical assistant) from Dist. Agri. Farm, Thaliparamba; Dr. Ajay Munj (Junior Entomologist) from Regional Fruit Research Station, Vengurla; Dr. A. Benniamin (Scientist E and Head), Dr. Jayanthi, Dr. Rashmi Dubey (Scientist E), Mr. Sameer Patil from BSI, Pune; Prof. S. R. Yadav (INSA Senior Scientist), Dr. M. M. Lekhak (Assistant Professor), Mr. Sandip Gavade (Research Scholar), Ms. Vaishali Patil (Research Scholar), Dr. Avinash Gholave, Dr. Avinash Adsul from Shivaji University, Kolhapur; Dr. W. Gastmans (Herbarium Keeper) from AURO, Villupuram; Dr. P. Ravichandran (Professor and Head), Dr. Pandian (Post Doctoral Fellow); Mr. M. Arun Kumar (M.Phil. student); Mr. Evitex (Research Scholar) from Manonmaniam Sundaranar University, Tirunelveli; Dr. Manimekalai (Assistant Professor and Head) from Sri Parasakthi College for Women, Courtallam; Dr. V. Arunachalam (Principal Scientist) from ICAR-Central Coastal Agricultural Research Institute, Goa; Dr. Deepal Ghadge from Ramnarain Ruia College, Mumbai; Dr. Praveen Karanth (Professor) from IISC, Bengaluru; Mr. U. Suresh Kumar (Technical Assistant) from RGCB, Thiruvananthapuram; Dr. M. Latha (Principal Scientist) from ICAR-NBPGR Regional station, Thrissur; Mr. Vishal Ahale (Product Specialist) from Spinco Biotech, Navi Mumbai for the help extended during the research work.

I am immensely grateful to Mr. V. Ganesan (Rtd.) (Principal Chief Conservator of Forest); Mr. V. Saktivel (Range Forest Officer) and their team from Pollachi Division, Tamil Nadu Forest Department for their precious help and accompanying during field trip to Anamalai Tiger Reserve. Also, I would like to thanks Mr. Sandeep (Range Forest Officer), Mr. Gandhiraj & Mr. Palraj (Forest Guards), Mr. Vijayan from Eravikulam National Park, Munnar.

I would like to acknowledge Dr. S. G. Tilve (Rtd.) (Senior Professor), Ms. Lima (Research Scholar) from Department of Chemistry; Dr. S. K. Shyama (Rtd.) (Professor) from Department of Zoology for their help during the study.

I am thankful to Kshitija (sister), Shrikrishna & Jaydeep (brothers) and Mr. Hrishikesh for accompanying with me for local field trips. I owe my deep gratitude to local people from the states of Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu and union territory of Puducherry for their valuable help during field trips.

I am indebted to my colleagues and friends from Goa University for their help and support during the research work: Dr. Ravikiran Pagare, Dr. Anup Deshpande, Dr. Mayur Nandikar, Dr. Sharad Kambale, Dr. Govind Parab, Dr. Surya Nandan Meena, Dr. Maria Vera Da Costa, Dr. Nisha Kevat, Dr. Rosy D'Souza, Dr. Sidhesh Naik, Dr. M. Baskaran, Dr. Bharat Patil, Dr. Cassie Rodrigues, Dr. Kim Rodrigues, Dr. James D'Souza, Ms. Flozil Dias, Ms. R. Archana, Ms. Maria Cineola Fernandes, Ms. Nikita Verenkar, Ms. Aditi Naik, Ms. Prabha Pillai, Ms. Rutuja Kolte, Mr. Shivaprakash Nedle, Mrs. Pallavi Konge-Randive, Ms. Sankrita Gaonkar, Mr. Kiran Gaude, Mr. Dhillan Velip, Ms. Sheela Pal, Ms. Tanvi Prabhu and Ms. Apurva Shet. I also extend my thanks to all the M.Sc. dissertation students worked in laboratory during 2013-2020 for their help.

It would not have been possible for me to undertake the strenuous research work without the financial support. Hence, I would like to acknowledge the financial assistance provided by the Council of Scientific & Industrial Research (CSIR), New Delhi [38(1348)/12/ EMR-II dated 16/10/2012] (2013-2016) and [38(1471)/18/EMR-II dated 04/04/2018] (2018 to till date) under the research project sanctioned to Prof. S. Krishnan and Goa University Research Studentship (2016-2018).

At the end, I owe my gratitude and love to my late grandmother (Smt. Shailaja), Shri. Eknath (grandfather), Shri. Satish (father) and Smt. Suvarna (mother) for their continued and unconditional support in my life. My special thanks to my sister (Kshitija), Shrikrishna & Anurag (brothers) for their co-operation, moral support and for keeping my spirits high always. I would like to thank my uncle (Shri. Ravindra), all relatives and friends for their support and encouragement during the course of my work.

Rutuja Satish Palkar

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Abbreviations

AAS : atomic absorption spectroscopy

ABTS : 2,2'- azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)

AUC : area under ROC curve

BSA : bovine serum albumin

DAF : number of days after fertilisation

DMSO : dimethyl sulfoxide

DNA : deoxyribonucleic acid

dNTPs : deoxynucleotide triphosphates

DPPH : 2,2- diphenyl-1-picrylhydrazyl

EDTA : ethylenediaminetetraacetic acid

EI-MS : electron ionization mass spectrometry

EI/ESI-MS : electron impact electrospray ionization mass spectrometry

ESIMS : electrospray ionization mass spectrometry

Eng. : English

ENM : ecological niche modeling

FID : flame ionization detector

FRAP: ferric reducing antioxidant power/ potential

FTIR : fourier transform infrared spectrometry

FTMS : fourier transform mass spectrometry

FTC : ferric thiocyanate

GBSSI : granule-bound starch synthase I

GC-MS : gas chromatography mass spectrometry

GC/TEA : gas chromatography thermal energy analyser

GI_{50} : growth inhibitory 50%

GLC : gas liquid chromatography

g : gram

GPS : global positioning system

HCA : hydroxycitric acid

HPLC : high performance liquid chromatography

HPLC-PDA : high performance liquid chromatography photodiode-array detection

hr/hrs : hour/ hours

HRESI-MS : high resolution electrospray ionization mass spectrometry

HREMS : high resolution electrospray mass spectrometry

HR-EI-MS : high-resolution electron ionization mass spectrometry

HRESI-ToF-MS : high resolution electrospray ionization time-of-flight mass spectrometry

IC_{50} : the half maximal inhibitory concentration

I.D. : internal diameter

IR : infrared spectrometry

ISSR : inter simple sequence repeat

ITS : internal transcribed spacer

IUCN : international union for conservation of nature

Kan. : kannada

km^2 : square kilometre

Kon. : konkani

kV : kilovolt

LC_{50} : 50% cell kill

LC/ESI-MS/MS : liquid chromatography - electrospray ionization - mass spectrometry

LC-MS/MS : liquid chromatography with tandem mass spectrometry

LC-ToF-MS : liquid chromatography time-of-flight mass spectrometry

LD_{50} : lethal dosage 50

L. S. : longitudinal section

LSC : liquid-solid chromatography

*mat*K : maturase K

Mar. : marathi

Mal. : malyalam

ML : maximum likelihood

m/z : mass to charge ratio

m : metre

µg : microgram

µl : microliter

µm : micron/ micrometre

µM : micromolar

mg : milligram

ml : millilitre

mm : millimetre

mM : millimolar

min/mins : minute/ minutes

M : molar

MS : mass spectrometry

NE : no-evaluable data

ng : nanogram

nm : nanometre

NCBI : National Center for Biotechnology Information

NIST : National Institute of Standards and Technology

NMR : nuclear magnetic resonance

ORAC : oxygen radical absorbance capacity

PCR : polymerase chain reaction

pM : picomolar

Port. : portuguese

ppm : parts per million

PTLC : preparative thin layer chromatography

RAPD : random amplified polymorphic DNA

R. T. : retention time

*rbc*L : ribulose-bisphosphate carboxylase

rcf : relative centrifugal force

Rf value : retention factor

ROC curve : receiver operating characteristic curve

RPMI : Roswell Park Memorial Institute

SDM : species distribution model

SDS : sodium dodecyl sulphate

sec : second

SRB : sulforhodamine-B

SWG : southern Western Ghats

Tam. : tamil

TBARS : thiobarbituric acid reactive substance assay

TCA : trichloroacetic acid

TGI : total growth inhibition

TIC : total ion chromatogram

TLC : thin layer chromatography

T. S. : transverse section

UPLC-QTOF-MS : ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry

UPLC/PDA/MS : ultra-pressure liquid chromatography with photodiode array and mass spectrometry

UV : ultraviolet

v/v : volume/ volume

WHO : world health organization

w/v : weight/ volume

Chapter 1

Introduction

India is known for its traditional medicinal systems such as Ayurveda, Siddha and Unani. The Ayurvedic practice is around 3000 years old and it is becoming popular with a long history of treating diseases (Hankey, 2001; Subhose *et al*., 2005). Plant-based formulations play a vital role in the Ayurvedic healing process. According to World Health Organization (WHO) estimated data, more than 80% of the world population from developing countries for their basic health care are dependent on herbal medicine (Vines, 2004). India possess vast repository of medicinal plants useful in traditional medical treatments and it has become an ancient practice. About 70% of rural population is depends on the traditional Ayurvedic system of medicine in India. However, approximately 40% of people are using herbal medicine in the Western countries to cure various diseases (Ballabh & Chaurasia, 2007; Pandey *et al*., 2013). In India around 20,000 medicinal plants have been recorded for their uses in different traditional medicinal systems among them only 1200 to 1500 plants have been incorporated into the official Ayurvedic pharmacopeia (Pandey *et al*., 2008; Manohar, 2012). As a dietary supplements or ethnic traditional medicines more than 1500 herbals are sold (Patwardhan *et al*., 2005). Despite the fact that plants are used as potential medicine their safety depends on method of administration and specific disease conditions. Hence, an adequate awareness of plants w.r.t. its nomenclature, identity, properties and applications is needed so that it can be incorporated into the Ayurvedic pharmacopeia (Manohar, 2012; Kumar *et al*., 2017).

India is one of the mega biodiversity centres possessing 8% of the global biodiversity in 2.4% land. Worldwide 32 ecologically sensitive areas (hot spots) are present, among them two hotspots such as the Western Ghats and Eastern Himalayas are present in India (Myers, 1988). The Arabian sea to the western side, Vindhya & Satpura mountain ranges to the northern side and the semi-arid Deccan plateau to the eastern side confines the Western Ghats which includes the states of Gujarat,

Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala. The forest of the Western Ghats represents finest non-equatorial tropical evergreen forests in the world (Pascal, 1988; 1991). It covers only 5% total land area of the country, but occupies around 27% total plant species of the country (MacKinnon & MacKinn, 1986; Daniel, 1997). It is unique biogeographic area and centre of high endemism in India due to varied tropical and subtropical climates, altitudinal ranges and many edaphic conditions (MacKinnon & MacKinn, 1986; Pushpangadan *et al*., 1994). The Western Ghats forests harbour large number of endemic medicinal plants which are useful for traditional and folk medicinal practices (Joy *et al*., 2001). But, only 3-5% higher plant species from the region have been fully documented, prospected and thoroughly investigated (Gilani, 2005). Hence, there is a need for an intensive research on medicinal plants from the region.

The genus *Garcinia* L. belongs to the family Clusiaceae (earlier name: Guttiferae), order Malpighiales of angiosperms. Clusiaceae is one of the economically and medicinally important family due to its edible fruits, gums, resins and timber. The genus *Garcinia* L. includes about 260 species worldwide predominantly distributed in tropics of the Old World especially in Asia and Africa (Mabberley, 2017). In India, the genus is represented by 41 species with 7 varieties, of which 17 species with 5 varieties are endemic (Anderson, 1874; Maheshwari, 1964; Singh, 1993; Srivastava, 1994; Pandey & Diwakar, 2008; Sabu *et al*., 2013; Nayar *et al*., 2014; Sarma *et al*., 2016; Shameer *et al*., 2017).

The genus *Garcinia* L. named in honour of the French-Swiss physician and naturalist Laurent Garcin (1681-1751) by Carl Linnaeus. Laurent Garcin was a Dutch East India company ship's surgeon. Along with medical duties he made observations on plants of the places and collected plant materials for European gardens and herbaria during his voyages to Asia. He was an honorary member of the Royal Society of London and corresponding member of the Paris Academy of Sciences due to his contributions in many scientific subjects especially tropical Asian flora (Briquet, 1940; Maheshwari, 1964; Cook, 2016).

The genus *Garcinia* is found as evergreen trees or shrubs in tropical evergreen and semi-evergreen forests. They prefer wet or low rainfall areas and altitude below

1000 m some species exceptions up to 1830 m. They are the slow growing trees and mode of dispersal is through arboreal mammals. Trees usually found as symmetrical, straight-stemmed, glabrous and medium-sized with horizontal branches. Wood found as moderately hard or hard, yellowish-white, red or grey; exudate yellow, sometimes white, thick, sticky, resinous mostly from branches, fruits and bark; leaves opposite, simple, entire, glabrous, coriaceous, lanceolate or oblong with prominent midrib; flowers small, solitary or fascicled, polygamo-dioecious or pseudo-hermaphrodite, female flowers larger than male flowers, flowers beautiful with yellow, pinkish-white, greenish-white, red colour, flowers of some wild species nocturnal; fruit with coriaceous rind, whitish, juicy pulp (aril), 1-12 seeds (Anderson, 1874; Maheshwari, 1964; Singh, 1993).

Garcinia indica is well-known and widely used species in the Konkan region of the Western Ghats. Ripe fruits are edible, anthelmintic, anti-oxidant, cardiotonic. Also, fruits are helpful to relieve gastric problems like indigestion, acidity, flatulence, constipation etc. HCA a bio-active compound from fruits lowers cholesterol level and curbs lipogenesis leads to weight loss. Dried fruit rind of the species sold in different markets under the name Kokum/ Amsul an Indian spice with dark purple to black colour when added to food gives pink to purple colour and sweet-sour taste; used in many parts of country as garnish to give acid flavour in vegetarian and non-vegetarian curries. Also, it is used for preparation of solkadhi which is spicy, sweet and sour drink. It is used as cooling syrups and possesses anticancer and antiulcer properties. From fruit rinds dyes has been extracted and evaluated for biological & textile staining. An acidic juice from the fruits is used for melting iron by blacksmiths. It is also used as mordant. Fruit juice is effective as coolant against dehydration and sunstroke also brings down fever and allergic reactions. As compared to other commercial bottled drinks, the fruit juice is more healthier and refreshing option. It acts as appetite stimulant, anti-helmintic; in Ayurvedic medicine Kokum extracts were used to treat infections, piles, dysentery, strengthen cardio-vascular system and to stabilize liver function. Seeds yield edible oil known as Kokum butter is used as an adulterant of ghee and in confectionary. It is also useful in soap and candle manufacturing; in cosmetic (especially to prepare face cream, sun screen lotion and lipsticks) and pharmaceutical industry as an ointment, emollient, moisturizer on dry, burnt, sensitive, chapped and wound infected areas of skin and fissures of lips. It is

used as remedy in dysentery and scrofulous diseases. Root bark, fruit and seed oil proved to be cure for abdominal disorders, piles, mouth diseases, cardiac diseases and worm infestation. Wood is useful in paper pulp industry. Some of the commercial products from the species available in markets are Kokum rind powder; dehydrated salted rind (Kokum, Amsul); Kokum sugar syrup (Amrit Kokum); salted juice (Kokum agal); sarbat mixture; solkadhi mixture; seed extract (Kokamtel); Kokum squash; wine; Kokum soda; Kokum juice; soft drinks; honey; jams and red coloured pigment anthocyanin as food colourant replacement for synthetic colours & in cosmetics. Due to all these properties and applications, *Garcinia* products have been traded at local, national & global levels and exported from India at large scale in recent years. Also, several patents has been granted related to HCA; methods for producing HCA salts; various processes and formulations in food industry and in confectionary; several other products and their bioactivities (Anderson, 1874; Maheshwari, 1964; Singh, 1993; Ravikumar & Ved, 2000; LathanKumar *et al*., 2003; Kamat, 2005; Thakor *et al*., 2012; Braganza *et al*., 2012; Korikanthimath & Desai, 2012; Nayak & Rastogi, 2012; Patil, 2012; Verenkar & Krishnan, 2017).

G. gummi-gutta tree yields yellow, insoluble gum known as Gamboge or gummi-gutt can be used as pigment in miniature-paintings and water colours, good varnish and as a purgative. Fruits of the species can be eaten raw or pickled, acidic fruit juice used as condiment, coolant and helps to reduce body weight. Dried fruit rinds are used as condiment for garnishing and flavouring curries in Malabar region; for gold and silver polishing, for rubber latex coagulation; in Ayurveda for ailments of rheumatism, rickets, uterine complaints, enlargement of spleen and animal disorders. Seed oil is useful in medicine. Wood of the species is suitable for preparation of posts, match boxes and splints. Leaves and fruits are sour, thermogenic, astringent and useful in prevention of *vata*, *kapha*, haemorrhoids, dysentery and hyperdipsia. Leaves, fruits and seed oil are reported to be purgative, emetic and curing ulcers, bleeding piles, indigestion, inflammation and diarrhoea. Large scale export of *G. gummi-gutta* extract and fruit rinds were recorded from India in recent years. ***G. mangostana*** is considered one of the most delicious fruit among tropical fruits; it is used as dessert and preserve. Fruit rind of the species is useful in preparation of juice, jellies and in tanning and dying purposes; useful in treatment of skin diseases, chronic diarrhoea, dysentery, cystitis and gonorrhoea. Tea from young leaves and bark of the tree helps

to reduce fever and urinary disorders. Young leaves, bark and rind can be used as gargle for sore mouth. Wood is suitable for building purposes, cabinet work and spear handles. ***G. hombroniana*** fruits are edible and acidic in nature; roots and leaves are useful against itch; timber can be used for preparation of oars and house construction. The pigment exudes from ***G. morella*** tree is indigenous source of yellowish orange coloured gamboge but it is not yet explored at commercial scale; it is useful in preparation of water colours, dyeing silken fabrics, golden yellow ink and golden-coloured spirit varnishes for metals. It has astringent, anthelmintic, aphrodisiac, amenorrhoea, cathartic, abortifacient and antibacterial properties. It is helpful to lower the blood pressure quickly in ulcer and in cerebral congestion. Also, it relieves inflammation with burning sensation during *pitta* and *kapha*. It is used for treating leprosy, blisters and other skin infections. Fruit rind used for tanning. Stem is helpful for local application on pimples and boils. Seeds are used to relieve catarrh affections of the throat, pulmonary mucous membrane, cough and asthma. Seed oil or butter can be used in candle making, medicine, confectionary and cooking as a substitute for ghee; preventive in ulcer, skin diseases and herpetic eruptions. Wood is useful in cabinet work. The fruits of ***G. cowa*** are edible and useful for making jams and preserves. In Assam cooked young leaves of the species are eaten as a vegetable and dried fruit rinds are used for treating dysentery. Tree yields inferior gamboge resin which is useful in preparation of yellow varnish for metallic surfaces. Acidic fruits of ***G. xanthochymus*** are useful for making sherbets, jams, medicaments and in curries as substitute for tamarind. In bilious condition, sherbet prepared from dried fruit is used as medicine. Leaves are used in treatment of tongue diseases. Gamboge or gum resins from fruit, bark and stem are useful to make water colour and dyes. ***G. spicata*** plant wood is strong timber for construction. Fruit pulp gives chrome-yellow pigment. Bark contains colouring matter called as Fukuji works as mordant. Fruits of the ***G. livingstonei*** are edible and fermented beverages are prepared from fruit pericarp and pulp. ***G. travancorica*** trees yields yellow gamboge. Stem bark and fruits are used as medicine by Kani tribal people in southern Western Ghats. Seed oil from ***G. rubro-echinata*** seeds useful in soap-making and stearine preparation for candle making. Leaves and bark used as vermifuge and for treating dropsical affections. Also ***G. wightii*** tree produces gamboge which is soluble and yields good pigments (Anderson, 1874; Maheshwari, 1964; Singh, 1993; Ravikumar & Ved, 2000; LathanKumar *et al*., 2003; Mahapatra & Panda, 2009; Warrier *et al*., 2010; Patil, 2012).

Along with traditional uses *Garcinia* is one of the medicinally and economically important genera due to the presence of two major bioactive compounds namely hydroxycitric acid (HCA) and garcinol. HCA and its derivatives are ascertained to be potent metabolic regulators of obesity and lipid abnormalities which inhibits ATP-citrate lyase an important enzyme in Kreb's cycle involved in conversion of carbohydrates into fats (Ishihara *et al*., 2000; Jayaprakasha & Sakariah, 2000; Jena *et al*., 2002). As HCA have been extensively studied for its regulatory effect on lipogenesis, fatty acid synthesis, appetite & weight loss; proved to be safe and efficient molecule which could be used as an intervention to overcome hypercholesterolemia, obesity, diabetes, inflammation, insulin resistance and other oxidative stress associated disorders (Evans & Scholz, 1977; Sullivan *et al*., 1984; Majeed & Rajendran, 1998; Hastings & Barnes, 1999; Ohia *et al*., 2002; Asgar *et al*., 2007). HCA and its derivatives have been used in anti-obesity, cardio protection, weight loss formulations in pharmaceutical industries (Yamada *et al*., 2007).

Garcinol is proved as a multi-therapeutic agent due to its anti-oxidant, anti-inflammatory, anti-microbial and anti-cancer properties (Nayak *et al*., 2010; Hemshekhar *et al*., 2011). Due to efficient inhibition of nitric oxide (NO) accumulation (Liao *et al*., 2005) and oxidative protein damage (Kolodziejczyk *et al*., 2009) garcinol proved to be a good antioxidant agent. Also it has activity against bacteria such as *Helicobacter pylori* (Chatterjee *et al*., 2005); methicillin-resistant *Staphylococcus aureus* (Rukachaisirikul *et al*., 2005). It showed anticancer property by induction of apoptosis (Matsumoto *et al*., 2003; Balasubramanyam *et al*., 2004); suppression of oxygen free radical ($O_2 \cdot^-$), NO radical generation and expression of iNOS, COX-2 proteins (Tanaka *et al*., 2000) and cell growth inhibition (Ito *et al*., 2003).

The angiosperm phylogeny group (APG) classification is usually used to establish phylogenetic relationships among plant species but it cannot distinguish species level evolutionary relationship which leads to low resolution of evolutionary trees (Kembel & Hubbell, 2006; Letcher, 2010). Hence, DNA barcoding is helpful to solve this task in which standard DNA fragments are prepared to construct evolutionary relationships of different plant species within same community (Erickson *et al*., 2014). DNA barcoding is a useful tool developing rapidly in recent

years for phylogenetic construction, species identification, population genetics and biodiversity investigation (Hebert *et al*., 2003; Hajibabaei *et al*., 2007; Hollingsworth *et al*., 2011; Pei *et al*., 2017). For DNA barcoding widely used regions are nuclear (ITS), chloroplast (*rbc*L, *trn*L-F, *mat*K, *psb*A, *trn*H, *psb*K) and mitochondrial (*COI*) (Purty & Chatterjee, 2016).

1.1 Objectives of the Present Investigation

Based on available literature it has been found that there is ambiguity in exact number of *Garcinia* species present in the Western Ghats and their correct taxonomic identification. Presently many companies are making health tablets out of HCA and garcinol being considered as potential anti-cancer compound, it is important to estimate the quantities of both the compounds from different species of the genus. Besides these compounds, there is need to explore other phytochemical compounds and also to evaluate their possible bioactivities. Hence, comparative biochemical analysis and bioprospecting for all the Western Ghats species is proposed to get their maximum benefits. Molecular phylogenetic studies are essential to understand the inter relationship among the species and solve uncertainties in species evolution and evolving conservation strategies.

In view of the above gaps, the following specific objectives are proposed:

1. Field survey, documentation and taxonomic characterisation of *Garcinia* species from the Western Ghats of India.

2. Quantitative estimation of bio-active compounds garcinol and hydroxycitric acid (HCA) during various developmental stages of fruits of *Garcinia indica* and matured fruits of other *Garcinia* species using HPLC system.

3. Phytochemical profiling of fruits of selected *Garcinia* species from the Western Ghats using GC-MS.

4. Evaluation of anti-oxidant and anti-cancer (cell line screening) activities of fruit extracts of selected *Garcinia* species.

5. Molecular phylogeny of *Garcinia* species from the Western Ghats using nuclear and chloroplast genes.

Chapter 2

Review of Literature

2.1 Genus *Garcinia* and *Garcinia indica*

The genus *Garcinia* L. belongs to the family Clusiaceae is found as evergreen tree and shrub. It represents about 260 species worldwide which are mainly distributed in Asian and African countries of the Old World (Mabberley, 2017). It is one of the medicinally and economically important genera due to the presence of two major bioactive compounds namely hydroxycitric acid (HCA) and garcinol. Some of the *Garcinia* species are endemic to India with high medicinal and economic values. *Garcinia indica* is one such most important species in the genus (Padhye *et al*., 2009; Koppad & Shivanna, 2010; Baliga *et al*., 2011; Ramachandran, 2014). It occurs particularly in the Konkan regions of Maharashtra, Goa, Karnataka and some extent to Kerala in natural populations as well as in the cultivated form and popularly known as Kokum in these regions. Kokum is now recognized as a medicinal plant by National Medicinal Plant Board, Ministry of health and family Welfare, Government of India and a unique food by Spice Board, Government of India. Along with Kokum, studies on other *Garcinia* species which are endemic to India and especially to the Western Ghats needs to be carried out thoroughly before it is claimed by any other countries to get their benefits (Braganza *et al*., 2012).

2.1.1 Taxonomic studies, documentation and identification of the genus *Garcinia*

The taxonomic work of *Garcinia* was carried out by Hooker (1875), Engler (1925), Robson (1961), Whitmore (1973) and Bamps (1978) in different parts of the world. Recently taxonomic revision of *Garcinia* in Australia was carried out with four new species additions from tropical Queensland (Cooper, 2013).

First report on Indian *Garcinia* was carried out by Anderson (1874) and described 30 species from the region. The pioneering work on enumeration of wild types of Indian *Garcinia* producing gamboge was carried out by Maheshwari (1964).

According to the study 35 species were documented in India among them 31 species are found to be wild species. All species are described in detail and key to the species is presented in the study. Another important taxonomic study on the genus *Garcinia* under the family Clusiaceae was carried out by Singh (1993). The study revealed the presence of 17, 7, 6 and 4 endemic species respectively from India, Western Ghats, Andaman & Nicobar Islands and north-east India. The study reported presence of 35 species in India and 11 species in the Western Ghats. All the species are described in detail including key to species. Gunaga *et al*. (2010) reported 10 species of *Garcinia* from Western Ghats of India. Nayar *et al*. (2014) listed 12 species of *Garcinia* from Western Ghats.

Another important taxonomic revisionary work carried out by Shameer *et al*. (2016) revealed 9 species, among them 7 species and 2 varieties are endemic to the Western Ghats. In this study, morphological features, distribution and conservation aspects for *Garcinia* species of the region were discussed. In the revisionary work, two new *Garcinia* species namely *G. pushpangadaniana* and *G. gamblei* from southern Western Ghats were described and illustrated which are endemic to the region. Former species shows close resemblance with *G. xanthochymus* however, both species are different and their morphological differences are discussed in the publication (Sabu *et al*., 2013). Another species *G. gamblei* which is allied to *G. pushpangadaniana* found as new species with distinguishing morphological characters (Shameer *et al*., 2017). Another new species namely *G. assamica* from Assam, north-east India was described under the same revisionary work (Sarma *et al*., 2016).

The comparative vegetative anatomical study of 10 *Garcinia* species from Sri Lanka was carried out using leaf lamina, petiole, stem and wood (Pathirana & Herat, 2004). Another systematic study of genus *Garcinia* from Sri Lanka was carried out to investigate the relationships among the species and to construct an identification key based on the vegetative morphological characters (Nimanthika & Kaththriarachchi, 2010). A study on morphology and ethnobotany of six *Garcinia* species from the Brahmaputra Valley of Assam was carried out (Baruah & Borthakur, 2012).

2.1.2 Ecological niche modeling

For ecological niche modeling study several species distribution models (SDMs) are available. These are the numerical tools which combines observations of species occurrence or abundance with environmental estimates. These are widely used to gain ecological and evolutionary perceptions and to predict distributions (Elith & Leathwick, 2009). The species distribution modeling was used to estimate the probability of occurrence of species with climate data based on presence-only (or absence) data for many plant species (Ortega-Andrare *et al*., 2015; Thriveni *et al*., 2015). Ethnobotany, management and niche modeling of nine *Garcinia* species from southern China namely *G. bracteata*, *G. cowa*, *G. esculenta*, *G. multiflora*, *G. oblongifolia*, *G. paucinervis*, *G. pedunculata*, *G. xanthochymus* and *G. yunnanensis* were studied to understand their use as edible fruit, medicine, wood, ornamental plants, extracting oil and sustainable utilization & species distribution (Liu *et al*., 2017).

2.2 Quantification of bio-active compounds hydroxycitric acid (HCA), garcinol and other compounds

Hydroxycitric acid (HCA) is the major acid of fruits of *G. indica*, *G. cambogia*, *G. mangostana* and *G. atroviridis*. The biochemical and medicinal studies of HCA revealed that it is potential anti-obesity agent. It curbs appetite without negatively affecting nervous system unlike other fat reducing drugs. It lowers the production of cholesterol, improving the energy level and also cardio-protective in nature (Jena *et al*., 2002; Padhye *et al*., 2009). Another important compound present in *Garcinia* species is garcinol. It is polyisoprenylated benzophenone derivative which is an anticancer agent (Pan *et al*., 2001). In case of garcinol several researchers have evaluated its biological activities. Garcinol chelates iron, scavenges DPPH, superoxide anion, hydroxyl radical and suppress protein glycation. Thus, garcinol is beneficial as a potent antioxidant and a glycation inhibitor (Yamaguchi *et al*., 2000). Also garcinol shows the biological activities such as anti-inflammatory, anti-HIV and gastro-protective. Due to presence of HCA, garcinol, several acids, phenols and other phytochemicals *Garcinia* genus has pharmaceutical importance. However, their biological properties are only beginning to be elucidated and at present many

companies are making health tablets out of these compounds. Considering the above importance of HCA and garcinol it is needed to estimate the quantities of both the compounds from different species of the genus.

A review on plant profile, phytochemistry and pharmacology of *Garcinia indica* (Kokum) revealed nutraceutical properties of the plant due to presence of several compounds along with two major active compounds such as garcinol and hydroxycitric acid (Ramachandran, 2014). Hydroxycitric acid is also called as garcinia acid and it has hydroxyl groups at the second and third carbon atom. Since, it has two asymmetric carbons and thus has two pairs of diastereoisomers. The two lactones for HCA are (-)-hydroxy citric acid lactone and (+)-allohydroxy citric acid lactone. HCA is commercially available in markets in salt forms such as calcium, sodium and potassium due to unstable nature in free acid form. HCA demand is increased since it is effective against weight management. HCA is present in two forms as free acid and lactone. HCA free acid form is biologically active but highly unstable hence to attain higher stability it is usually converted to its less active lactone form by combining with various counter ions (Louter-Van De Haar *et al*., 2005).

A report on quantification of organic acids from *G. cambogia* showed presence of HCA with high concentration (16-18%) however, other acids such as citric and malic acids in minor quantities (Jayaprakasha & Sakariah, 1998). An additional study on determination of HCA in commercial samples of *G. cambogia* was performed by using HPLC showed HCA as a major organic acid (51-55%) (Jayaprakasha & Sakariah, 2000).

For domestication purpose, identification of superior genotypes with desired characters is necessary. Hence, it is needed to investigate the species physico-chemical characters in available germplasm which will be having effect on productivity. Such kind of report is available for the species *G. indica* which indicated correlation between fruit morpho-types and chemical parameters. The study showed highest amount of HCA present in green and red fruit morpho-types however, all the morpho-types indicated significant variation for different traits such as moisture content, anthocyanin content, fatty acid content, total sugar content, total acidity etc. (Krishna, 2014).

The study on chemical constituents of Kokum (*G. indica*) ripe fruit rind showed presence of 22.8% HCA and 2.4% mixture of two anthocyanins as a pigment suggested that red and yellow dyes can be prepared for foodstuffs using fruit rind (Krishnamurthy *et al*., 1982). Extraction of HCA from dried fruit rinds of the species *G. oblongifolia* was carried out using microwave and quantified using HPLC. The chromatographic conditions used were phosphoric acid as mobile phase, UV detector wavelength at 210 nm. From 100 g of dried rind extract, the amount of HCA found was 10.137 g (Vinh *et al*., 2011).

With increasing market demand for commercial products of *G. cambogia* as anti-obesity drugs it can be adulterated with similar lower priced products from other species. Hence, HPLC and spectrometric methods were carried out to identify and quantify adulteration in commercial products of the species. No adulteration was found in the samples and to detect adulteration HPLC was used as precise analytical technique (Jamila *et al*., 2016).

Garcinol, is polyisoprenylated benzophenone derivative and potent bioactive compound. It contains phenolic hydroxyl groups and also called as camboginol which is a triisoprenylated chalcone. The melting point of garcinol is 122°C and molecular weight is 602 dalton. Garcinol is fat soluble compound with yellow colour found in Kokum rinds at 2-3% level (Mishra *et al*., 2006; Ramachandran, 2014). It resembles to the structure of curcumin since it contains both phenolic hydroxyl groups and diketone moiety (Pan *et al*., 2001).

Isolation of two new molecules polyisoprenylated benzophenone & acylphloroglucinol derivative and two known molecules namely garcinol & isogarcinol was done by large scale extraction from the fruits of *G. indica* using chromatography (Kaur *et al*., 2012). Among all four compounds, garcinol was isolated as a major compound. In another study along with several guttiferones, garcinol was isolated from the fruits of *Garcinia cambogia* by using semi-preparative HPLC/DAD method (Masullo *et al*., 2008).

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are special group of compounds mainly present in the family Guttiferae. The simultaneous quantification of 16 PPAPs from different plant parts of 10 *Garcinia* species was carried out using

ultra performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS). Results indicated variation in contents of PPAPs in different plant parts from different species and showed richness of the compounds in the genus (Zhou *et al*., 2010). Identification and quantification of benzophenones and bioflavonoids from eight *Garcinia* species was carried out using HPLC-PDA method. During the study three benzophenones such as guttiferone-A, guttiferone-E & xanthochymol and four biflavonoids such as amentoflavone, fukugeside, fukugetin and volkensiflavone were identified and quantified in comparison with standards. *G. spicata* and *G. intermedia* showed the highest amount of xanthochymol and guttiferone-A respectively. Also, among all species, highest antioxidant activity was observed in *G. intermedia* (Acuna *et al*., 2012).

The method developed for identification and quantification of two biologically active polyisoprenylated benzophenones such as xanthochymol and isoxanthochymol from the fruit rinds, stem bark, seed pericarps and leaves of *G. indica* and fruit rinds of *G. cambogia* using liquid chromatography-tandem mass spectrometry (LC/ESI-MS/MS). The method developed was found to be useful for identification and quantification of both the compounds and indicated that fruit rinds of both the species possesses high amount of mentioned bioactive compounds (Chattopadhyay & Kumar, 2006). Another report on identification and quantification of two isomeric polyisoprenylated benzophenones isoxanthochymol and camboginol in different extracts of *Garcinia* species by HPLC & LC-ESI-MS methods showed presence of both the compounds in *G. cambogia* fruits however, absent in stem bark, seeds and seed pericarp of *G. indica* species (Kumar *et al*., 2009).

2.3 & 2.4 Phytochemistry, antioxidant, anticancer and other bio-activities of different *Garcinia* species.

Several researchers have been working on phytochemistry and various aspects of bioprospecting of different *Garcinia* species. The studies were mainly focused on isolation and characterisation of bioactive compounds from different plant parts and their bio-activities. Studies were carried out using various phytochemical methods and different bioassays. The comprehensive account of work carried out on phytochemistry and bio-activities are provided below:

Species	Plant part	Methods	Compounds/ phytochemicals	Bio-activities	References
G. afzelii	Stem bark	Column chromatography, TLC, UV, NMR, MS	Afzeliixanthones-A, afzeliixanthones-B, β-sitosterol, stigmasterol, xanthone	Antioxidant: DPPH	Waffo *et al*., 2006
G. amplexicaulis	Stem bark	Column chromatography, TLC, HPLC, UV, NMR, MS	Xanthones, tocotrienol derivatives, triterpenes, phenolic compounds	Antioxidant: ORAC, TBARS	Lavaud *et al*., 2015
G. atroviridis	Fruit rind, pulp	GC-MS	Sesquiterpenoids: β-caryophyllene, β-caryophyllene alcohol, α-humulene	Antibacterial; anti-inflammatory: cyclooxygenase	Tan *et al*., 2012
G. bancana	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. bifasculata	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. brasiliensis	Fruit epicarp	Column chromatography, UV, IR, NMR, MS	Morelloflavone-4-O-β-D-glycosyl, fukugiside, morelloflavone	Leishmanicidal; antiproteolytic; antioxidant: DPPH, reducing power	Gontijo *et al*., 2012

G. buchananii	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. cantleyana	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. cochinchinensis	Pericarp	TLC, column chromatography, NMR	Polyisoprenylated benzophenones, guttiferones, xanthones, trimethyl citrate	Cytotoxicity: MCF-7, HeLa, NCI-H460	Nguyen *et al*., 2011
G. cowa	Twig	Column chromatography, TLC, HPLC, IR, NMR, HRESI-MS	Polyprenylated acylphloroglucinol derivative: garcicowin-A, garcicowin-B, garcicowin-C, garcicowin-D	Cytotoxicity: HT-29, HCT-116	Xu *et al*., 2010
	Fruits	Column chromatography, TLC, FTIR, UV, NMR, HREMS	Cowaxanthone-A, cowaxanthone-B, cowaxanthone-C, cowaxanthone-D, cowaxanthone-E	Antibacterial	Panthong *et al*., 2006
	Fruit rind	-	-	Antioxidant: phosphomolybdenum, reducing power assay; anti-aflatoxigenic	Joseph *et al*., 2005
	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-	Jabit *et al*., 2009

				inflammatory	
G. edulis	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. eugenifolia	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. ferrea	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. forbesii	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. griffithii	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. gummi-gutta/ cambogia	Fruit rind	LC/ESI-MS/MS	Xanthochymol, isoxanthochymol	-	Chattopadhyay & Kumar, 2006
	Leaf	Qualitative tests	Major: Alkaloids, tannins, phenolic flavonoids, carbohydrates, proteins; Minor: steroids, terpenoids, phlobatannin, cardiac glycosides	-	Madappa & Bopaiah, 2012

	Fruit rind	Qualitative tests	Flavonoids, terpenoids, phenols, tannins, glycosides, saponin, sterols, coumarin, phlobatannin, carbohydrates, amino acids	-	Krishnamoorthy *et al*., 2014
	Fruit peel	Qualitative tests	Triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate, vitamin-C	Antioxidant: DPPH, hydroxyl radical scavenging, FRAP	Sherly *et al*., 2015
	Root, bark	Column chromatography, EI-MS, IR, NMR, UV spectroscopy	Garbogiol, rheediaxanthone-A, garcinol, isogarcinol	-	Iinuma *et al*., 1998
	Seed	TLC, UV, FTIR, NMR, GC-MS	Fatty acid methyl esters: saturated, mono-unsaturated, poly-unsaturated	-	Choppa *et al*., 2015
G. hombroniana	Bark	TLC, column chromatography, NMR, UV, IR, EI/ESI-MS	2,30,4,50-tetrahydroxy-6-methoxybenzophenone; 2,30,4,40-tetrahydroxy-6-methoxybenzophenone; 2,30,4,6-tetrahydroxybenzophenone; 1,3,6,7-tetrahydroxyxanthone; 3,30,40,5,7-	Cytotoxicity: MCF-7, DBTRG; antioxidant: DPPH, ABTS, FRAP	Jamila *et al*., 2014

			pentahydroxyflavone; 3,30,5,50,7-pentahydroxyflavanone; 3,30,40,5,50,7-hexahydroxyflavone; 40,5,7-trihydroxyflavanone-7-rutinoside; 18(13-17)-abeo-3b-acetoxy-9a,13blanost-24E-en-26-oic acid; garcihombronane-B; garcihombronane-D; friedelan-3-one; lupeol; stigmasterol; stigmasterol glucoside		
	Twig	Column chromatography, PTLC, NMR, UV, HREIMS	Garcihombronanes-A, garcihombrononones-B, garcihombronone-C, garcihombronone-D, garcihombronone-K, garcihombronone-L	Antibacterial	Klaiklay *et al*., 2013
	Bark	Quantitative tests	Total phenols, total flavonoids	Antioxidant: DPPH, FRAP, ABTS; anticholinesterase; antibacterial	Jamila *et al*., 2013
G. huillensis	Fruit	GC-MS	Monoterpenes, sesquiterpenes,	-	Chagonda &

			aromatic hydrocarbons		Chalchat, 2005
	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. imbertii	Stem bark	GC-FID, GC-MS	Terpenoids: Humulene, β-caryophyllene, caryophyllene oxide, humulene oxide	Antimicrobial	Rameshkumar *et al*., 2005
	Leaves, stem bark	Qualitative tests	Alkaloids, flavonoids, steroids, glycosides, phenols, saponins, terpenoids, resins, tannins, carbohydrates	Antioxidant: DPPH, ABTS	Rajkumar *et al*., 2015
G. indica	Fruit rind, bark, leaves, seed pericarp	LC/ESI-MS/MS	Xanthochymol, isoxanthochymol	-	Chattopadhyay & Kumar, 2006
	Leaves	GC-MS	Fatty acids	-	Mahadkar *et al*., 2013
	Fruit	HPLC	HCA (2.338%)	Antioxidant: total phenolic content, DPPH, superoxide dismutase, nitric oxide inhibition; cell viability: THP-1	Minakshi *et al*., 2015
	Fruit rind	Column	Garcinol	Antioxidant: DPPH, β-	Selvi

		chromatography, IR, NMR		carotene-linoleate; antifungal	*et al*., 2003
	Fruit	TLC, HPLC, GC-MS	Phenols, alkaloids	Antioxidant: FRAP	Gayathri & Govindaraju, 2012
	Fruit rind	Column chromatography, UV, NMR, EI-MS	Garcinol	Antioxidant: linoleic acid peroxidation, DPPH, superoxide anion scavenging; anti-glycation	Yamaguchi *et al*., 2000
	Fruit rind	-	-	Antioxidant: ABTS, ORAC, FRAP, lipid peroxidation	Mishra *et al*., 2006
	Fruit rind	Column chromatography, UV, IR, NMR, EI-MS	Garcinol	Antioxidant: superoxide anion, hydroxyl radical scavenging, reactive oxygen species; antiulcer: Wistar rats	Yamaguchi *et al*., 2000
	Fruit rind	-	Garcinol	Cytotoxicity: HL-60; induction of apoptosis	Pan *et al*., 2001
	Fruit rind	LC/ESI-MS/MS, HPLC	Xanthochymol, isoxanthochymol	Cytotoxicity: Colo-320-DM, MCF-7, WRL-68	Kumar *et al*., 2007
	Fruit rind	-	Garcinol, cambogin, garcim-1, garcim-2	Cell growth inhibition: HT-29, HCT-116, IEC-	Hong *et al*., 2007

				6, INT-407; apoptosis induction; autoxidation dependent growth stimulatory effect	
G. kingaensis	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. kola	Seeds	HPLC	Total phenols, epicatechin	-	Niemenak *et al*., 2008
	Bark, leaves, seeds	Qualitative tests	Alkaloids, tannins, flavonoids, coumarin, triterpenoids, steroids	Antibacterial; antifungal; antioxidant: DPPH, ABTS	Dah-Nouvlessounon *et al*., 2015
	Seed	Qualitative tests, AAS, spectrophotometer	Crude fat, crude protein, crude fibre, total carbohydrates, minerals, tannins, saponin, steroid, terpenoids, cardiac glycoside, alkaloid, flavonoid, phlobatannins	-	Alaje *et al*., 2014
	Seed	GLC, LSC, GC-MS	Monoterpenes, sesquiterpenes, diterpenes, hydrocarbons	-	Onayade *et al*., 1998
	Seed	Column chromatography, TLC, GC-MS	Sterols, fatty acid derivatives	Anti-Listeria	Penduka *et al*., 2014
	Seeds	GC/TEA	Primary: methylamine,	-	Atawodi

			ethylamine, isobutylamine, isopentylamine; Secondary: dimethylamine		*et al*., 1995
G. lanceifolia	Bark	Qualitative tests, quantitative standards, TLC	Alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, phenolic compounds	Toxicity: Wistar rats	Bora *et al*., 2017
	Fruit pericarp	Folin-Ciocalteu	Phenols, flavonoids	Antioxidant: DPPH, ABTS, nitric oxide inhibition, total ion reducing power	Gogoi *et al*., 2015
G. livingstonei	Fruit	TLC, HPLC, LC-ToF-MS	Guttiferone-A, guttiferone-K, xanthochymol, guttiferone-E, cycloxanthochymol, isoxanthochymol, gambogenone, amentoflavone, biapigenin, volkensiflavone, morelloflavone, fukugiside, alloathyriol	Cytotoxicity: HCT-116, HT-29, SW-480	Yang *et al*., 2010
	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. lucida	Fruits, bark	Qualitative tests	Polyphenols, steroids, saponins, tannins, flavonoids,	Antioxidant: DPPH, nitric radical	Sylvie *et al*., 2014

			alkaloids, glycosides	scavenging, hydroxyl radical scavenging, reducing power assay, total phenols, FRAP	
G. macrophylla	Leaves, flowers	GC-MS	Terpenes, fatty acid derivatives, benzenoids, total oil	-	Andrade *et al*., 2007
G. maingayi	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. malaccensis	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. mangostana	Fruit	HPLC	Xanthone	Cytotoxicity: Hepa lclc 7; Quinone reductase; Hydroxyl radical scavenging	Chin *et al*., 2008
	Fruit peel, rind, pulp	GC-FID	Phenolic acids	-	Zadernowski *et al*., 2009
	Pericarp	-	-	Apoptosis & cell cycle alterations: MDA-MB231	Kurose *et al*., 2012
	Pericarp	-	-	Antitumor: NL-17; *in vivo* toxicity: BALB/c	Kosem *et al*., 2013

				mice	
	Stem bark	Column chromatography, EI-MS, FTIR, NMR, UV spectroscopy	Mangaxanthone, mangaphenone, mangostanin, mangostenol	-	See *et al*., 2014
	Fruit	Qualitative tests, HPLC, GC-MS	Phenols, flavonoids, triterpenoids	Anti-proliferative: HepG2	Manimekalai *et al*., 2016
	Pericarp	TLC, NMR, HREIMS	α-mangostin; β-mangostin; 1-hydroxy-3,6,7-trimethoxy-2,8-bis (3-methylbut-2-enyl) xanthone; 9-hydroxycalabaxanthone; tovophyllin-A; catechin	Cytotoxicity: MRC-5	Al-Massarani *et al*., 2013
	Pericarp	LC-ESI-MS	Epigallocatechin, garcinone-C, 3-isomangostin, mangostanol, 8-desoxygartanin, α-mangostin, garcinone-E, β-mangostin	Antioxidant: DPPH, lipid peroxidation, hadroxyl radical, cytochrome-C reduction	Zarena & Sankar, 2009
	Fruit rind	GC-MS	Organic acids, fatty acid derivative, phenols	Antimicrobial; anticancer: MCF-7; antioxidant: DPPH	Wei *et al*., 2014
	Fruit hull	Column chromatography, UV, IR, NMR	Phenols: epicatechin	Antioxidant: DPPH, linoleic acid peroxidation,	Yu *et al*., 2007

				superoxide anion scavenging	
	Fruit pericarp	-	α-mangostin, γ-mangostin, 8-deoxygartanin	Cytotoxicity: SK-MEL-28; apoptosis induction	Wang *et al*., 2011
	Stem bark	Column chromatography, HPLC, UV, IR, NMR, ESIMS, HRESI-TOF-MS	11-hydroxy-3-*O*-methyl-1-isomangostin; 11-hydroxy-1-isomangostin; 11R-mangostanin; 3-isomangostin; R-mangostin; β-mangostin; garcinone D; 9-hydroxycalabaxanthone; 8-deoxygartanin; gartanin; cratoxyxanthone	Cytotoxicity: HT-29	Han *et al*., 2009
	Fruit rind	LC-MS	α-mangostin, γ-mangostin	Cytotoxicity: HCT-116, apoptosis induction, anti-tumourigenicity, effect on cell signalling pathways; *in vivo* anti-tumour: mice	Aisha *et al*., 2012
	Fruit pericarp	Column chromatography, TLC, UV, NMR	Phenols: epicatechin	Antioxidant: FTC; cytotoxicity: MCF-7, HELF;	Yu *et al*., 2009

				immunomodulatory effect: BALB/c mice	
	Fruit pericarp	Column chromatography, TLC, HPLC, UV, FTIR, NMR, FTMS	8-hydroxycudraxanthone-G; mangostingone [7-methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone]; cudraxanthone-G; 8-deoxygartanin; garcimangosone-B; garcinone-D; garcinone-E; gartanin; 1-isomangostin; α-mangostin; γ-mangostin; mangostinone; smeathxanthone-A; tovophyllin-A	Antioxidant: peroxynitrite scavenging	Jung *et al*., 2006
G. merguensis	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. morella	Latex	GC-MS	Terpenoids, flavonoids, saponins, tannins, cardiac glycosides, phenols, alkaloids	Antioxidant: DPPH, phosphomolybdate, hydrogen peroxide scavenging, reducing power; larvicidal	Murthy *et al*., 2017
	Leaf, bark,	-	-	Antioxidant: DPPH,	Choudhury *et al*.,

	fruit			reducing power assay, lipid peroxidation; anticancer: DLA cells, Swiss albino rat	2016
	Fruit	Quantitative tests	Total phenol, total flavonoid	Antioxidant: DPPH, ABTS, nitric oxide inhibition; hepatoprotective: Albino rats	Gogoi *et al*., 2017
G. multiflora	Leaves	Column chromatography, TLC, NMR, ESI-MS, HRESI-MS, UV	Garcinianin-A; garcinianin-B; 3,30,5,50,7-pentahydroxyflavan; mahuannin-A	-	Jiang *et al*., 2014
	Root	Folin-Ciocalteu method, Column chromatography, NMR, MS	Total phenols	Antioxidant: DPPH, superoxide radical scavenging, reducing power, ferrous ion-chelating ability	Wu *et al*., 2008
G. nervosa	Leaves, bark	-	-	Antioxidant: DPPH; anti-inflammatory: lipoxygenase, hyaluronidase, xanthine oxidase; cytotoxicity: MCF-7, HT-29, HeLa	Seruji *et al*., 2013

	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. nigrolineata	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. oblongifolia	Bark	Column chromatography, TLC, UV, NMR, LC-MS/MS, HPLC	Xanthones: oblongixanthones-A, oblongixanthones-B, oblongixanthones-C; polyprenylated benzoylphloroglucinols: oblongifolin-E, oblongifolin-F, oblongifolin-G	Apoptosis inducing effect: HeLa	Huang *et al*., 2009
G. opaca	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. parvifolia	Fruit peel, flesh	Quantitative tests	Total phenolic, flavonoid, anthocyanin, carotenoid content	Antioxidant: DPPH, FRAP, ABTS, acetylcholinesterase inhibition	Hassan *et al*., 2013
	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G.	Fruit rind	-	-	Antioxidant:	Joseph

pedunculata				phosphomolybdenum, reducing power assay; anti-aflatoxigenic	*et al*., 2005
	Fruit rind	Column chromatography, UV, IR, NMR	-	Antioxidant: β-carotene–linoleate, DPPH; anti-mutagenic	Jayaprakasha *et al*., 2006
G. penangiana	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. polyantha	Leaves	Column chromatography, TLC, IR, NMR, EI-MS, HR-EI-MS	Banganxanthone; echinocystic acid; isoxanthochymol; β-sitosterol; 4-[(2E)-3,7-dimethylocta-2,6-dien1-yl]-1,5,8-trihydroxy-3-methoxy-9H-xanthen-9-one; 2-hydroxy-1,7-dimethoxyxanthone	Cytotoxicity: A-549, MCF-7, PC-3, HeLa, THP-1	Lannang *et al*., 2014
G. prainiana	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. preussii	Fruit, leaf	Column chromatography, TLC, HPLC, NMR, HRMS	Garciniagifolone, garcimultiflorone-E, garcinialiptone-B, garcinol, 7-epi-clusianone, garcimangosone, astragalin,	Antioxidant: DPPH, ABTS, ALP, ORAC; cytotoxicity: DU-145, HeLa, HT-29, A-431; antimicrobial	Messi *et al*., 2014

			eriodictyol		
G. pyrifera	Fruit	Column chromatography, HPLC, UV, IR, NMR, MS	Xanthochymol, guttiferone-E, cycloxanthochymol, isoxanthochymol	Anticancer: tubulin assay; cytotoxicity: KB	Roux *et al*., 2000
G. rostrata	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. semseii	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. smeathmannii	Root bark	TLC, UV, IR, NMR, EI-MS	Cheffouxanthone, guttiferone-I, isoxanthochymol, triacontanyl caffeate, smeathxanthones	Antioxidant: DPPH	Lannang *et al*., 2006
G. subelliptica	Seed	Column chromatography, UV, IR, NMR, MS	Garcinielliptones-Q, garcinielliptones-S, phloroglucinol, garcinielliptone-R	Cytotoxicity: NTUB1; antioxidant: DPPH, ABTS	Lin *et al*., 2012
G. urophylla	Fruit, stem, leaf	-	-	Cytotoxicity: MCF-7, NCI-460, DU-145; anti-inflammatory	Jabit *et al*., 2009
G. vieillardii	Stem bark	TLC, HPLC, UV, NMR, ESI	6-*O*-methyl-2-deprenylrheediaxanthone-B;	Antioxidant: DPPH	Hay *et al*., 2004

			vieillardixanthone		
G. volkensis	Root, fruit, fruit hull, stem, seed	Qualitative tests	Phenols, tannins, steroids	Anti-HIV; cytotoxicity: A-549, DU-145, KB, Kbvin	Magadula & Suleimani, 2011
G. xanthochymus	Seeds, seed oil	GC-FID	Fatty acids	Antimicrobial; antioxidant: DPPH	Manohar *et al*., 2014
	Twig	Column chromatography, UV, IR, NMR, HRESI-TOF-MS	Xanthochymones-A; xanthochymones-B; xanthochymones-C; garcinexanthone-A; volkensiflavone; morelloflavone; amentoflavone; angelicoin-B; 1,4,6-trihydroxy-5-methoxy-7-prenylxanthone	Antibacterial	Trisuwan *et al*., 2014
	Wood	Column chromatography, EI-MS, IR, NMR, UV spectroscopy	Prenylated xanthone	Nerve growth factor potentiating cytotoxicity	Chanmahasathien *et al*., 2003
	Twig bark	Column chromatography, IR, NMR, HR-ESI-MS	Phenolic compounds: xanthones with prenyl or geranyl substituents	Cytotoxicity: MDA-MB-435S, A-549	Han *et al*., 2007
	Fruit	Folin-Ciocalteu	Phenols, flavonoids	Antioxidant: DPPH,	Gogoi

	pericarp			ABTS, nitric oxide inhibition, total ion reducing power	*et al*., 2015
	Fruit	Column chromatography, TLC, HPLC, UV, NMR, ESIMS, HRESI-MS	Guttiferone-H; gambogenone; aristophenone-A; alloathyriol; amentoflavone; 3,8''-biapigenin; cycloxanthochymol; (±)-fukugetin; (±)-fukugiside; guttiferone-E; isoxanthochymol; (±)-volkensiflavone; xanthochymol	Cytotoxicity: SW-480; antioxidant: DPPH	Baggett *et al*., 2005
	Stem bark	Column chromatography, UV, NMR, EIMS, HREIMS	Xanthones	Antioxidant: DPPH	Chen *et al*., 2010
	Fruit	Qualitative tests	Flavonoids, glycosides, steroids, triterpenoids	Anti-inflammatory: Wistar rats	Winata *et al*., 2018
G. yunnanensis	Pericarp	UPLC/PDA/MS, NMR	Guttiferone-K; oblongifolin-A, B, C, D; garciyunnanin-A, B; diethylhexyl phthalate	Apoptosis: HeLa	Xu *et al*., 2008

2.5 Molecular phylogeny

In molecular phylogeny, analysis was carried out on the basis of comparison of DNA or protein sequences. DNA barcodes involve sequencing of standard region of DNA and can be used for species identification. The nuclear and chloroplast locus are most commonly used for plant phylogenetic studies at species level because of high level of interspecific divergence (Kress *et al*., 2005; Hollingsworth *et al*., 2009). Molecular phylogeny has clarified many uncertainties in species evolution and taxonomic issues which was proved helpful for conservation purpose.

2.5.1 *Garcinia* molecular phylogeny

The genus *Garcinia* possesses extreme diversity of floral form and there are many unresolved taxonomic issues in the genus. To solve morphology-based taxonomic issues involving *Garcinia* and its relatives and to examine floral diversity in the genus molecular phylogenetic study was carried out. Phylogenetic relationship was inferred among 70 sample of *Garcinia* and close relatives by conducting Bayesian, parsimony and likelihood analyses with two nuclear genes, granule-bound starch synthase (GBSSI) and the internal transcribed spacer (ITS) sequence data (Sweeney, 2008). It revealed that all species of *Garcinia* fall into two major lineages based on presence or absence of nectariferous floral organs such as central disks, antesepalous lobes and intrastaminal ring-shaped disks.

Another study on genetic relationships of 16 *Garcinia* species was revealed by ITS sequence data by using maximum likelihood and neighbour-joining method. The phylogenetic trees showed consistent results with morphological classification and separation of *Garcinia* species distribution with exotic species in China (Liu & Liu, 2016). Molecular phylogeny has been used to clarify several uncertainties in species evolution and evolve conservation strategies. ITS sequence data was used to study phylogenetic relationships among 5 locally cultivated and 12 wild relatives of *Garcinia* species using parsimony and Bayesian approaches (Nazre *et al*., 2007). The study displayed identical tree topology showing five main monophyletic clades congruent with earlier taxonomic classification using both approaches.

Phylogenetic relationship of *Garcinia mangostana* and 17 *Garcinia* species were analysed by using internal transcribed spacer (ITS) region of nuclear ribosomal DNA sequence data (Yapwattanaphun & Subhadrabandhu, 2004). The analysis revealed that *G. mangostana* was closely related to its progenitor *G. malaccensis* however, another progenitor *G. hombroniana* showed more distant phylogenetic relation. The identification of possible parents of *Garcinia mangostana* by comparing its relationship to other species within the same section was carried out based on the genetic analysis of the internal spacer, chloroplast and microsatellite regions (Abdullah *et al*., 2012). The study indicated that *G. malaccensis* was one of the parents and another possible parent is *G. opaca* not *G. hombroniana* as it showed distant relationship with *G. mangostana* using molecular phylogeny. The report on phylogenetic relationship among *G. mangostana* and its relatives using morphological and inter simple sequence repeat (ISSR) markers indicated close relationship among *G. mangostana*, *G. malaccensis* and *G. celebica* (Sulassih *et al*., 2013). Another study revealed the new evidence on the origin of *G. mangostana* which was earlier considered as combination of *G. malaccensis* and *G. hombroniana*. However, field and molecular studies using ITS sequence data of 22 samples showed high resemblance with *G. malaccensis*. So author merged both species together as a one species (Nazre, 2014).

The genetic relationship among nine commonly available species from the Western Ghats and north-east Himalayan foot hills of India was revealed using ISSR molecular markers and evolutionary cluster analysis in MEGA5 software. Two major clusters were formed based on floral morphology and availability of hydroxycitric acid in their fruits (Parthasarathy *et al*., 2016). The genetic profile of nine *Garcinia* species from southern Western Ghats was analysed using *mat*K gene and used to resolve issues related to species identification (Sivu *et al*., 2016).

To assess adulteration of morphologically similar samples of *Garcinia* in herbal markets from south India DNA barcoding was used. DNA barcoding showed usefulness in herbal product authentication (Seethapathy *et al*., 2018). To boost breeding and conservation programmes for six *Garcinia* species phylogenetic relationship was investigated based on random amplified polymorphic DNA (RAPD)

markers. The study revealed genetic relatedness among the species (Bheemaiah *et al*., 2018).

The genetic relationship among the 12 accessions of *G. cambogia* from different geographical accessions of Kerala was evaluated using RAPD markers (Tharachand *et al*., 2015). Priya Devi *et al*. (2010) evaluated genetic diversity and relationship among *Garcinia* species such as *G. indica, G. mangostana, G. hombroniana* and *G. tinctoria* in the region of Goa using RAPD and ISSR markers. It suggested the use of above mentioned molecular markers in genetic fingerprinting and development of species specific markers as well as to develop markers linked with the sex trait. Supporting the later work, sex determination in micro-propagated plants and natural population of *G. indica* was carried out by Dias & Krishnan (2015) using ISSR and RAPD markers which suggested the use of molecular markers to distinguish male or female plants at seedling stage.

2.5.2 Malpighiales and Clusiaceae molecular phylogeny

Phylogenetic analysis of Clusioid clade (Malpighiales) was carried out using plastid and mitochondrial nucleotide sequence data by maximum likelihood and Bayesian methods. The study included 194 species representing all 5 families revealed well resolved, taxon rich phylogeny and provided the foundation for improving the classification of the group and for exploring the biogeography of tropical angiosperms (Ruhfel *et al*., 2011). Phylogeny of the family Clusiaceae was carried out using chloroplast gene *rbc*L. For the study, 26 species representing all genera of the family were used (Gustafsson *et al*., 2002). Another study on family Guttiferae from peninsular Malaysia was carried out based on plastid sequences. Chloroplast DNA sequence data and 21 taxa from 4 genera of the family were used. The study showed that *Calophyllum*, *Mammea* and *Garcinia* are monophyletic genera while *Mesua* is polyphyletic genus (Zakaria *et al*., 2007).

Chapter 3

Materials and methods

3.1 Field survey, documentation and taxonomic characterisation of *Garcinia* species from the Western Ghats of India.

3.1.1 Study area

The study area was the entire Western Ghats of India which includes six states namely Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu.

3.1.2 Data collection

Initial data collection was carried out by visiting various herbaria such as AURO: Auroville, Villupuram; BSI: Botanical Survey of India, Pune (Western Regional Centre); CALI: Calicut University, Calicut; FRLH: FRLHT (Foundation of Revitalization of Local Health Traditions), Bengaluru; Goa University, Goa; Madras Herbarium (MH): Botanical Survey of India, Coimbatore (Southern Regional Centre); SUK: Shivaji University, Kolhapur and TBGT: JNTBGRI (Jawaharlal Nehru Tropical Botanical Garden and Research Institute), Thiruvananthapuram for documentation of location, distribution and taxonomic information of different *Garcinia* species.

3.1.3 Field survey, collection and identification of plant material

Based on the data collected from different herbaria, floras and previous literature; systematic field trips and survey were carried out in around Western Ghats regions of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu to collect the specimens of *Garcinia* species. During field survey latitude, longitude and altitude were recorded using hand-held GPS (Model: GARMIN MONTANA 650). Photographic documentation of all collected species was done by using digital camera (Nikon D-90, SLR). Taxonomic identification and characterization of collected

species was done with the help of taxonomists, herbarium specimens, available literature, type specimens and using anatomical methods. Free-hand transverse sections of petiole were stained with 1% Safranin and photographed under bright-field microscope with image analysing system (Nikon E-800, Nikon Corporation, Tokyo, Japan). The herbarium specimens of all collected species was prepared with standard protocol (Jain & Rao, 1977) and submitted in MH & Goa University herbarium. Along with the above specimens, fruits and leaves of different *Garcinia* species were also collected. Collected fruit samples were preserved in deep freezers and leaf material was dried using silica crystals for further studies.

3.1.4 Ecological niche modeling study

During field survey along with other *Garcinia* species, distributional data for the species *Garcinia indica* from wild populations were also collected. Considering the importance and threat of overexploitation of the species, the prediction of potential distribution and understanding the climatic factors responsible for the niche of the species in wild including areas for its cultivation was worked based on primary occurrence data by using ecological niche modeling (ENM). ENM study was done using MaxEnt and BIOCLIM algorithms as described in Palkar *et al*. (2020) publication.

3.2 Quantitative estimation of bio-active compounds garcinol and hydroxycitric acid (HCA) during various developmental stages of fruits of *Garcinia indica* and matured fruits of other *Garcinia* species using HPLC system.

3.2.1 Plant material

During collection trips, various developmental stages of fruits of *Garcinia indica* and matured fruits of different *Garcinia* species were collected. Further the fruits were washed with tap water as well as distilled water. These were wiped with 70% alcohol. Fruits were cut open to remove inside part which contains seeds and pulp. Outermost part of the fruit that is fruit rind was taken and cut into small pieces. Small pieces of rinds were dried using hot air oven (Meta-lab Scientific, Mumbai, India) at 60°C. Moisture loss was achieved 70-80% by using loss on drying basis.

3.2.2 Extraction

Ten gram of dried fruit rind of different *Garcinia* samples were weighed and extracted with 500 ml of methanol (Merck, HPLC grade) solvent using Soxhlet extraction method. Soxhlet extraction was carried out at 40°C for 48-72 hrs.

3.2.3 Preparation of extract and extraction yield

After complete extraction, the extracts were filtered through laboratory filter paper to remove pectic substances. The filtrate was kept overnight at room temperature to settle down pectinaceous material present in it. Further it was filtered with laboratory filter paper and then with Whatman filter paper no. 1 to remove impurities. Filtered extracts were concentrated with the help of vacuum rotary evaporator (Heidolph Instruments, Germany) at 20-25°C and made it to 50 ml. The concentrated extracts were transferred to 100 ml beakers and kept in hot air oven at 30-40°C to evaporate the solvent. After complete evaporation of solvent, semi-solid extracts were stored in refrigerator until the use. Extract yield of the above samples was calculated using following formula:

Extraction yield = Weight of beaker and extract after solvent evaporation – Weight of empty beaker

3.2.4 Thin-layer chromatography (TLC) for confirmation of hydroxycitric acid

For TLC, aluminium sheets of silica gel 60 (Merck) were used. Samples were dissolved in methanol and loaded at respective loading point using fine capillary tubes for chromatographic study. Solvent system n-butanol:formic acid:water in proportion with 4:1:5 was used. 1% sodium metavanadate spray was used to confirm the presence of HCA and Rf values were calculated (Jena *et al*., 2002; Ferrara, 2014).

3.2.5 High performance liquid chromatography (HPLC) analysis

This study was carried out with two separate methods for quantification of each compound hydroxycitric acid (HCA) and garcinol.

3.2.5.1 Method development, validation and quantification of hydroxycitric acid (HCA)

3.2.5.1.1 Method development and equipment conditions

For quantification of HCA the method was developed by changing different mobile phases and different chromatographic conditions such as elution pattern, flow rate etc. Final procedure was followed as described by Jayaprakasha *et al*. (2002) with partial modification. All solvents used were of HPLC/AR grade. The high performance liquid chromatographic system (HPLC) Prominence-i (Shimadzu, Japan) with LC-2030 UV detector and C18 column (150 × 4.6 mm I.D. packed with 5 µm particle size; Phenominex, USA) was used for analysis. (-)-Hydroxycitric acid calcium salt (HCA) standard was obtained from Sigma Aldrich Chemicals Pvt. Ltd., U.S.A. Standard HCA solution was prepared by dissolving in 8 mM sulfuric acid then filtered through 0.22 µm membrane syringe filter and further used for the analysis. The elution was carried out with mobile phase 8 mM sulfuric acid which was vacuum filtered using 0.45 µm nylon membrane filter (Ultipor®, Pall Life Sciences). The flow rate was maintained at 1 ml/min under isocratic condition. Detection was done at wavelength of 210 nm with 5 mins runtime.

3.2.5.1.2 Method validation

For method validation following parameters such as carry over test, system suitability and linearity was checked.

3.2.5.1.2.1 Carry over test

This test was carried out to check the appearance of an analyte in a run when a blank containing no analyte is injected. Methanol was used as a blank.

3.2.5.1.2.2 System suitability

The system suitability test was checked using reproducibility (% RSD) with six injections of standard solutions of HCA.

3.2.5.1.2.3 Linearity and calibration curve

To assess the linearity, various concentrations of standard HCA in 8 mM sulfuric acid (5, 10, 20, 30, 50 µg/ml) were used and the calibration curve for HCA was prepared by plotting concentration of HCA versus peak area (average of three runs were taken).

3.2.5.1.3 Sample preparation and analysis

Purified and concentrated semi-solid extracts of different *Garcinia* species and different developmental stages of fruits of *G. indica* were dissolved in methanol to make 1000 ppm concentration. Dissolved samples filtered through 0.22 µm Acrodisc membrane syringe filter (Pall Life Sciences) and further used for quantification of HCA. A 1.5 ml of filtered extract samples were taken in 2.0 ml of auto-sampler vial (Borosil) and 20 µl of injection volume were used for analysis using above HPLC conditions.

3.2.5.2 Method development, validation and quantification of garcinol

3.2.5.2.1 Method development and equipment conditions

To quantify the garcinol method was developed by changing elution patterns and wavelengths. Final analysis was carried out with partial modification of procedure described by Praveen Kumar *et al.* (2013) and Kumar *et al.* (2013). All solvents used were of HPLC/AR grade. The high performance liquid chromatographic system (HPLC) Prominence-i (Shimadzu, Japan) with LC-2030 UV detector and C18 column (150 × 4.6 mm I.D. packed with 5 µm particle size; Phenominex, USA) was used for analysis. The mobile phases were acetonitrile:water (90:10 v/v, solvent-A) and methanol:acetic acid (99.5:0.5 v/v, solvent-B). Before carrying into the system, solvents were filtered and degassed using vacuum filtration assembly containing 0.45 µm membrane filter (Ultipor®, Pall Life Sciences). Standard garcinol (Biogenuix Medsystems Pvt. Ltd., India) was dissolved in methanol, filtered through 0.22 µm membrane syringe filter and used for analysis. The flow rate was maintained at 1

ml/min under isocratic condition. Detection was done at wavelength of 215 and 276 nm with 8 mins runtime.

3.2.5.2.2 Method validation

For method validation following parameters such as carry over test, system suitability and linearity was checked

3.2.5.2.2.1 Carry over test

This test was carried out to check the appearance of an analyte in a run when a blank containing no analyte is injected. Methanol was used as a blank.

3.2.5.2.2.2 System suitability

The system suitability test was checked using reproducibility (% RSD) with six injections of standard solutions of garcinol.

3.2.5.2.2.3 Linearity and calibration curve

Different concentrations of standard garcinol in methanol (0.5, 1, 2, 5, 10 µg/ml) were used to check linearity. Calibration curve for garcinol was obtained by plotting concentration of garcinol versus peak area (average of three runs were taken).

3.2.5.2.3 Sample preparation and analysis

For quantification of garcinol from purified and concentrated semi-solid extracts of different *Garcinia* species and different developmental stages of fruits of *G. indica*, 1000 ppm concentrations of the same were prepared in methanol and filtered through 0.22 µm Acrodisc membrane syringe filter (Pall Life Sciences). A 1.5 ml of filtered extract samples were taken in 2.0 ml of auto-sampler vial (Borosil) and 20 µl of injection volume were used for analysis using above HPLC conditions.

3.3 Phytochemical profiling of fruits of selected *Garcinia* species from the Western Ghats using GC-MS (Zadernowski *et al*., 2009; Mahadkar *et al*., 2013).

3.3.1 Plant material, extraction and sample preparation

To check the phytochemical profiling of different *Garcinia* species gas chromatography mass spectrometry (GC-MS) an analytical method was used. Methanolic extracts of dried mature fruit rinds as prepared in previous experiment for all *Garcinia* species from the Western Ghats were used for the analysis. All the extracts were re-suspended in methanol (Merck, HPLC grade) solvent and made up to 1000 ppm concentration. All dissolved extracts were filtered through 0.45 µm nylon syringe filter (Merck) and used for further analysis.

3.3.2 Equipment parameters and sample analysis

GC-MS analysis of the samples was carried out using Shimadzu Make QP-2010 with non-polar Rxi-1 MS column (30 m length × 0.25 µm thickness × 0.25 mm diameter). Helium was used as the carrier gas. The temperature programming was set with initial oven temperature at 60°C held for 2 mins then injection temperature increased to 260°C held for 5 mins and 280°C held for 5 mins at a rate of 15°C and 2°C per mins respectively. A 2 µl of sample was injected with 15.0 split ratio. The interface temperature for GC-MS was set to 240°C. The mass spectrometer was operated with detector voltage of 0.9 kV, a scan time of 0.30 sec with mass range from 40 m/z to 500 m/z. The total runtime was 35.33 mins. The chemical components from the samples were identified by comparing the retention times of chromatographic peaks using flame ionization detector (FID) with National Institute of Standards and Technology (NIST) mass spectral library to relative retention indices. Quantitative determinations were made by relating respective peak areas to total ion chromatogram (TIC) areas from the GC-MS.

3.3.3 Data analysis

The results obtained from GC-MS analysis were further evaluated to check nature and chemical properties of each compound in different samples using various chemical databases.

3.4 Evaluation of anti-oxidant and anti-cancer (cell line screening) activities of fruit extracts of selected *Garcinia* species.

3.4.1 Anti-oxidant activity

Anti-oxidant activities of fruits of different *Garcinia* species was evaluated using two different spectrophotometric assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)]. All solvents used for the study were of HPLC/AR grade and standard chemicals used were obtained from Sigma Aldrich Chemicals Pvt. Ltd., U.S.A.

3.4.1.1 Plant material, extraction and sample preparation

To check the anti-oxidant potential of different *Garcinia* species, methanolic extracts of dried mature fruit rinds as used in previous experiment were further re-suspended in methanol solvent and made up to 1000 ppm concentration. Serial dilutions of all dissolved extracts were used for further analysis.

3.4.1.2 DPPH assay

Protocol for screening of antioxidant activity using DPPH assay was standardized by using Brand-Williams *et al*. (1995) and Sharma & Bhat (2009) methodology. L-ascorbic acid in methanol was used as reference standard. Serial dilutions were made by taking different aliquots of 1000 ppm concentration of methanolic solution of L-ascorbic acid and different plant extracts separately. Final volume was made to 3 ml using methanol. Reaction was started by addition of 200 µl of methanolic DPPH (200 µM) solution. The reaction mixture was kept at 30°C for 30 mins in water bath and after incubation period the absorbance was measured at 517

nm wavelength using spectrophotometer (Shimadzu). Mixture of methanol and DPPH was used as a control. All operations were carried out in dark condition. Inhibition percentage was calculated by using following formula-

$$\text{Inhibition \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Assay was done in triplicate and results were expressed as mean. IC_{50} values were calculated using linear regression equation by plotting concentration of sample (µg/ml) against percent inhibition.

3.4.1.3 ABTS assay

Protocol for screening of antioxidant activity using ABTS assay was determined by using Re *et al*. (1999) methodology. ABTS stock solution of 7 mM concentration was prepared in distilled water. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hrs before use. For the assay $ABTS^{\cdot+}$ solution was diluted with distilled water to an absorbance of 0.70 at 734 nm wavelength and equilibrated at 30°C. Trolox in methanol was used as reference standard. Different aliquots of 1000 ppm concentration of methanolic solution of trolox and different plant extracts were taken separately. Final volume was made to 2 ml using methanol. Reaction was started by addition of 1 ml of diluted $ABTS^{\cdot+}$ solution (A_{734nm} = 0.70). The reaction mixture was kept at 30°C for 4 mins and after incubation period the absorbance was measured at 734 nm wavelength. Mixture of methanol and diluted $ABTS^{\cdot+}$ solution was used as a control. All reactions were carried out in dark condition. Inhibition percentage was calculated by using following formula-

$$\text{Inhibition \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Assay was done in triplicate and results were expressed as mean. To calculate IC_{50} values linear regression equation method was used by plotting concentration of sample (µg/ml) against percent inhibition.

3.4.2 Anti-cancer (cell line screening) activity

To accomplish this activity dried mature fruit rinds of all *Garcinia* species collected from the entire Western Ghats were subjected for extraction. Extract preparation was carried out with absolute ethanol as solvent by using same method as described in objective no. 2. Concentrated extracts were sent to Anti-cancer Drug Screening Facility, Advanced Centre for Treatment, Research & Education in Cancer, Tata Memorial Centre, Navi Mumbai, Maharashtra (ACTREC) to check anti-cancer activity against different human cancer cell lines such as MCF-7 (breast), HeLa (cervix), HT-29 (colon) and A-549 (lung). To check the activity, SRB (sulforhodamine-B) protein stain assay was used as described by Skehan *et al*. (1990) and Vichai & Kirtikara (2006). The experimental procedure is as per the following:

3.4.2.1 Cell line preparation

The cell lines were grown in RPMI (Roswell Park Memorial Institute) 1640 medium (Appendix 1) containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C with 5% CO_2, 95% air and 100% relative humidity for 24 hrs prior to addition of experimental drugs (extracts).

3.4.2.2 Compound preparation and exposure of cells to test compound

Experimental drugs were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, aliquots of frozen concentrate (1 mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. An aliquots of 10 µl of these different drug dilutions were added to the

appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. Adriamycin was used as positive control. After compound addition, plates were incubated at standard conditions for 48 hrs.

3.4.2.3 Cell fixation and staining

After incubation period, assay was terminated by the addition of cold TCA (trichloroacetic acid). Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA to produce final concentration of 10% TCA and incubated for 60 mins at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. 50 µl of 0.4% (w/v) sulforhodamine-B (SRB) solution in 1% acetic acid was added to each of the wells, and plates were incubated for 20 mins at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM tris base solution.

3.4.2.4 Optical Density measurement and analysis of results

An absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Using the six absorbance measurements i.e. time zero (Tz), control growth (C) and test growth in the presence of drug at the four concentration levels (Ti); the percentage growth was calculated at each of the drug concentration levels. Percent Growth was expressed as the ratio of absorbance of the test well to the absorbance of the control wells x 100.

Percentage total cell growth inhibition (TGI) was calculated as: [Ti/C] x 100.

For all test samples each compound concentration was tested in triplicate and average value was used to calculate GI_{50} value i.e. concentration of drug required to inhibit 50% cell growth. GI_{50} values were determined by plotting drug concentrations used verses percent control of growth using regression analysis in Microsoft Excel. GI_{50} value showing value $\leq$10 and $\leq$20 µg/ml was considered to demonstrate activity for pure compounds and plant extracts respectively.

3.5 Molecular phylogeny of *Garcinia* species from the Western Ghats using nuclear and chloroplast genes.

3.5.1 Source of material

Leaf samples of different *Garcinia* species collected from the entire Western Ghats of India were used for sequencing. Silica dried as well as fresh leaf materials were used to isolate DNA. Gene sequencing was carried out using universal primers of nuclear spacer (ITS) and chloroplast genes (*rbc*L, *mat*K) (Table 3.1) at Regional Facility for DNA Fingerprinting, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala (RGCB) as per the procedure provided in 3.5.2 & 3.5.3 (White *et al*., 1990; CBOL Plant Working Group, 2009):

3.5.2 DNA isolation, PCR analysis and DNA sequencing

3.5.2.1 DNA isolation using NucleoSpin® Plant II kit (Macherey-Nagel)

About 100 mg of the tissue was homogenized using liquid nitrogen and the powdered tissue was transferred to a micro centrifuge tube. Four hundred microliters of CTAB lysis buffer (PL1) (Appendix 2) was added and vortexed for 1 min. Ten microliters of RNase-A solution was added and inverted to mix. The homogenate was incubated at 65°C for 10 mins. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 rcf for 2 mins. The flow through liquid was collected and the filter was discarded. Four hundred and fifty microliters of binding buffer (PC) (Appendix 3) was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 min and the flow through liquid was discarded. Four hundred microliter of washing buffer (PW1) (Appendix 4) was added to the column, centrifuged at 11000 rcf for 1 min and flow though liquid was discarded. Then 700 µl of washing buffer (PW2) was added, centrifuged at 11000 rcf and flow through liquid was discarded. Finally 200 µl of buffer PW2 was added and centrifuged at 11000 rcf for 2 mins to dry the silica membrane. The column was transferred to a new 1.7 ml tube to that 50 µl of low salt elution buffer (PE) was added and incubated at 65°C for 5 mins. The column was then centrifuged at 11000 rcf for 1

min to elute the DNA. The eluted DNA was stored at 4°C. Quality of isolated DNA was checked with the help of agarose gel electrophoresis.

3.5.2.2 Polymerase chain reaction (PCR) analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 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, 0.5 M Betaine, 5 pM of forward and reverse primers. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using amplification profile as per Table 3.2.

Table 3.1: Primers used during the present study.

Loci	Name	Sequence	Annealing Temperature (°C)	Ref.
ITS (Internal Transcribed Spacer)	ITS-5F	GAAGTAAAAGTCGTAACAAGG	58	White *et al*., 1990
	ITS-4R	TCCTCCGCTTATTGATATGC		
***rbc*L (RuBisCo large subunit)**	a-f	ATGTCACCACAAACAGAGACTAAAGC	55	Levin *et al*., 2003
	724-r	TCGCATGTACCTGCAGTAGC		Fay *et al*., 1997
***mat*K (Maturase K)**	3F-KIM-F	CGTACAGTACTTTTGTGTTTACGAG	50	Ki-Joong Kim, unpublished
	1R-KIM-R	ACCCAGTCCATCTGGAAATCTTGGTTC		

Table 3.2: Polymerase chain reaction parameters used for amplification of nuclear and chloroplast genes.

Parameters	Temperature : Time	No. of cycles
Initial denaturation	98°C : 30 sec	1
Denaturation	98°C : 5 sec	40
Annealing	AT°C : 10 sec	
Extension	72°C : 15 sec	
Final extension	72°C : 60 sec	1
Storage	4°C : ∞	

3.5.2.3 Agarose gel electrophoresis of PCR products

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

3.5.2.4 ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) 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. Five microliters of PCR product was mixed with 2 µl of ExoSAP-IT and incubated at 37°C for 15 mins followed by enzyme inactivation at 80°C for 15 mins.

3.5.2.5 Sequencing using BigDye Terminator v3.1

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 manufactures protocol. The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (forward or reverse)
Sequencing Mix	-	0.28 µl
5X Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10 µl

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

3.5.3 Sequence analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010).

3.5.4 Construction of phylogenetic tree (Baldauf, 2003; Lemey *et al*., 2009)

Resulted sequences from ITS region, *rbc*L and *mat*K genes for different *Garcinia* species were deposited to NCBI (GenBank) and further used for the construction of phylogenetic trees with the help of bioinformatics tools to understand the relationship among species. Also the highly similar ITS region, *rbc*L and *mat*K gene sequences from different species of the genus *Garcinia* and an out-group *Pentadesma butyracea* were downloaded from NCBI (BLAST-n) database for analysis. The ITS and chloroplast DNA datasets were analysed individually as well as in combination. All sequences were aligned in ClustalX v2.1 (Larkin *et al*., 2017). MEGA v7.0.26 (Kumar *et al*., 2016) software was used to construct the Maximum Likelihood (ML) trees. Details of the parameters used in ML method are as following table:

Table 3.3: Details of the parameters used for phylogenetic analysis using maximum likelihood method and tree statistics for *Garcinia*.

MEGA v7.0.26 (Maximum Likelihood method)					
Parameters	**ITS**	***rbc*L**	***mat*K**	**ITS+*rbc*L**	**ITS+*mat*K**
No. of taxa	54	43	38	43	33
Best fit nucleotide substitution model	T92+G(5)	GTR+G(5)+ I	GTR+G(5)+ I	T92	GTR+G(5)+ I
Bootstrap replications	1000	1000	1000	1000	1000
No. of Characters	968	1425	1531	2300	2849
Conserved sites	154	859	382	495	851
Variable sites	677	547	805	1785	1638
Parsim-informative sites	554	482	440	1594	1175
Singleton sites	114	64	361	187	425

Chapter 4

Results

4.1 Field survey, documentation and taxonomic characterisation of *Garcinia* species from the Western Ghats of India.

4.1.1 Study area

The study area covers entire Western Ghats of India which harbour one of the finest tropical forests in the world. It is geographically located between 08°19'18" to 21°16'24" N and 72°56'24" to 78°19'40" E with an average elevation 1200 m ranging from 300 m to 2695 m. It covers an area of 1,64,280 km^2 which includes six states namely Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu.

4.1.2 Data collection

Data collection was carried out by visiting various herbaria, regional floras and previous literature yielded information about different *Garcinia* species location, distribution and taxonomic characters.

4.1.3 Field survey, collection and identification of *Garcinia* species

Based on the data collection, systematic field trips and survey were carried out in around the Western Ghats regions of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu revealed the presence and distributional status of 16 different *Garcinia* species with 3 varieties. Twelve species with three varieties were found to occur as natural populations while remaining species such as *G. cowa*, *G. hombroniana*, *G. livingstonei* and *G. mangostana* were found as cultivated and introduced species in the area. Among 16 species, 9 species with 3 varieties namely *G. gamblei*, *G. gummi-gutta* var. *conicarpa*, *G. gummi-gutta* var. *gummi-gutta*, *G. gummi-gtta* var. *papilla*, *G. imbertii*, *G. indica*, *G. pushpangadaniana*, *G. rubro-echinata*, *G. talbotii*, *G.*

travancorica and *G. wightii* are endemic to the region. Details of localities and geographic co-ordinates of all the 16 species with 3 varieties collected and documented from different parts of the entire Western Ghats are provided in the Table 4.1. Different plant parts such as twigs, mature fruits and leaves were also collected for the preparation of herbarium specimen, biochemical & bioprospecting study and molecular analysis.

Table 4.1: *Garcinia* species collected and documented from different parts of the entire Western Ghats and other locations.

Species name	Location	Latitude	Longitude	Altitude (m)
Garcinia cowa	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32**
	JNTBGRI garden, Palode, Kerala	N08°45'02.95"	E77°01'41.73"	84
Garcinia gamblei	Ponmudi, Thiruvananthapuram, Kerala	N08°46'11.28"	E77°06'32.22"	1034**
Garcinia gummi-gutta* var. *conicarpa	Eravikulam National Park, Munnar, Idukki, Kerala	N10°08'36.66"	E77°02'19.38"	1883**
	Akkamalai Forest area, Valparai, Coimbatore, Tamil Nadu	-	-	1636-1700
Garcinia gummi-gutta* var. *gummi-gutta	Regional Fruit Research Station, Vengurla, Sindhudurg, Maharashtra	N15°52'13.50"	E73°38'50.04"	18**
	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32
	IISR farm, Calicut, Kerala	N11°17'57.99"	E75°50'27.75"	57
	Anmode ghats, Bhagwan Mahavir Sanctuary and Mollem National Park, Dharbandora, Goa	N15°26'19.82"	E74°18'02.54"	663
	Jog falls, Sagar, Shimoga, Karnataka	N14°13'51.12"	E79°04'40.45"	305
	Botanical garden of Calicut University, Kozhikode, Kerala	N11°08'02.12"	E75°53'26.18"	77

	ICAR campus, Old Goa, Goa	N15°29'52.41"	E73°55'03.80"	09
	FRLHT campus, Bangalore, Karnataka	N13°07'24.65"	E77°33'00.70"	921
	Mallasery, Pathanamthitta, Kerala	N9°13'44"	E76°48'32"	225
Garcinia gummi-gutta* var. *papilla	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32
	Eravikulam National Park, Munnar, Idukki, Kerala	-	-	**
	JNTBGRI garden, Palode, Kerala	N08°45'02.95"	E77°01'41.73"	84
Garcinia hombroniana	IISR farm, Calicut, Kerala	N11°17'57.99"	E75°50'27.75"	57**
	Regional Fruit Research Station, Vengurla, Sindhudurg, Maharashtra	N15°52'13.50"	E73°38'50.04"	18
	JNTBGRI garden, Thiruvananthapuram, Kerala	N8°45'02.96"	E77°01'41.73"	84
Garcinia imbertii	Chemmunji hills, Bonaccord, Nedumangad, Thiruvananthapuram, Kerala (Agasthyamalai range)	N08°39'43.02"	E77°10'45.54"	1006
	Ponmudi, Thiruvananthapuram, Kerala	N08°45'49.26"	E77°06'52.62"	980**
Garcinia indica	Regional Fruit Research Station, Vengurla, Sindhudurg, Maharashtra	N15°52'13.50"	E73°38'50.04"	18
	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32
	Dandeli Wildlife Sanctuary, Karnataka (Kali Tiger Reserve)	N15°08'55.26"	E74°25'58.86"	591
	IISR farm, Calicut, Kerala	N11°17'57.99"	E75°50'27.75"	57
	Cola, Canacona, Goa	N15°04'24.50"	E73°58'15.74"	48**
Garcinia livingstonei	Lalbagh Botanical garden, Bangalore, Karnataka	N12°57'13.99"	E77°35'07.00"	903**
	Botanical garden of	N11°08'02.12"	E75°53'26.18"	77

	Calicut University, Kozhikode, Kerala			
Garcinia mangostana	Regional Fruit Research Station, Vengurla, Sindhudurg, Maharashtra	N15°52'13.50"	E73°38'50.04"	18**
	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32
	Konni, Valicode, Kottayam	N15°26'19.82"	E74°18'02.54"	663
Garcinia morella	Anmode ghats, Bhagwan Mahavir Sanctuary and Mollem National Park, Dharbandora, Goa	N15°25'07.93"	E74°16'08.07"	552**
	Anshi National Park, Karnataka (Kali Tiger Reserve)	N14°58'22.92"	E74°22'10.92"	539
	Foothills of Chemmunji hills, Bonaccord, Nedumangad, Thiruvananthapuram, Kerala (Agasthyamalai range)	-	-	Approx. 400
	Thamarasser, Wayanad, Kerala	N11°49'68.4"	E76°02'44.7"	729
Garcinia pushpangadaniana	Akkamalai Forest area, Valparai,Coimbatore, Tamil Nadu	N10°19'39.54"	E77°01'28.92"	1636**
	Munnar, Idukki, Kerala	N10°08'43.7"	E77°01'74.1"	1416
Garcinia rubro-echinata	Chemmunji hills, Bonaccord , Nedumangad, Thiruvananthapuram, Kerala (Agasthyamalai range)	N08°38'50.28"	E77°12'05.10"	989**
Garcinia spicata	Puthupet, Cuddalore, Tamil Nadu	N12°03'00.00"	E79°51'60.00"	05
	FRLHT campus, Bangalore, Karnataka	N13°07'24.65"	E77°33'00.70"	921
	Kothattai, Cuddalore, Tamil Nadu	N11°30'59.46"	E79°42'45.53"	16**
Garcinia talbotii	Anmode ghats, Bhagwan Mahavir Sanctuary and Mollem National Park, Dharbandora, Goa	N15°26'19.82"	E74°18'02.54"	663

	Amboli, Sindhudurg, Maharashtra	N15°58'23.51"	E74°00'38.06"	691**
	Sada/ plateau area, Chorla ghat, Karnataka	N15°40'11"	E74°07'06"	806
Garcinia travancorica	Chemmunji hills, Bonaccord, Nedumangad, Thiruvananthapuram, Kerala (Agasthyamalai range)	N08°41'15.96"	E77°11'12.42"	1155**
Garcinia wightii	Achankovilar, Konni, Kerala	N09°07'41.3"	E76°01'74.1"	163
	JNTBGRI garden, Thiruvananthapuram, Kerala	N8°45'02.96"	E77°01'41.73"	84
	Athirappally, Thrissur, Kerala	-	-	**
Garcinia xanthochymus	Regional Fruit Research Station, Vengurla, Sindhudurg, Maharashtra	N15°52'13.50"	E73°38'50.04"	18
	District Agricultural Farm, Thaliparamba, Kannur, Kerala	N12°02'34.02"	E75°23'12.54"	32
	Navellim, Madgaon, Goa	N15°15'14.88"	E73°57'39.90"	09
	IISR farm, Calicut, Kerala	N11°17'38.22"	E75°49'13.37"	57
	Botanical garden of Calicut University, Kozhikode, Kerala	N11°08'02.12"	E75°53'26.18"	77**
	Punjabrao Deshmukh Agricultural college, Nagpur, Maharashtra	N21°08'35.73"	E79°04'40.45"	305
	ICAR campus, Old Goa, Goa	N15°29'52.41"	E73°55'03.80"	09
	FRLHT campus, Bangalore, Karnataka	N13°07'24.65"	E77°33'00.70"	921

Note: **The specimens deposited to herbarium (MH) and samples of different species used for further studies.

Photographic documentation and taxonomic characterisation of all the collected species were done to solve taxonomic ambiguities in species identification. Also, taxonomic identity and occurrence of some species in the region was worked out with the help of morphological characters using various floras and monographs.

The photo plates and notes depicting detailed information about each species morphological characters, anatomical characters, some key differentiating characters, flowering-fruiting period, habitat, distribution and local names are provided below:

4.1.3.1 *Garcinia cowa* Roxb. ex DC. (Plate 4.1)

Key differentiating characters: Latex yellow and after drying turning into orange colour; the colour of mature fruits is also orange.

Flowering & fruiting: December to June.

Habitat: Cultivated.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Cowa mangosteen (Eng.); Kattaphal (Hindi); Cowa, Kattukodapuli (Mal.).

4.1.3.2 *Garcinia gamblei* Shameer *et al*. (Plate 4.2)

Key differentiating characters: Fruit large in size weighing about 250 g, green in colour, irregularly ridged on surface, globose, depressed at distal end.

Flowering & fruiting: December to August.

Habitat: Shola forests at higher altitude (above 950 m).

Distribution: Kerala. Rare.

Local name: Not known.

4.1.3.3 *Garcinia gummi-gutta* var. *gummi-gutta* (L.) N. Robson (Plate 4.3)

Key differentiating characters: Fruits in round shape containing fine furrows, mature fruits yellow in colour.

Flowering & fruiting: Throughout the year.

Habitat: Evergreen, semi evergreen, moist deciduous forests, cultivated.

Distribution: Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu. Common.

Local names: Malabar gamboge (Eng.); Bilatti-amli (Hindi); Aradal, Dharambe (Konk.); Simai hunase, Kodagolu mande, Upagi-mara, Kadagulu-muruga (Kan.); Gorakkapuli, Kadumpuli, Kodampuli, Kodappuli, Marapuli, Meenpuli, Perumpuli, Pinaru, Pinenga (Mal.); Heela, Kodakkapuli, Penampuli (Tam.).

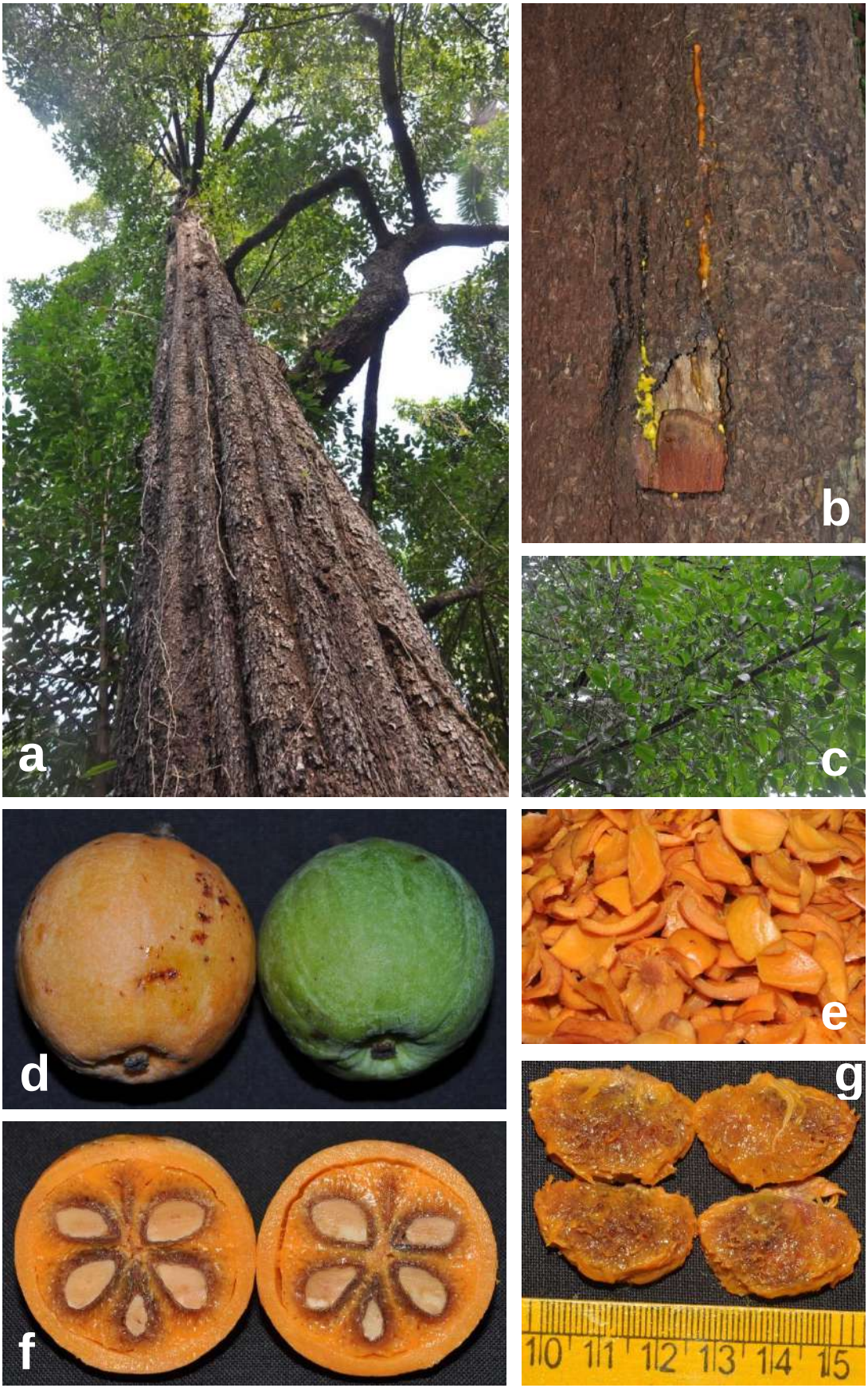


Plate 4.1: ***Garcinia cowa*****. a. Habit; b. Bark showing latex; c. Branch; d. Mature & immature fruits; e. Fresh fruit rind; f. T. S. of mature fruit; g. Seeds.**

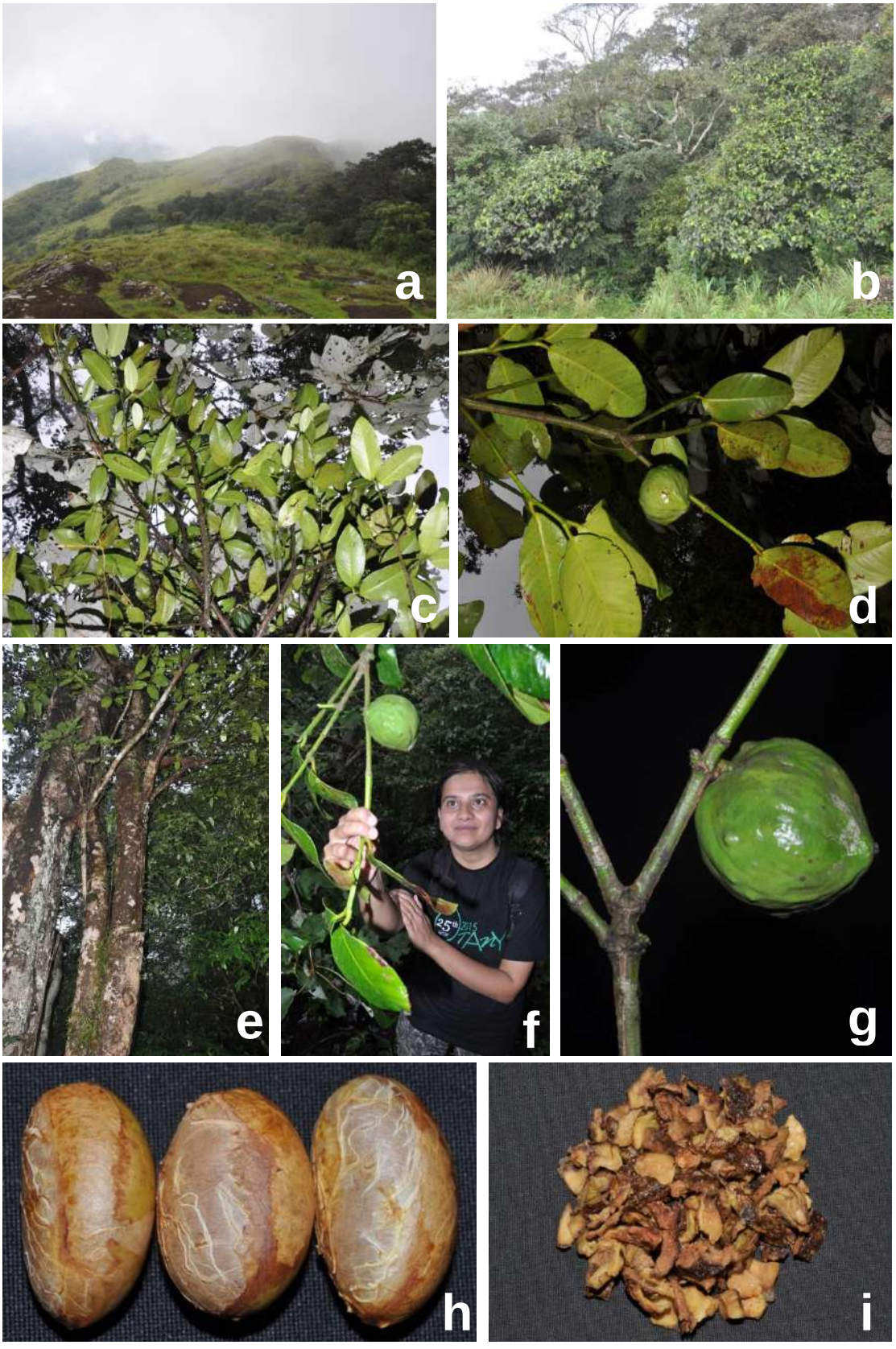


Plate 4.2: ***Garcinia gamblei*****. a. Habitat; b. Habit; c. Twig; d. Twig with closer view of leaf and fruit in natural habitat; e. Tree trunk; f & g. Closer view of fruit; h. Seeds; i. Fruit rind.**

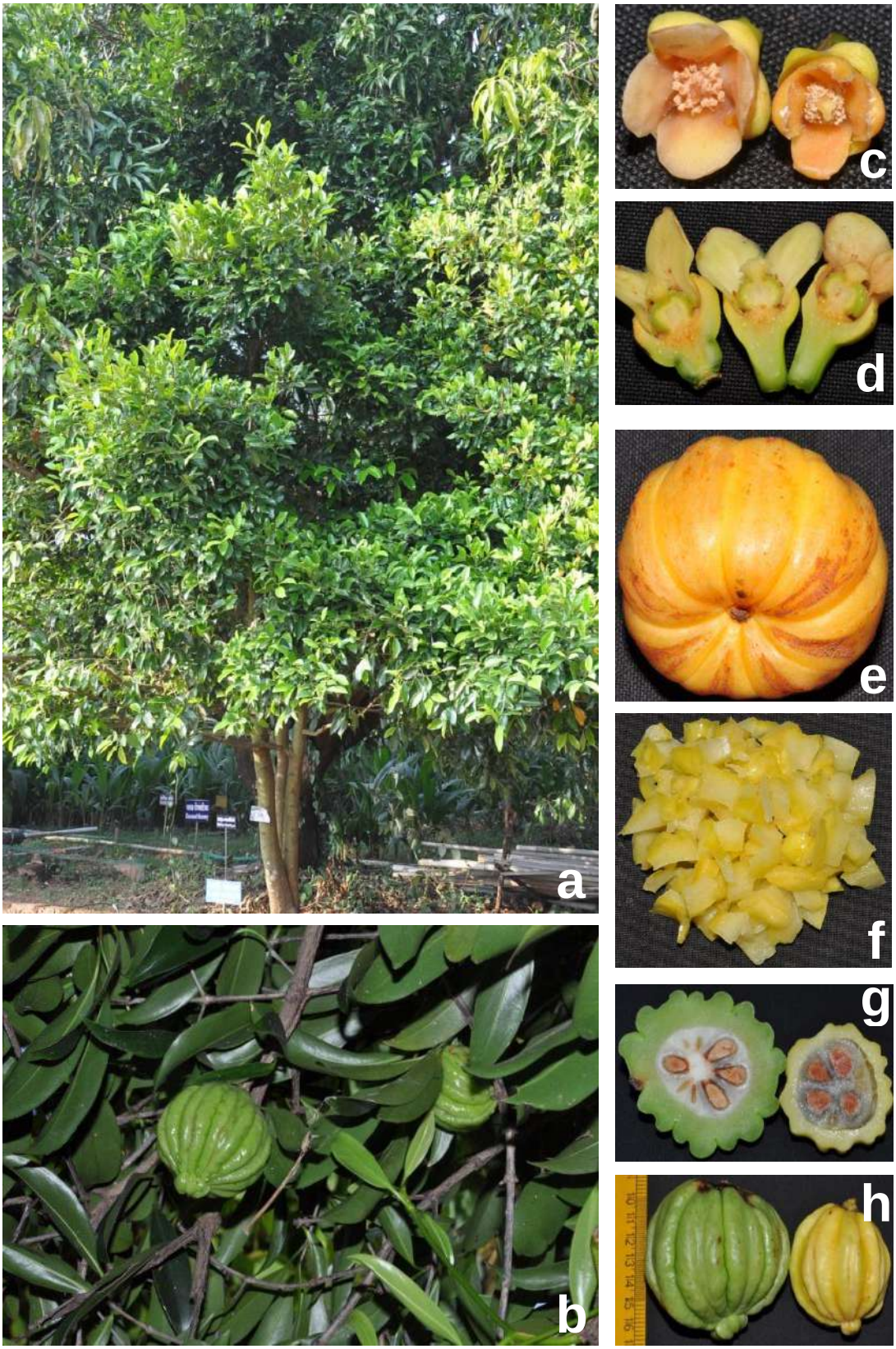


Plate 4.3: *Garcinia gummi-gutta* var. *gummi-gutta*. a. Habit; b. Fruiting branch; c. Male & bisexual flowers; d. L. S. of the younger developmental stage fruit showing latex (gum); e. Mature fruit; f. Fresh fruit rind; g. T. S. of fruits; h. Mature & immature fruits.

Following two varieties of *Garcinia gummi-gutta* namely *Garcinia gummi-gutta* var. *conicarpa* and *Garcinia gummi-gutta* var. *papilla* possess similar morphological characters however; both were identified with key differentiating characters.

4.1.3.4 *Garcinia gummi-gutta* var. *conicarpa* (Wight) N. P. Singh (Plate 4.4a-f)

Key differentiating characters: Leaf ligule absent; stamens 35; ovary 4-locular; stigmatic rays 4; mature fruits ovoid with conical shape containing angular furrows, brownish-purple in colour.

Flowering & fruiting: December to October.

Habitat: Shola, moist evergreen forests at high altitudes (above 1000 m).

Distribution: Kerala, Tamil Nadu. Rare.

Local name: Not known.

4.1.3.5 *Garcinia gummi-gutta* var. *papilla* (Wight) N. P. Singh (Plate 4.4g-j)

Key differentiating characters: Leaf ligule present; stamens 25; ovary 6-8 locular; stigmatic rays 4-8; fruits ovoid-oblong or globose with elongated beak.

Flowering & fruiting: October to February.

Habitat: Shola, moist evergreen forests at higher altitudes (above 1000 m).

Distribution: Kerala, Tamil Nadu. Rare.

Local name: Not known.

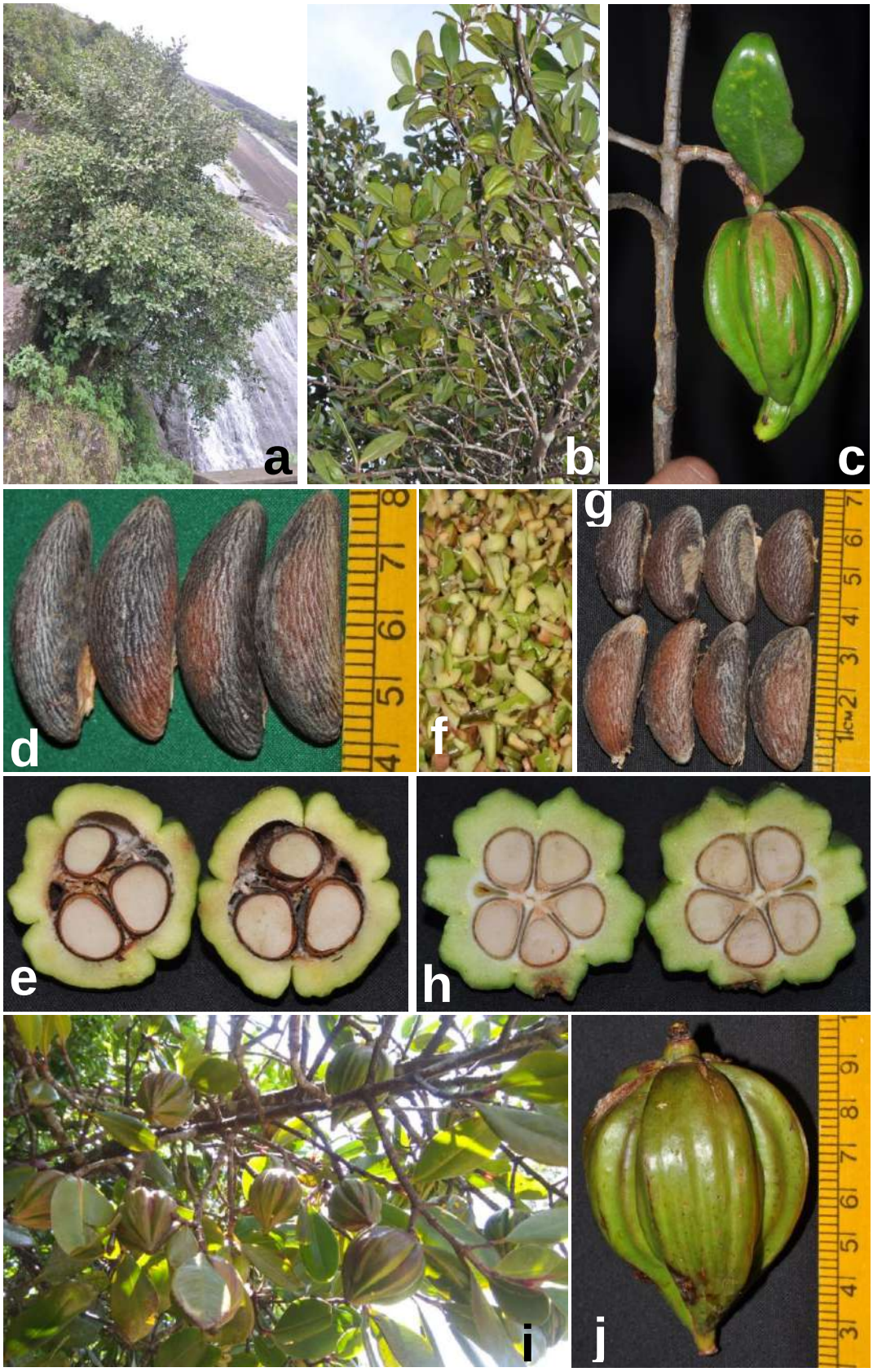


Plate 4.4: a-f. *Garcinia gummi-gutta* var. *conicarpa*. a. Habit; b. Branch showing fruits; c. Twig with conical fruit; d. Seeds; e. T. S. of fruit; f. Fresh fruit rind. g-j. *Garcinia gummi-gutta* var. *papilla*. g. Seeds; h. T. S. of fruit; i. Branch showing fruits; j. Fruit.

4.1.3.6 *Garcinia hombroniana* Pierre (Plate 4.5)

Key differentiating characters: Mature fruits with distinct red colour and sub-globose in shape.

Flowering & fruiting: Throughout the year.

Habitat: Cultivated.

Distribution: Maharashtra, Kerala, Tamil Nadu in botanical gardens. Rare.

Local names: Seashore mangosteen (Eng.).

4.1.3.7 *Garcinia imbertii* Bourd. (Plate 4.6)

Key differentiating characters: Mature fruit in greenish yellow colour with pleasant smell, smooth, globose, elongated at distal end.

Flowering & fruiting: April to December.

Habitat: Shola, moist evergreen forests at higher altitudes above 800 m.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Attupuli, Manja kanji (Mal.); Mania kanji (Tam.)

4.1.3.8 *Garcinia indica* (Thouars) Choisy (Plate 4.7)

Key differentiating characters: Male trees found to be flowering earlier than female trees; fruits smooth, spherical, acidic green when matured dark purple or wine or orange pink in colour.

Flowering & fruiting: November to June.

Habitat: Evergreen, semi evergreen, moist deciduous forests and also cultivated.

Distribution: Common- Maharashtra, Goa, Karnataka; Rare- Kerala, Tamil Nadu.

Local names: Kokum butter tree, The Brindonia tallow tree, Indian gamboge, Wild mangosteen, Red mango, Mangosteen, The mangosteen oil tree, The tomato tree (Eng.); Kokam, Kakam (Hindi); Amsol, Kokam, Kokambi, Bhirand, Bhiran, Ratamba, Bhinda, Katambi (Mar.); Brindall (Kon.); Brindon, Brindeos (Port.); Murgala, Murgal, Muringa-hulimara, Murganhuli, Ponpuli, Punerpuli (Kan.); Penampuli, Marappuli, Kattambi, Kokkam, Punampuli (Mal.); Murgali (Tam.).

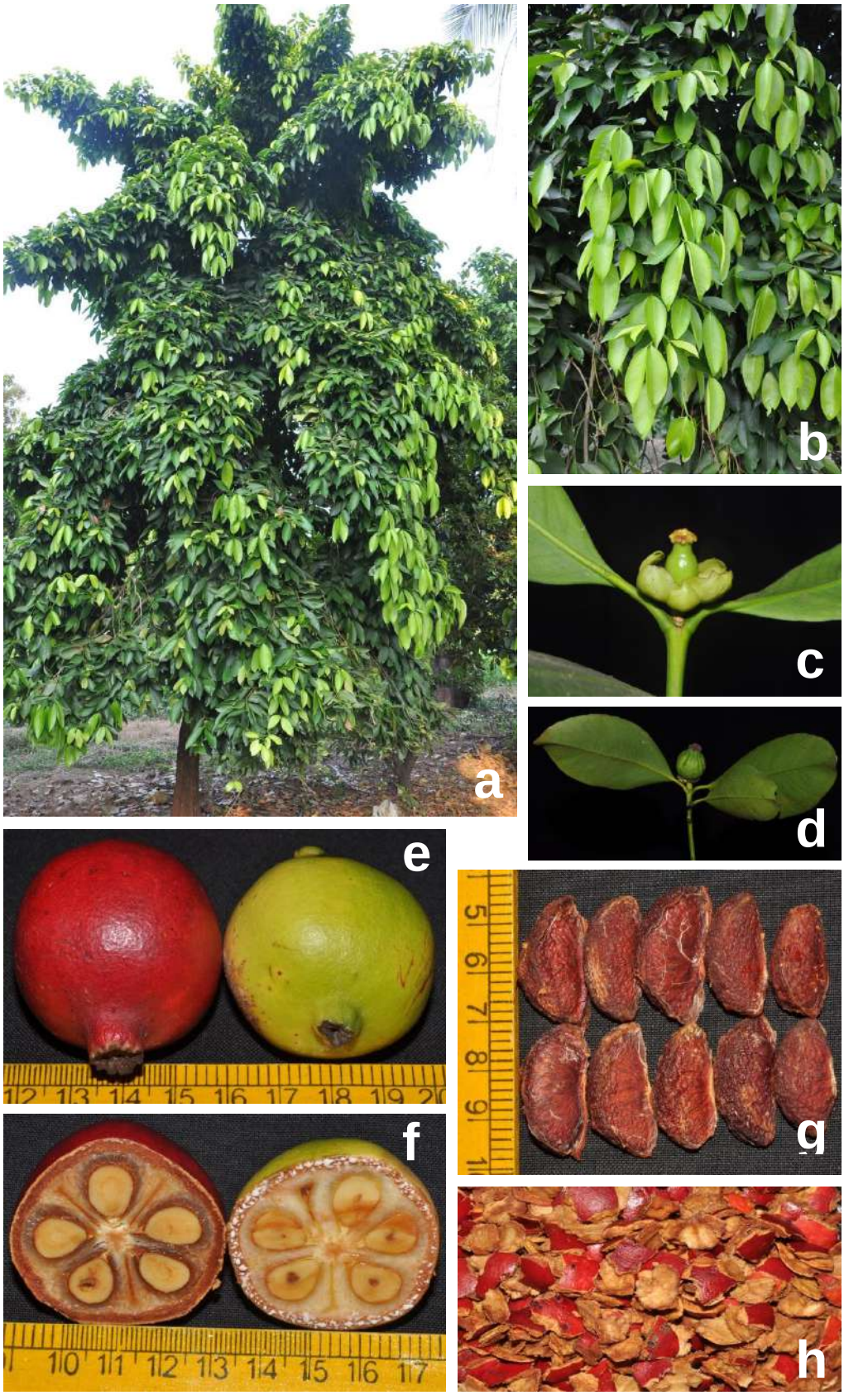


Plate 4.5: ***Garcinia hombroniana*****. a. Habit; b. Branch; c. Twig showing closer view of early developmental stage of fruit; d. Twig with immature fruit; e. Mature & immature fruits; f. T. S. of fruits; g. Seeds; h. Fruit rind.**

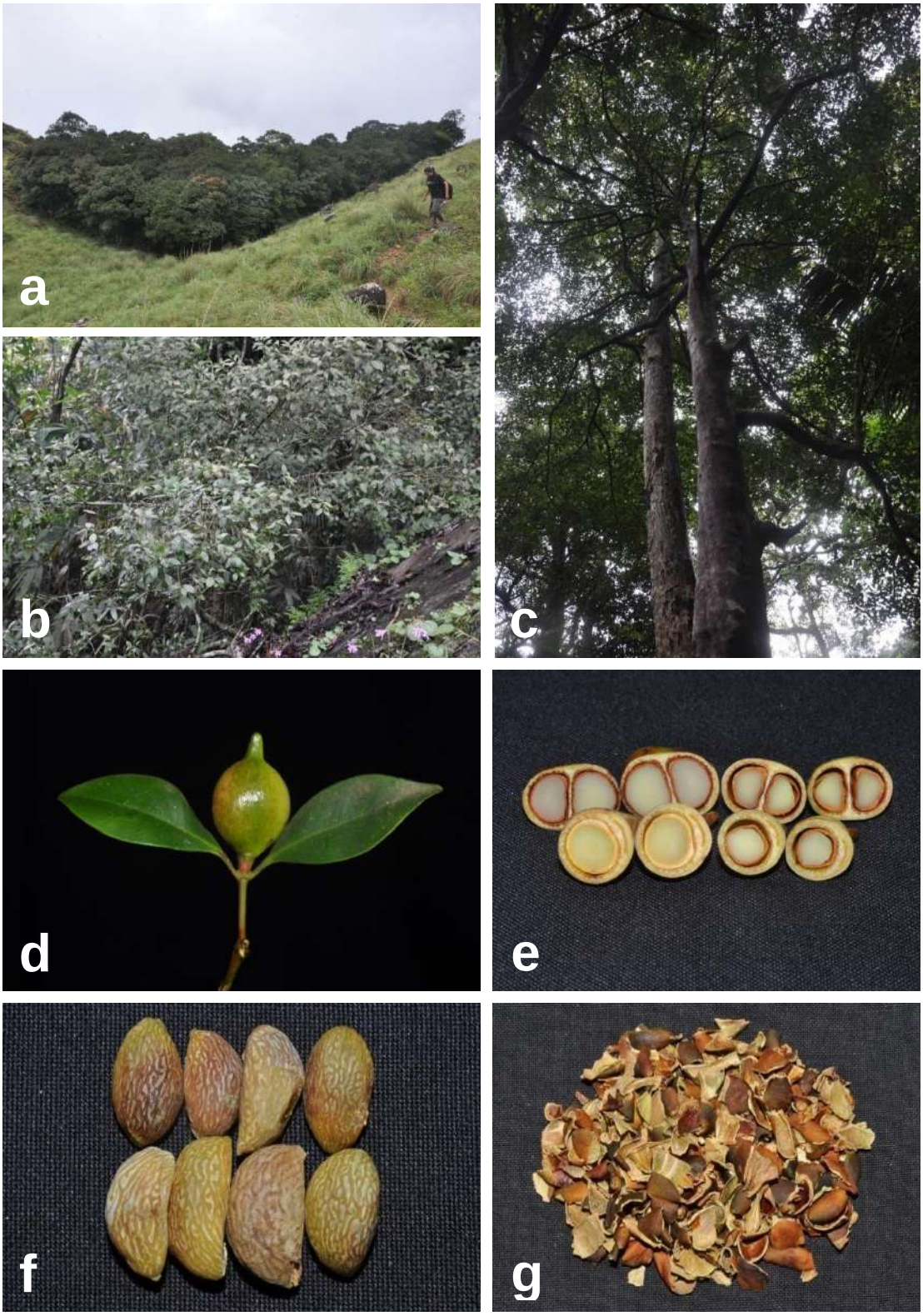


Plate 4.6: *Garcinia imbertii*. a. Habitat; b. Branch; c. Habit; d. Twig with fruit; e. T. S. of fruits; f. Seeds; g. Fruit rind.

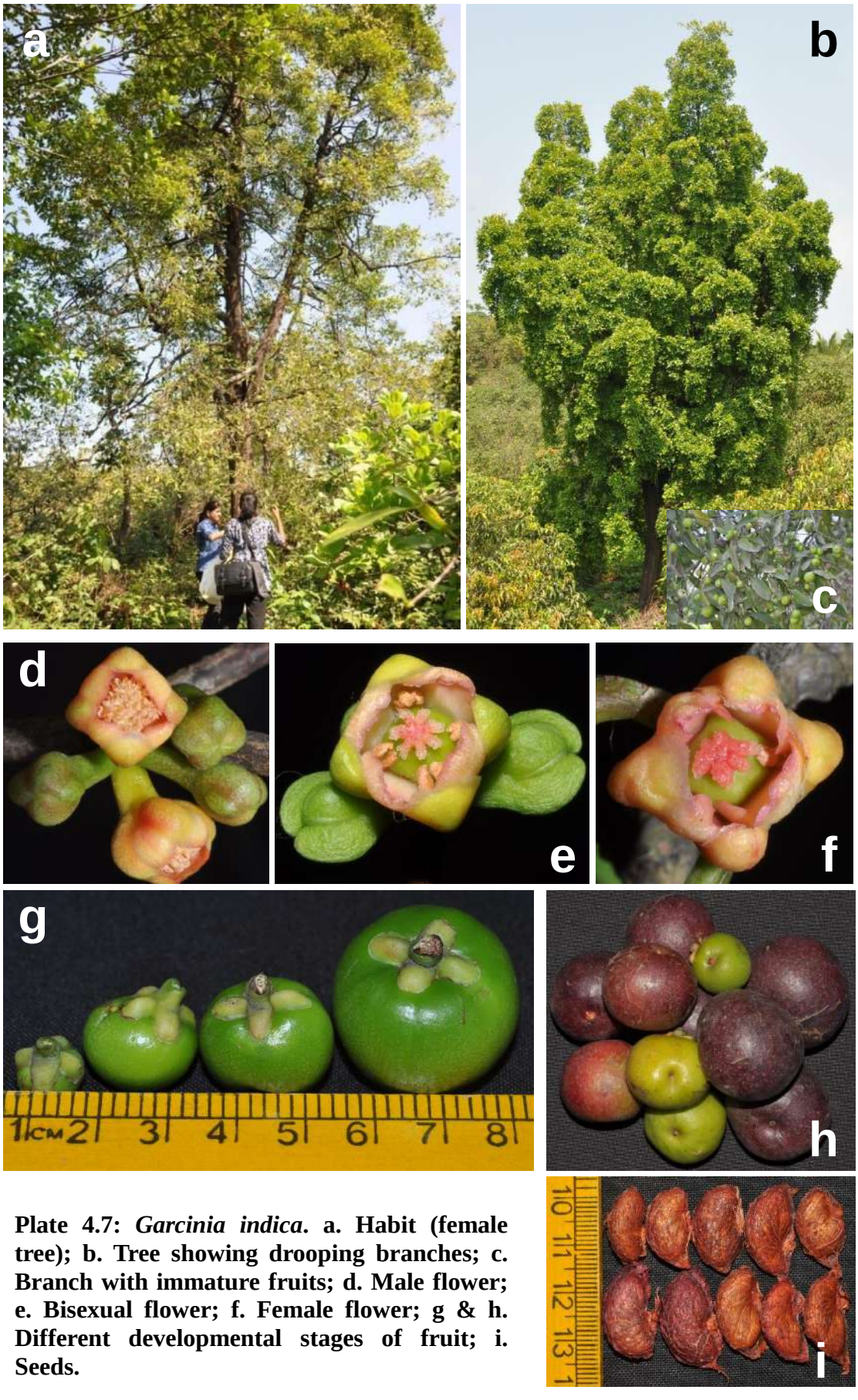


Plate 4.7: *Garcinia indica*. a. Habit (female tree); b. Tree showing drooping branches; c. Branch with immature fruits; d. Male flower; e. Bisexual flower; f. Female flower; g & h. Different developmental stages of fruit; i. Seeds.

4.1.3.9 *Garcinia livingstonei* T. Anderson (Plate 4.8)

Key differentiating characters: Leaves thick; mature fruits globose, smooth and shiny surface, orange in colour.

Flowering & fruiting: February to June.

Habitat: Cultivated.

Distribution: Maharashtra, Karnataka, Kerala in botanical gardens. Rare.

Local names: Not known.

4.1.3.10 *Garcinia mangostana* L. (Plate 4.9)

Key differentiating characters: Dark purple coloured fruit with hard rind.

Flowering & fruiting: January to October.

Habitat: Cultivated.

Distribution: Maharashtra, Goa, Kerala, Tamil Nadu. Common.

Local names: Mangosteen (Eng. & Tam.); Mangusta, Mangustan (Mal.).

4.1.3.11 *Garcinia morella* (Gaertn.) Desr. (Plate 4.10)

Key differentiating characters: Mature fruits globose, smooth, yellowish green to purple coloured with yellow gum.

Flowering & fruiting: November to June.

Habitat: Evergreen, semi evergreen, moist and dry deciduous forests.

Distribution: Goa, Karnataka, Kerala, Tamil Nadu. Common.

Local names: The Mysore gamboge tree, The Indian gamboge tree (Eng.); Tamal, Gotaghanba (Hindi); Devana huli, Murinahuli, Arsina gurgi, Hardala, Ponpuli, Jarize (Kan. & Konk.); Chigiri, Daramba, Iravi, Makki, Karukkampuli, Pinnarpuli, Pulinchikkai (Mal.); Makki, Solaippuli (Tam.).

4.1.3.12 *Garcinia pushpangadaniana* Sabu T. *et al*. (Plate 4.11)

Key differentiating characters: Leaves dark green, leathery; flowers pinkish white in red colour; mature fruits large in size, single fruit weighing around 500 g, greenish brown in colour, apex pointed with distinct mamilla.

Flowering & fruiting: May to December.

Habitat: Moist evergreen, semi evergreen forests at higher altitudes above 1000 m.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Not known.

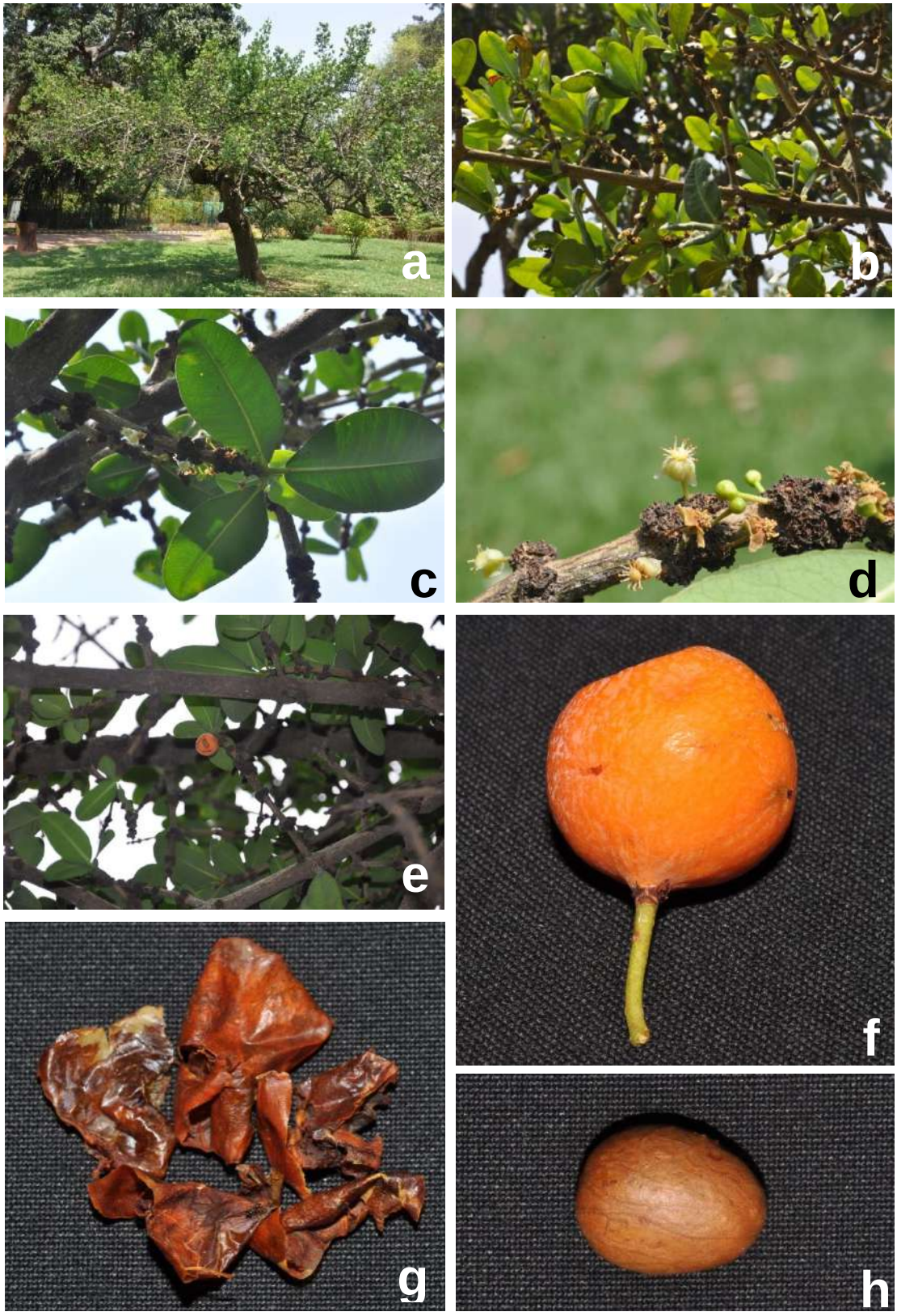


Plate 4.8: *Garcinia livingstonei*. a. Habit; b. Branch showing numerous flowers; c. Closer view of branch; d. Flower; e. Branch showing single fruit; f. Mature fruit; g. Fruit rind; h. Seed.

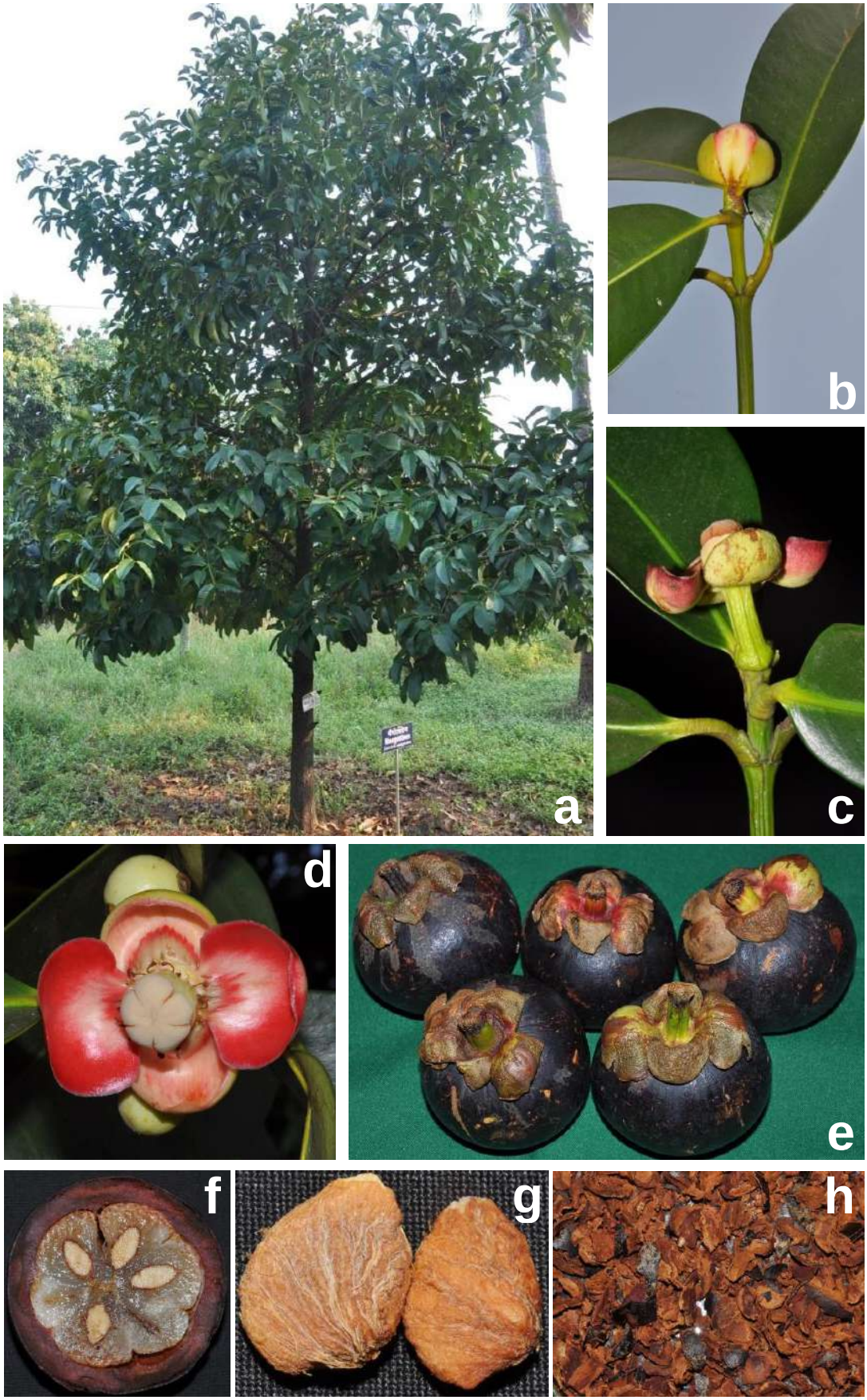


Plate 4.9: ***Garcinia mangostana*****. a. Habit; b. Flower bud; c. Twig bearing flower; d. Bisexual flower; e. Mature fruits; f. T. S. of fruit; g. Seeds; h. Fruit rind.**

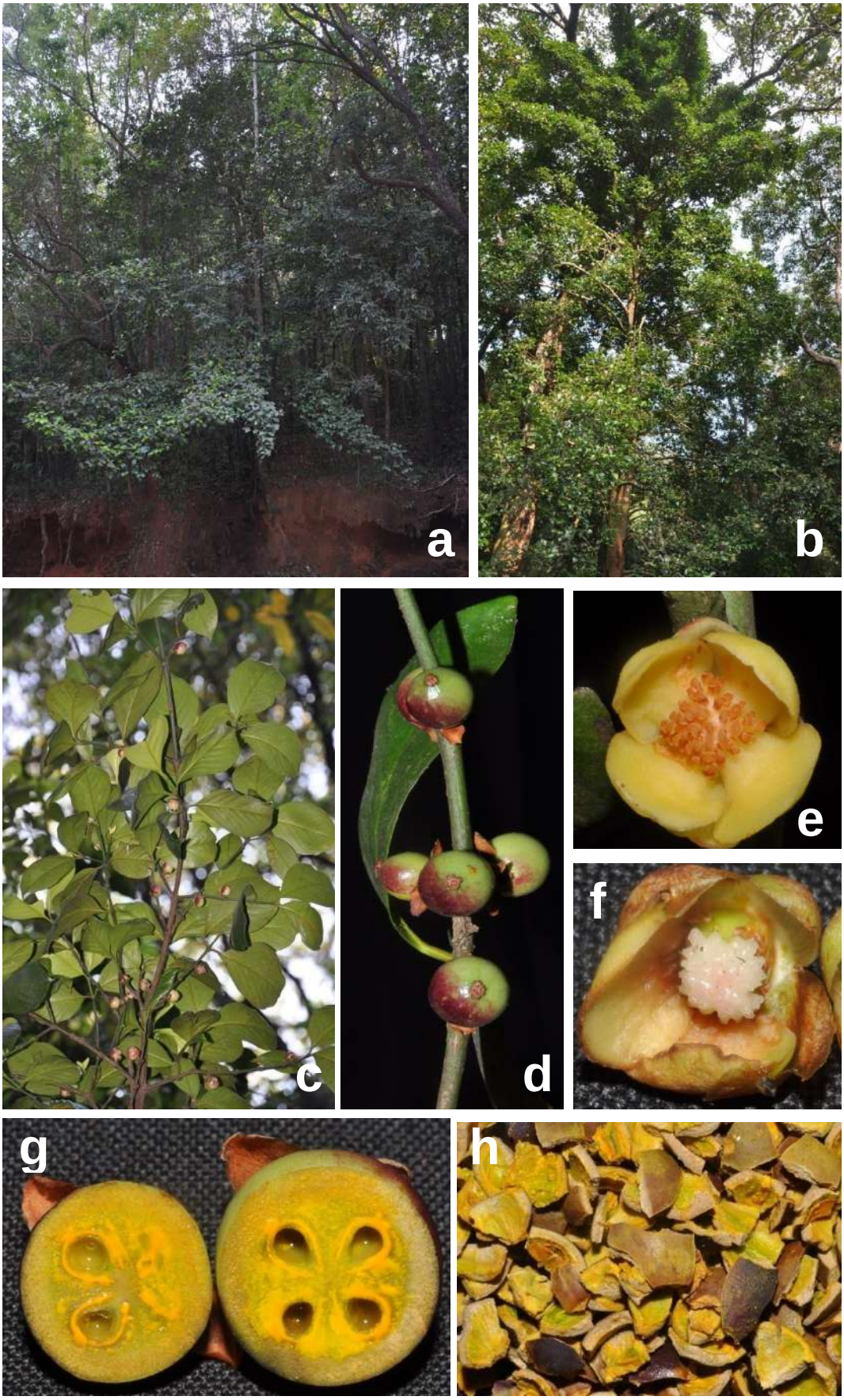


Plate 4.10: ***Garcinia morella*****. a & b. Habit; c. Branch with fruits; d. Fruits; e. Male flower; f. Bisexual flower; g. T. S. of fruit; h. Fruit rind.**

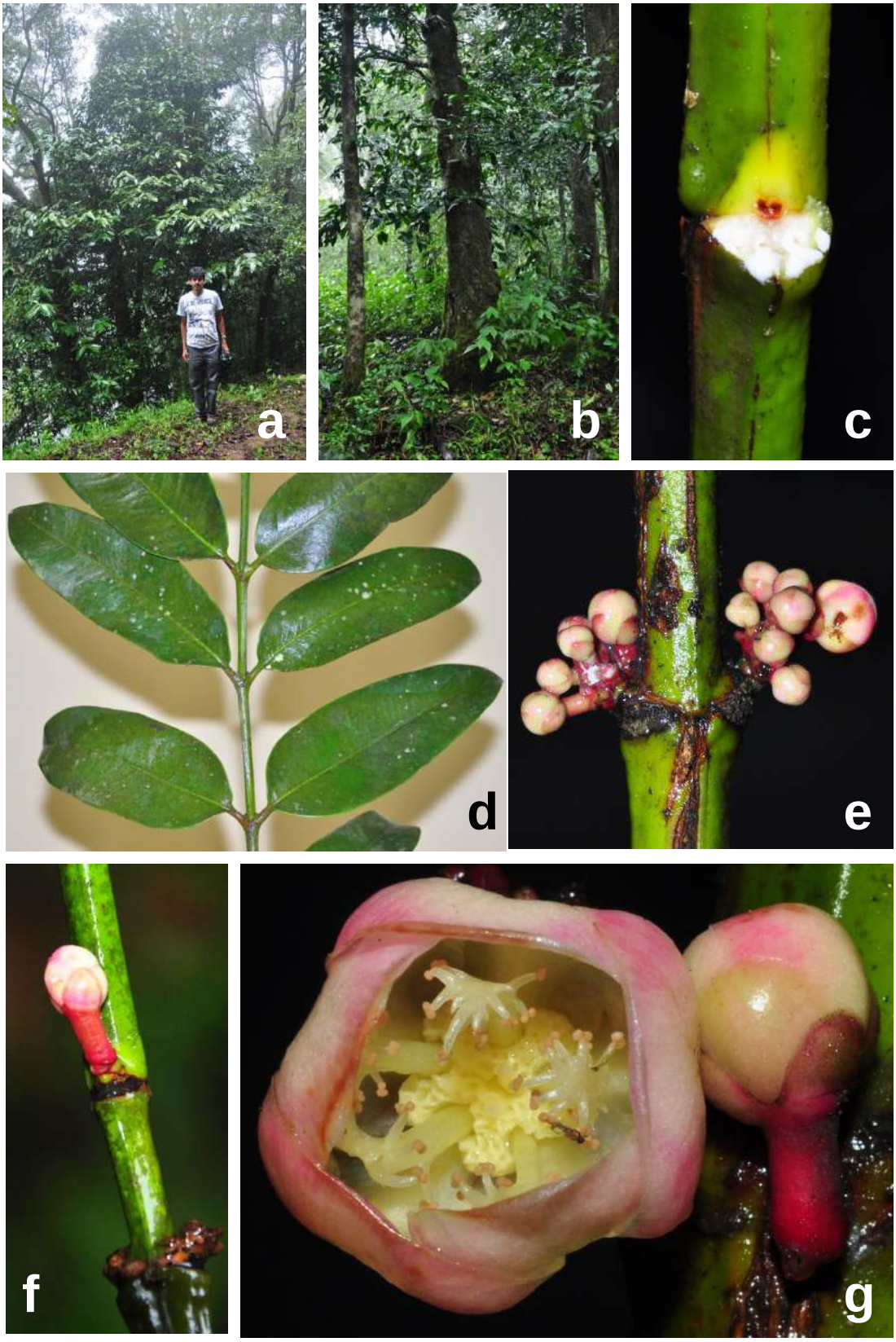


Plate 4.11: ***Garcinia pushpangadaniana*****. a. Habit (male tree); b. Habit (female tree); c. Milky white latex; d. Leaves; e. Male flower buds; f. Female flower bud; g. Male flower.**

Following two species namely *G. spicata* and *G. talbotii* were worked out for their taxonomic identity and occurrence in peninsular India (Palkar *et al*., 2017). As *G. talbotii* is often misidentified as *G. spicata* in the regional floras of northern Western Ghats. This study clearly delimits these two species with detailed description, differentiating characters, photographs depicting morphological and anatomical features (Fig. 4.1) and images of type specimens (Figs. 4.2 & 4.3) to facilitate the accurate identification.

4.1.3.13 *Garcinia spicata* (Wight & Arn.) Hook. f. (Plate 4.12)

Key differentiating characters: Branchlets 4-angled, angles flat; flowers in male plant spicate and fasciculate, spikes 1.4-9.5 cm long; flowers in female plant solitary or in pairs; length of pedicels in female flowers 1.2-3.5 cm; ovary 2-4 locular; stigmatic lobes 3-5; fruits shallowly angular; seeds broadly oblongoid, 1.5-2.5 × 1-1.3 cm, dark brown, black when dry; vascular strand in petiole incurved open with large gap.

Flowering & fruiting: January to August.

Habitat: Dry evergreen, semi evergreen forests at lower altitudes or coastal areas. Sacred groves, temple forests.

Distribution: Andhra Pradesh, Karnataka, Kerala, Tamil Nadu.

Local names: Manjanangu, Kokottai (Mal.); Pinnai maram, Pinnai tree, Kokottai, Perettai (Tam.).

4.1.3.14 *Garcinia talbotii* Raizada ex. Santapau (Plate 4.13)

Key differentiating characters: Branchlets 6-angled, angles concave; flowers in male plant fasciculate, also arranged in numerous pseudospikes of 0.5-0.8 cm long; flowers in female plant fasciculate; length of pedicels in female flowers 0.4-1 cm; ovary 2 or 3-locular; stigmatic lobes 3; fruits smooth; seeds narrowly oblongoid, 2.5-3 × 1.2-1.5 cm, brown; vascular strand in petiole curved open with no gap or minute gap.

Flowering & fruiting: January to June.

Habitat: Evergreen, semi evergreen forests at higher altitudes.

Distribution: Maharashtra, Goa, Karnataka, Kerala.

Local names: Talbot Garcinia, Monkey fruit tree (Eng.); Limboti, Pansara, Tavir, Poyandi, Phansada (Mar.); Haldi, Ont (Kan.)

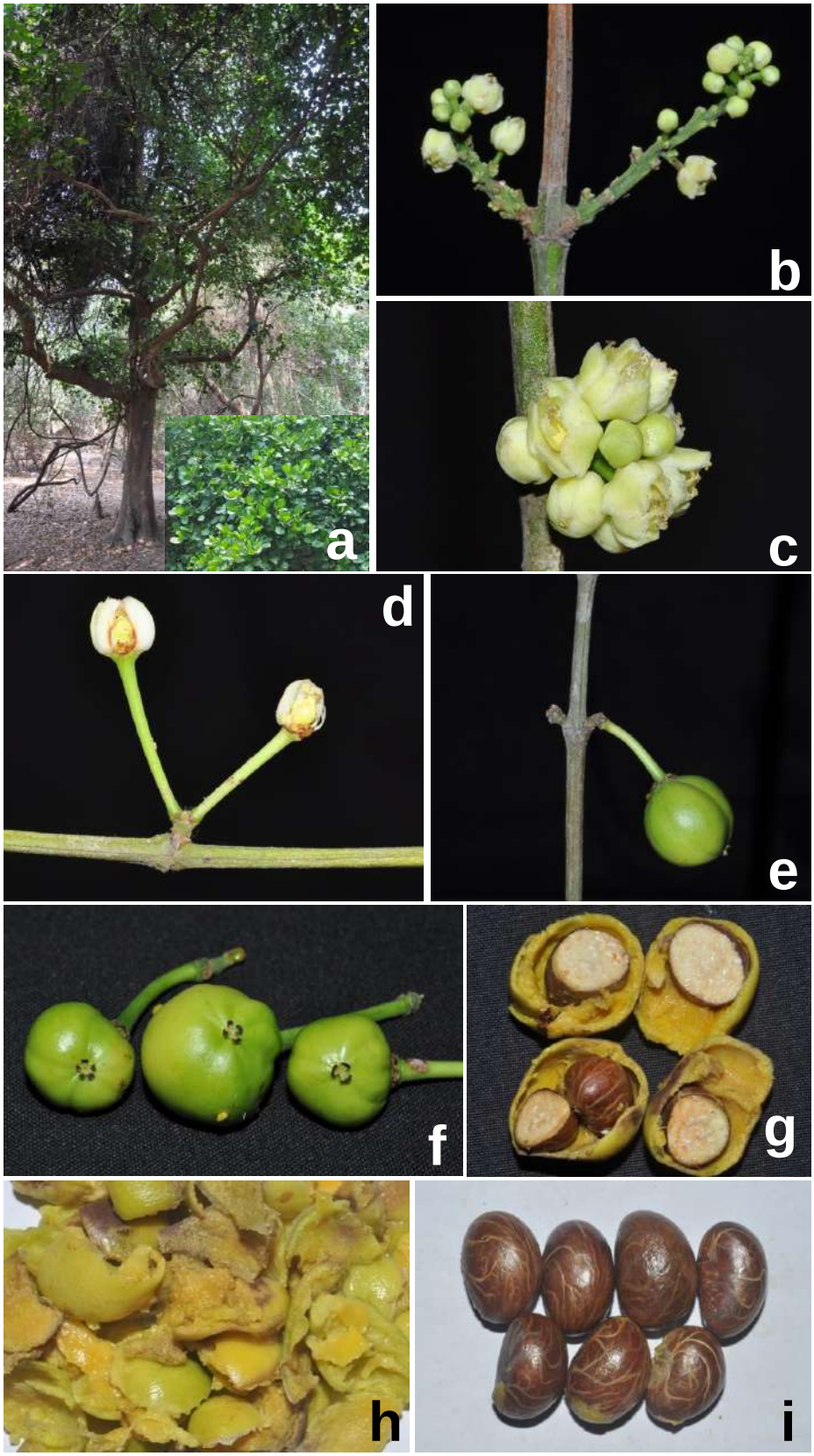


Plate 4.12: ***Garcinia spicata*****. a. Habit (insert- branch); b. Spicate inflorescence; c. Fasciculate inflorescence; d. Female flower with long pedicel; e. Immature fruit with ridges and furrows; f. Fruits with 3-5 stigmatic lobes; g. T. S. of fruits; h. Fresh fruit rind; i. Seeds.**

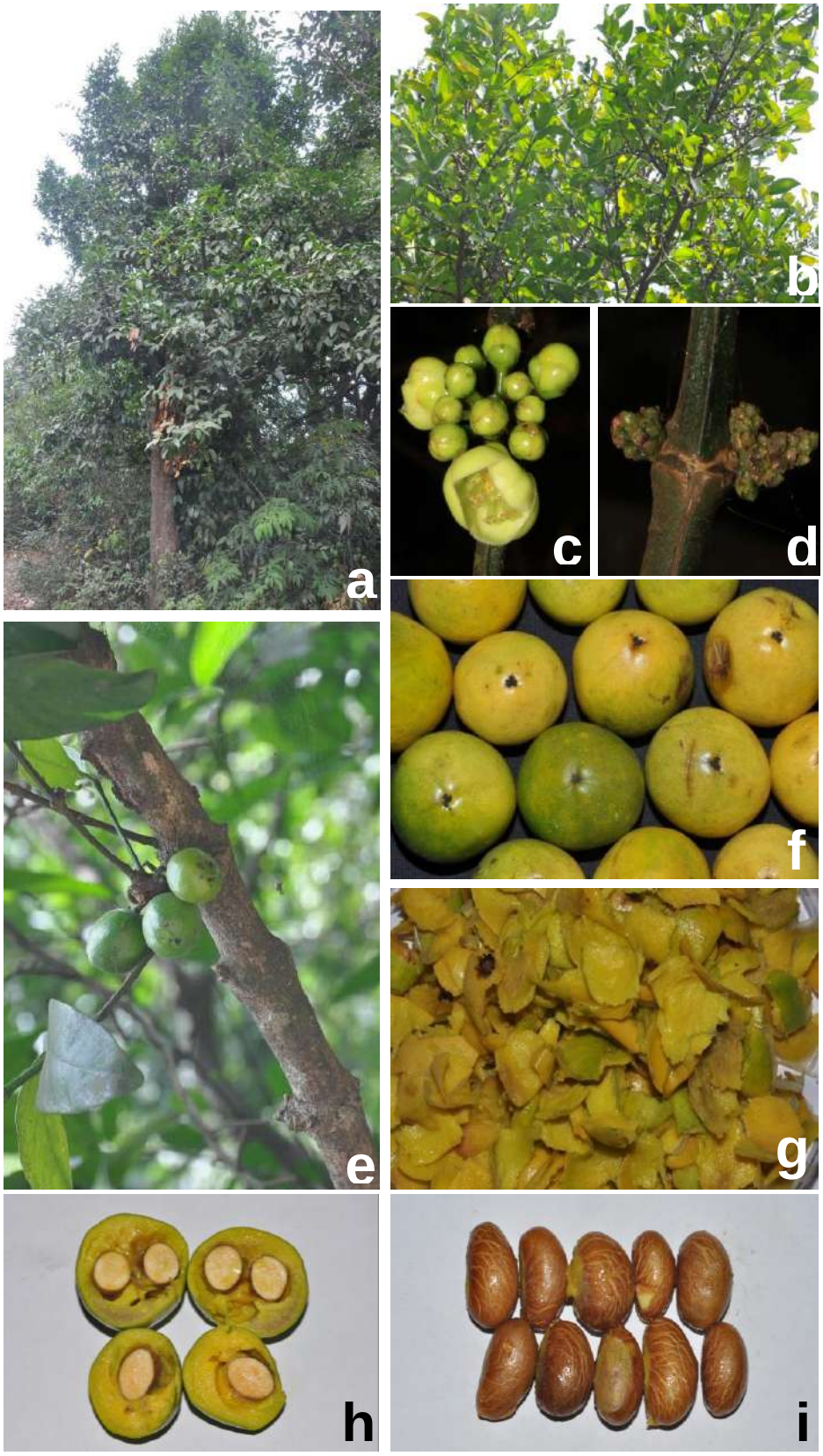


Plate 4.13: ***Garcinia talbotii*****. a. Habit; b. Branch showing numerous spikes in male plant; c. Fasciculate inflorescence (male); d. Closer view of spike; e. Immature fruit with smooth surface; f. Mature fruits showing 3-lobed stigma; g. Fresh fruit; h. T. S. of fruit; i. Seeds.**

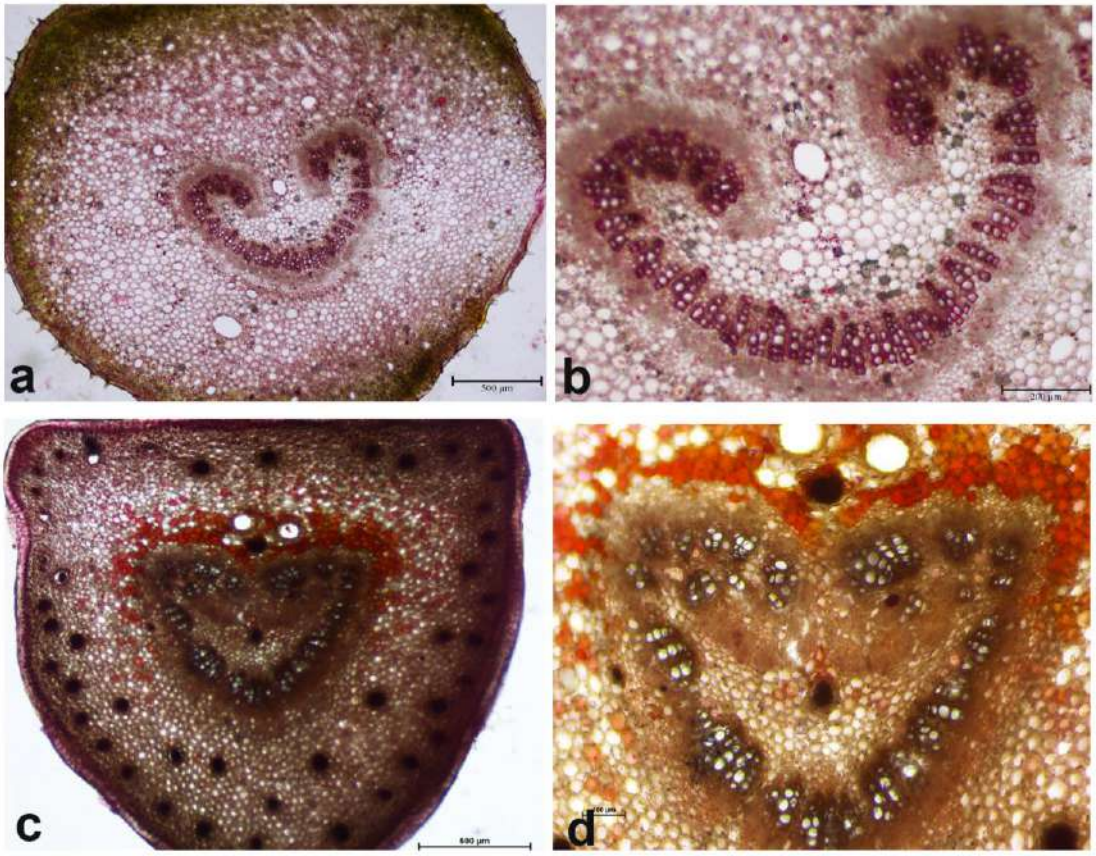


Figure 4.1: T. S. of petioles stained with Safranin. a & b. *Garcinia spicata* showing incurved open vascular strand with large distinct gap; c & d. *Garcinia talbotii* with curved vascular strand with very minute gap.



Figure 4.2: Syntype of *Garcinia spicata* (Wight & Arn.) Hook.f. (≡ *Xanthochymus spicatus* Wight & Arn. [R. Wight 346A, E00179065] @Royal Botanic Garden, Edinburgh.



Figure 4.3: Holotype of *Garcinia talbotii* Raizada ex Santapau (*Garcinia ovalifolia* var. *macrantha* Vesque) [W.A. Talbot 3713, BSI0000000003] @Botanical Survey of India, Western Regional Centre, Pune.

4.1.3.15 *Garcinia rubro-echinata* Kosterm. (Plate 4.14)

Key differentiating characters: Male flowers with fascicles in red colour however, female flowers are solitary; fruits spiny, yellowish pink when matured.

Flowering & fruiting: October to February.

Habitat: Moist evergreen forests at higher altitudes above 950 m.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Para, Pura, Malamkongu (Mal.); Madul (Tam.).

4.1.3.16 *Garcinia travancorica* Bedd. (Plate 4.15)

Key differentiating characters: Leaf upper surface dark green and lower surface pale green in colour; fruit dark green, sub-globose contracted into a short, thick style with a broad stigma, pleasant smell.

Flowering & fruiting: September to May.

Habitat: Shola, moist evergreen forests at higher altitudes above 1000 m.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Malampongu (Mal.); Malampunku (Tam.).

4.1.3.17 *Garcinia wightii* T. Anderson

Key differentiating characters: Leaves linear to lanceolate, thin, pale green in colour; fruits sub-globose, smooth.

Flowering & fruiting: October to February.

Habitat: Moist evergreen, semi evergreen forests.

Distribution: Kerala, Tamil Nadu. Rare.

Local names: Attukaruka, Pulimaranga, Kolivala (Mal.); Palimaranga (Tam.).

4.1.3.18 *Garcinia xanthochymus* Hook. f. (Plate 4.16)

Key differentiating characters: Very long, thick, shiny leaves; mature fruits dark yellow in colour, smooth, pulpy like mango.

Flowering & fruiting: Throughout the year.

Habitat: Evergreen, semi evergreen, moist deciduous forests and also cultivated.

Distribution: Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu. Common.

Local names: Mysore gamboge, Yellow mangosteen (Eng.); Dampel, Tamal, (Hindi); Jangali ramphal, Jharambi, Dharambo, Ota, Vilayati amba (Mar.); Dhanambe (Kon.); Tavir, Haldi, Deavkai, Devajaringe, Devajaarige, Gansargi, Janagi, Devangi, Jaarige (Kan.); Anavaya, Bhaviyam, Monthanpuli, Vairappuli (Mal.); Tamalam, Mukki, Kulavi, Malaippachai (Tam.).

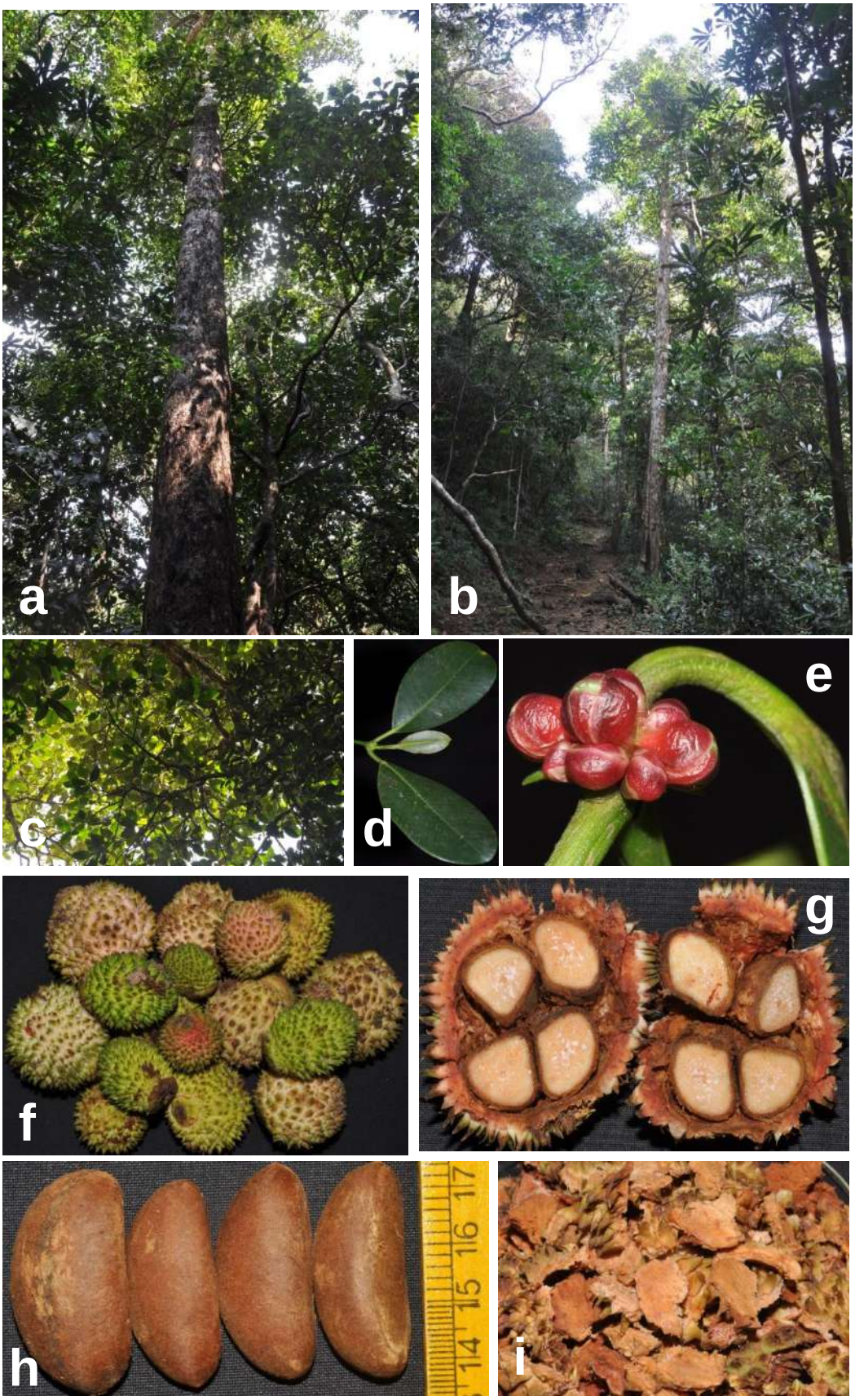


Plate 4.14: ***Garcinia rubro-echinata*****. a. Habit; b. Habitat; c. Fruiting branches; d. Leaves; e. Male flower buds; f. Mature & immature fruits; g. T. S. of fruit; h. Seeds; i. Fruit rind.**

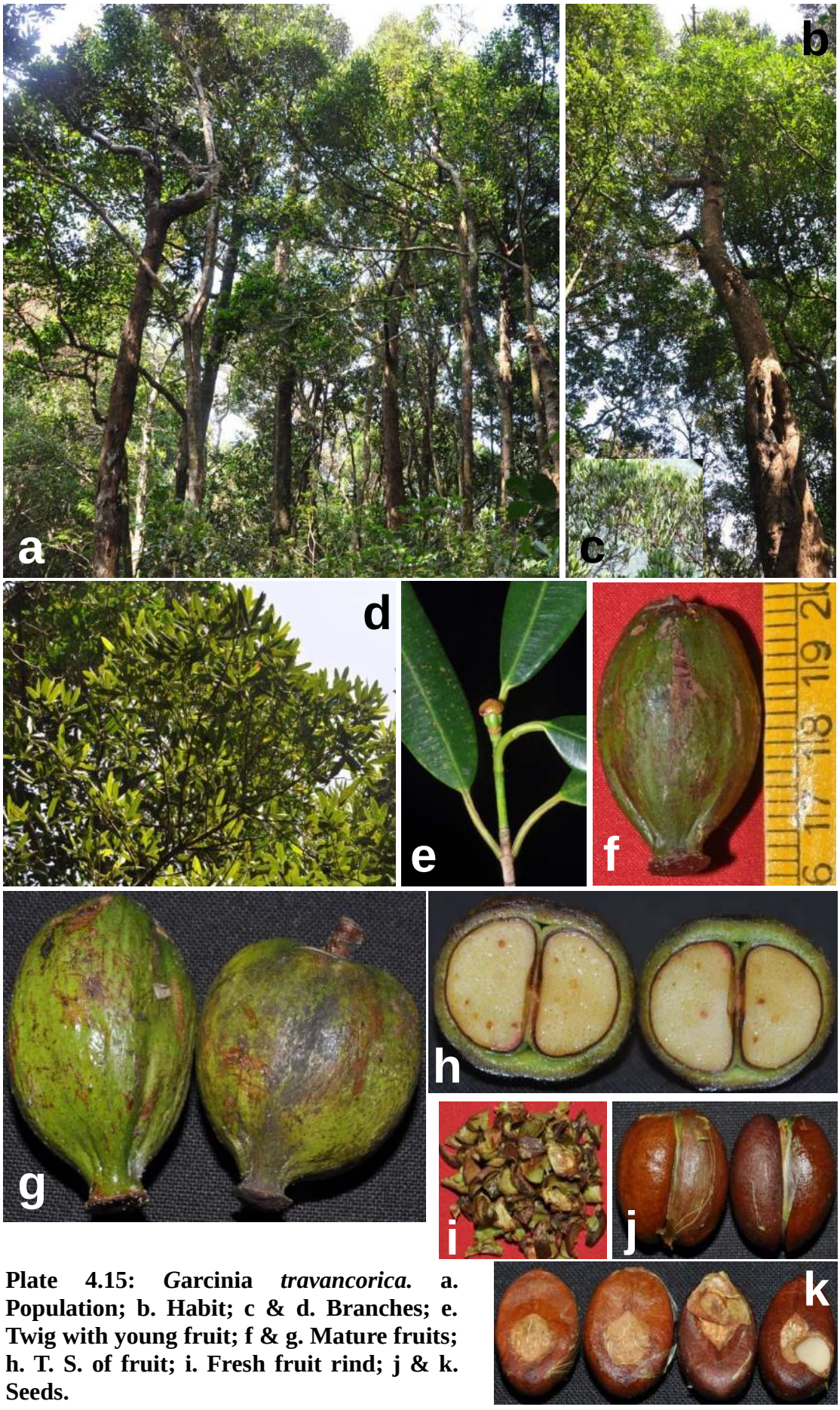


Plate 4.15: *Garcinia travancorica.* a. Population; b. Habit; c & d. Branches; e. Twig with young fruit; f & g. Mature fruits; h. T. S. of fruit; i. Fresh fruit rind; j & k. Seeds.

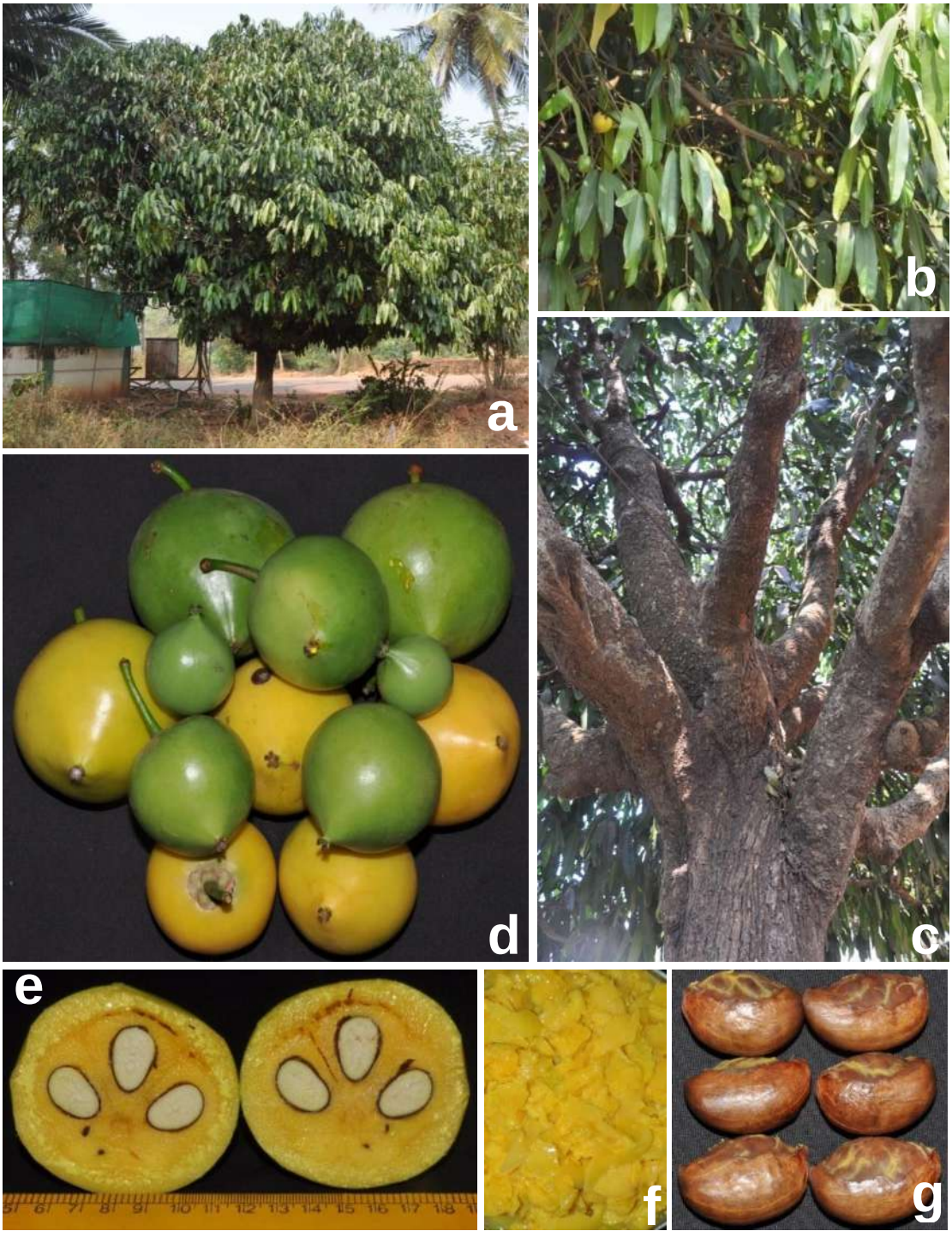


Plate 4.16: *Garcinia xanthochymus*. a. Habit; b. Fruiting branch; c. Tree trunk showing branching pattern; d. Mature & immature fruits; e. T. S. of fruit; f. Fresh fruit rind; g. Seeds.

A total of 20 different herbarium specimens containing 16 *Garcinia* species with 2 varieties were prepared with standard protocol and deposited in Madras Herbarium (MH) (Plate 4.17). Accession numbers provided by the MH for deposited herbarium specimens are incorporated in the letter no. BSI/SRC/5/23/2019/Tech./713 dated 9 July 2019.

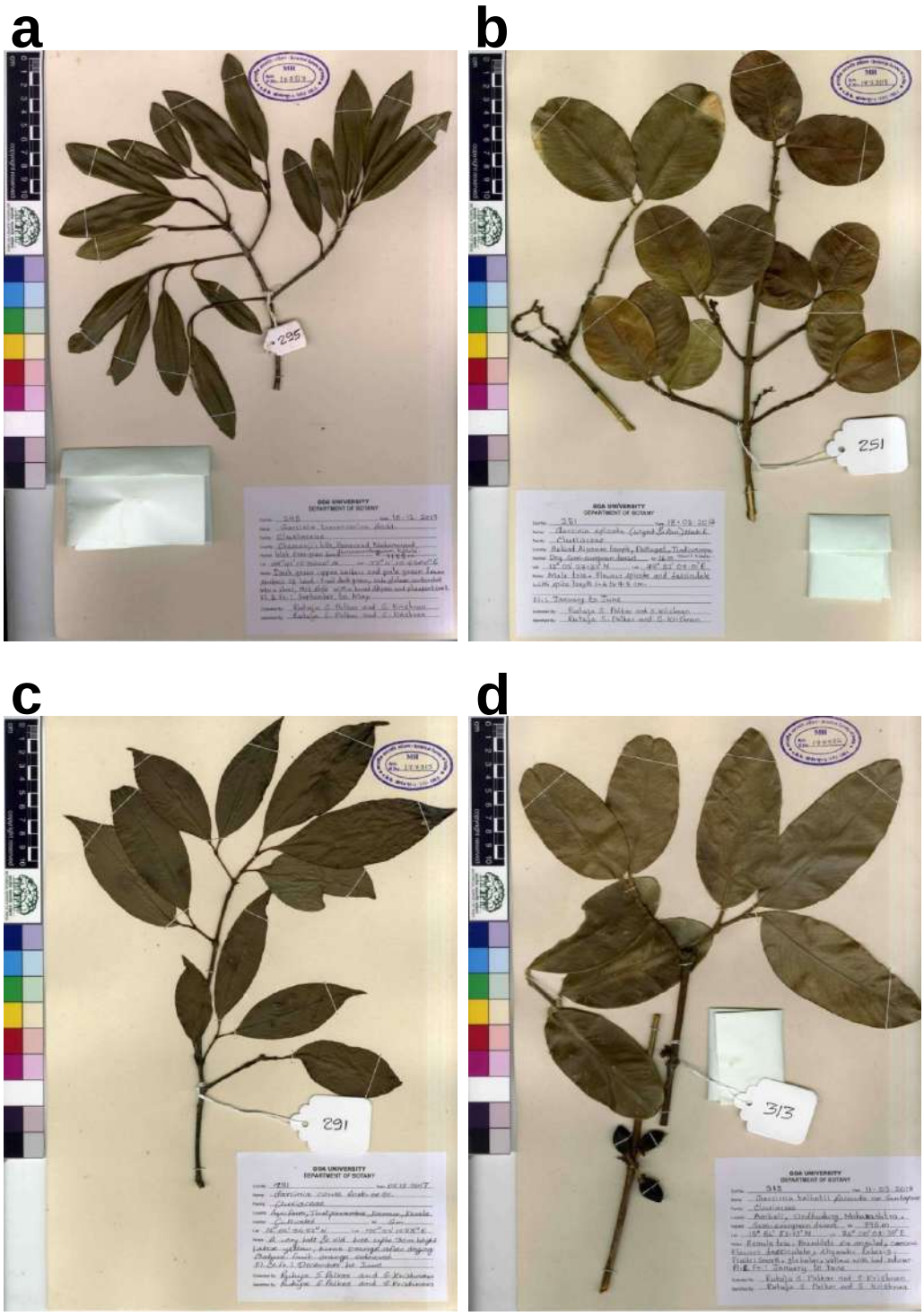


Plate 4.17: Representative herbarium specimens of *Garcinia* species submitted to MH. a. *G. travancorica*; b. *G. spicata*; c. *G. cowa*; d. *G. talbotii.*

4.1.4 Ecological niche modeling study (Palkar *et al*., 2020)

Distributional data for the species *Garcinia indica* from wild populations were collected during field survey and predicted for its potential distribution in the Western Ghats of India based on the species occurrence data and environmental variables using predictive modeling approaches in MaxEnt and BIOCLIM implemented in DIVA-GIS. Results of the analysis were interpreted based on the prediction maps and jackknife evaluation, percent contribution and permutation importance of each variable. Potential distribution of *G. indica* was predicted with the help of primary occurrence data using MaxEnt and BIOCLIM models and prediction of habitat suitability by both algorithms was found to be almost similar. The prediction maps obtained using both algorithms MaxEnt and BIOCLIM depicted the potential geographical distribution, range of richness and density of *G. indica* in the Western Ghats of India (Figs. 4.5 & 4.6). However, the model performance was evaluated based on AUC values which showed MaxEnt model with better performance of 0.99 AUC value compared with BIOCLIM model with 0.91 AUC value. High AUC value obtained during this study by using MaxEnt model indicates the significant prediction that is discrimination between the presence and absence areas for the species distribution. Hence, among the two algorithms used the best fit model was found to be MaxEnt and considered for elaborate interpretation in the study. In addition to threshold-independent AUC value (ROC curve), the MaxEnt algorithm was validated with omission/commission rate. The analysis of omission/commission revealed the lines of average omission and predicted area were close to each other which indicated that the model performed better than random and showed that the model is more accurate (Fig. 4.4a & b). The MaxEnt prediction revealed the potential range for richness of the species and habitat suitability is excellent at the junction of Northern-Central Western Ghats mainly comprising Konkan region of Maharashtra, Goa and Karnataka as indicated in the map as 80-100%. This part comprises Sindhudurg and Ratnagiri districts of Maharashtra; North and South Goa districts of Goa and Uttara Kannada and Udupi districts of Karnataka. Additionally, small part of Northern Kollam district of Kerala state also found to be excellent for the niche of the species. The range of high (40-60%) and very high (60-80%) habitat suitability was found to occur in the regions of Raigad district of Maharashtra; Dakshina Kannada district of Karnataka; and Kasargod, Alappuzha, Pathanamthitta and Idukki districts of Kerala in

a lesser extent. The suitability for niche of the species was found to be medium (20-40%) in the regions of Palghar, Thane and Northern part of Raigad districts of Maharashtra; Kodagu district of Karnataka; and Kannur, Wayanad and Northern part of Thiruvananthapuram district of Kerala. BIOCLIM algorithm also depicted similar prediction to MaxEnt and high habitat suitability was found to occur in the regions of Raigad, Ratnagiri and Sindhudurg districts of Maharashtra; North and South Goa districts; and Uttar Kannada district of Karnataka.

The richness and density of *G. indica* was significantly influenced by few environmental variables. The major environmental factors responsible for the species distribution and habitat suitability predicted by MaxEnt and BIOCLIM (Fig. 4.7) models were precipitation and temperature. The estimates of percent contribution and permutation importance of each variable were shown in Table 4.2. These results were consistent with jackknife evaluation (Fig. 4.4c). Considering the percent contribution, eight environmental variables out of 19 were contributed to the model output. Among them, precipitation of wettest month (BIO 13) (34.2%) and temperature seasonality (BIO 4) (16.2%) have maximum influence on the habitat suitability followed by precipitation in the month of June (PREC 6) (13.4%) and annual precipitation (BIO 12) (10.8%). However, the permutation importance revealed that the temperature seasonality (BIO 4) (32.7) was found to be major factor followed by precipitation in the month of July (PREC 7) (19.5), precipitation in the month of June (PREC 6) (6.4) and altitude influences the suitability in a lesser extent. Along with the above parameters, other parameters were also responsible in a lesser extent for the species distribution pattern.

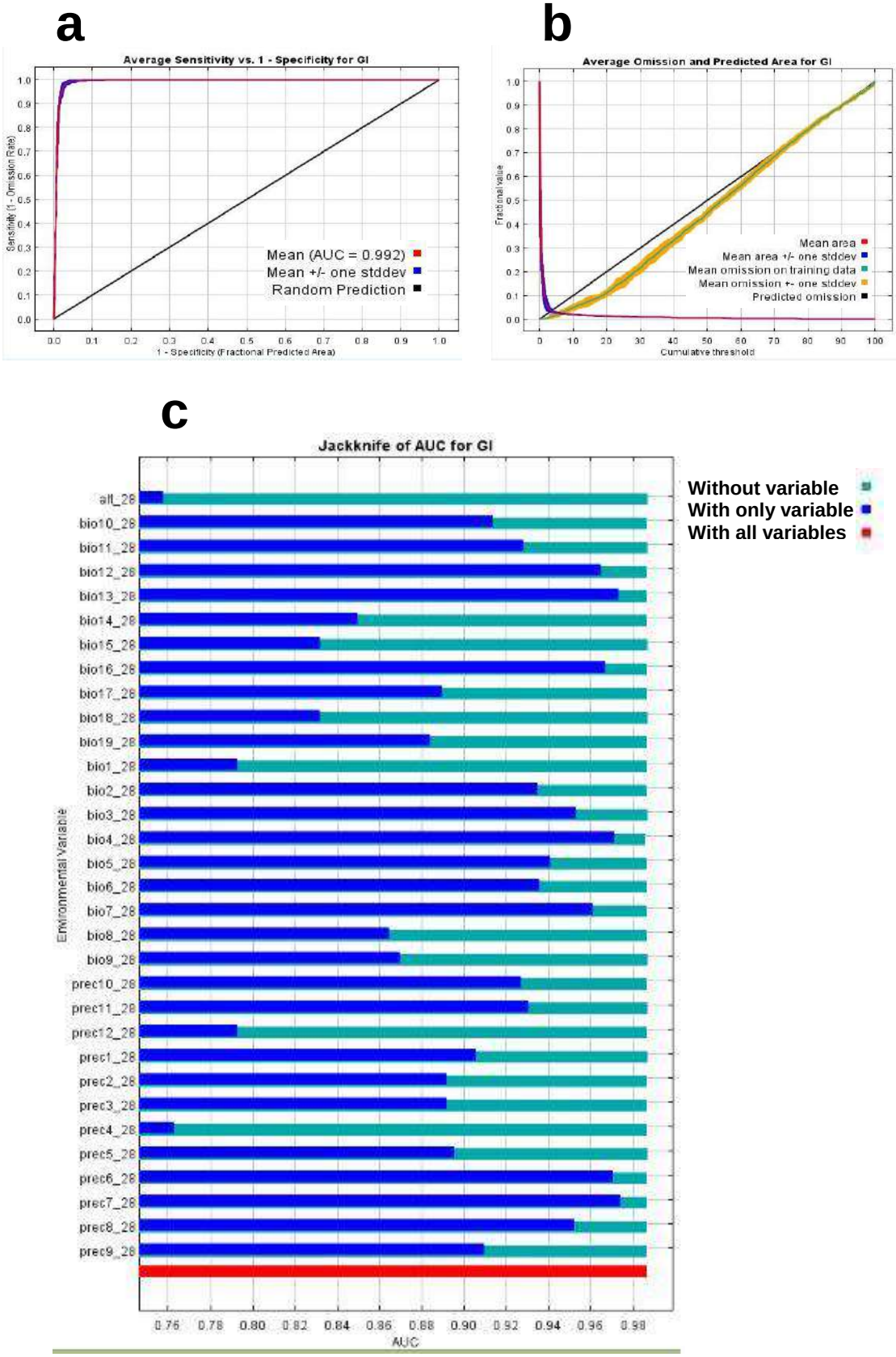


Figure 4.4: a. The receiver operating characteristic (ROC) curve; b. Omission versus predicted area; c. Jackknife test, using AUC showing importance of different environmental variables.

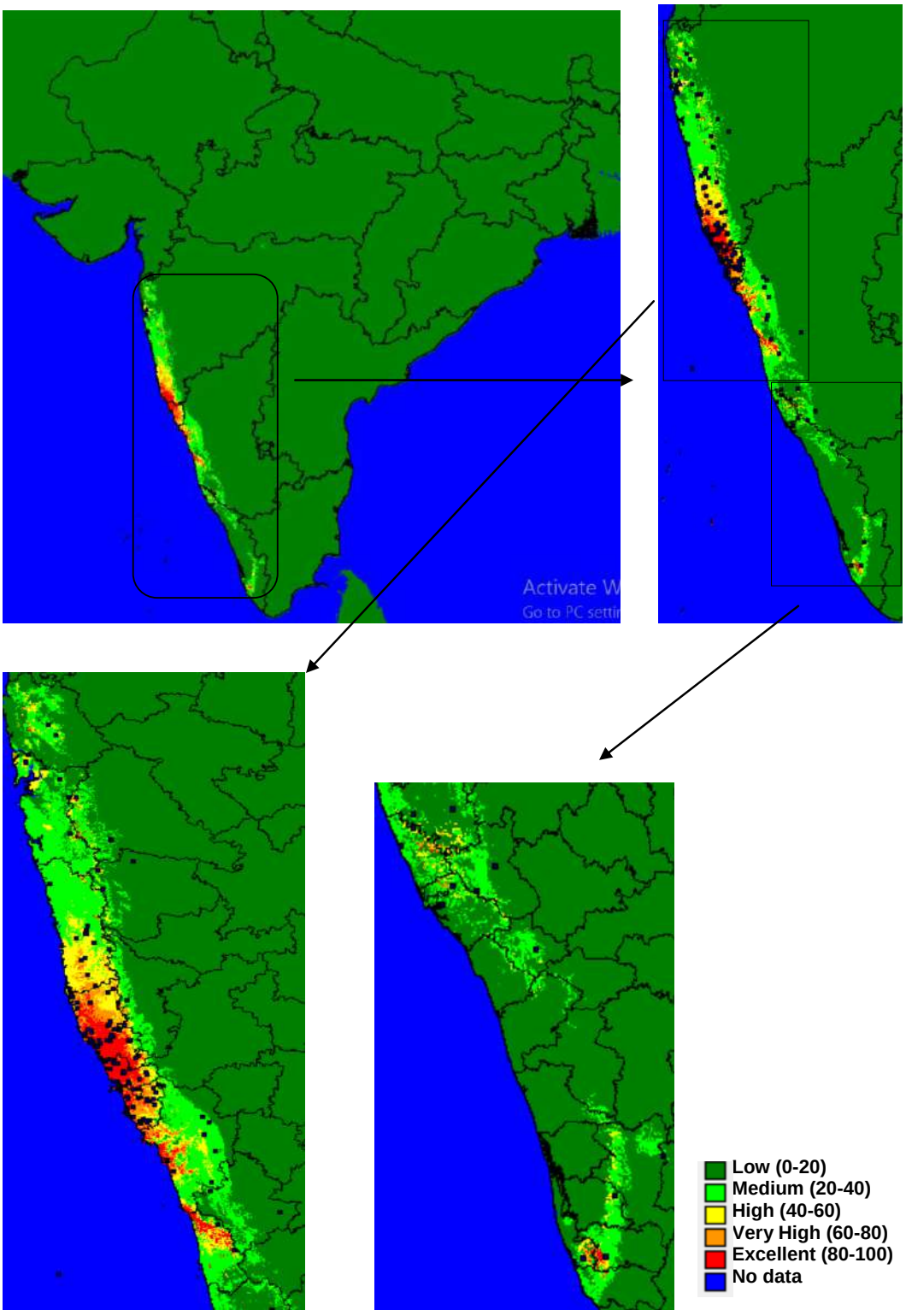


Figure 4.5: Predicted (maximum) potential distributional area of the Western Ghats of India which signifies different levels of probability of occurrence and actual occurrences of the *Garcinia indica* under current climatic conditions with MaxEnt.

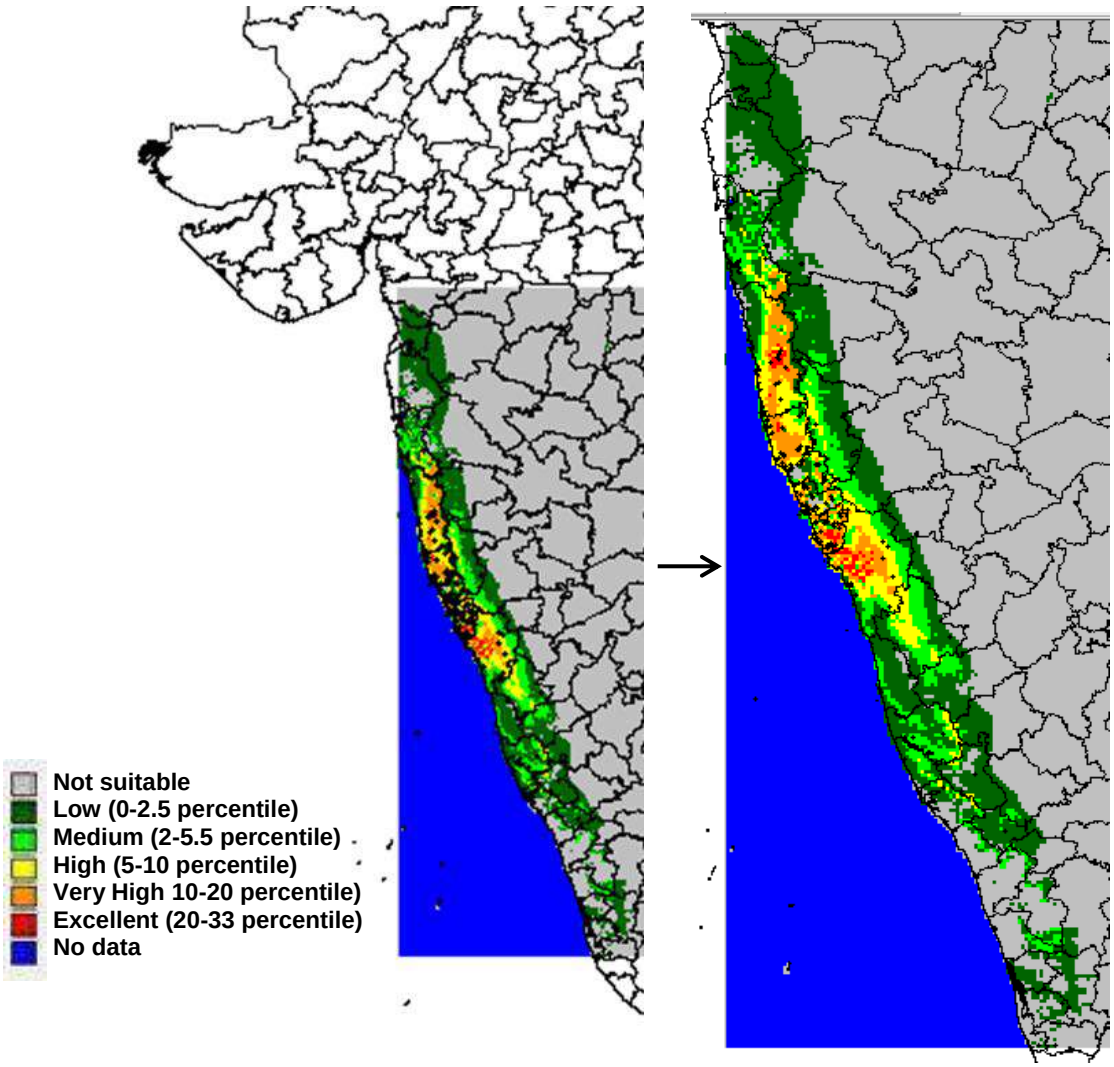


Figure 4.6: Predicted (maximum) potential distributional area of the Western Ghats of India which signifies different levels of probability of occurrence and actual occurrences of the *Garcinia indica* under current climatic conditions with BIOCLIM.

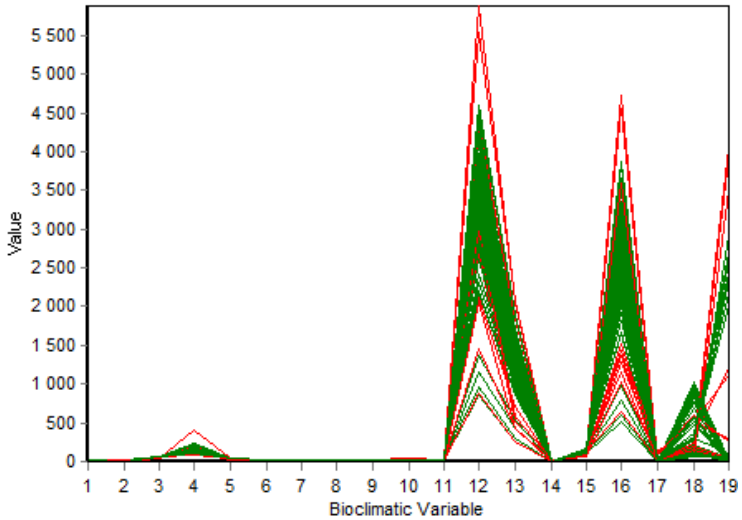


Figure 4.7: Relative contribution of the environmental variables with BIOCLIM.

Table 4.2: BIOCLIM layers used in the study with their percent contribution and permutation importance.

Code	Environmental Variable	Percent contribution	Permutation importance
bio13	Precipitation of wettest month	34.2	0.1
bio4	Temperature seasonality (standard deviation ×100)	16.2	32.7
prec6	Precipitation in the month of June	13.4	6.4
bio12	Annual precipitation	10.8	0.2
prec7	Precipitation in the month of July	5	19.5
bio19	Precipitation of coldest quarter	3.4	2.2
bio16	Precipitation of wettest quarter	3.2	0.3
bio7	Temperature annual range (Bio5 - Bio6)	1.4	0.4
bio11	Mean temperature of coldest quarter	1	3.5
bio9	Mean temperature of driest quarter	1	3
bio17	Precipitation of driest quarter	0.9	0.1
bio6	Minimum temperature of coldest month	0.9	0.2
prec5	Precipitation in the month of May	0.8	1.8
bio8	Mean temperature of wettest quarter	0.8	0.8
bio3	Isothermality ((Bio2/Bio7) × 100)	0.8	0.5
prec1	Precipitation in the month of January	0.7	0
prec2	Precipitation in the month of February	0.7	0.5
prec11	Precipitation in the month of November	0.6	4.9
bio15	Precipitation seasonality (CV)	0.6	1.4
bio10	Mean temperature of warmest quarter	0.6	0.6
bio5	Maximum temperature of warmest month	0.5	0.7
bio1	Annual mean temperature	0.5	0.7
prec4	Precipitation in the month of April	0.4	1.8
prec9	Precipitation in the month of September	0.4	2.4
prec10	Precipitation in the month of November	0.3	4.2
prec3	Precipitation in the month of March	0.3	0.9
bio2	Mean diurnal range (mean of monthly max. and min. temp.)	0.2	0.9
prec8	Precipitation in the month of August	0.2	0.2
bio18	Precipitation of warmest quarter	0.2	2.3
Alt	Altitude	0.1	4.3
prec12	Precipitation in the month of December	0.1	1.4
bio14	Precipitation of driest month	0.1	0.8

4.2 Quantitative estimation of bio-active compounds garcinol and hydroxycitric acid (HCA) during various developmental stages of fruits of *Garcinia indica* and matured fruits of other *Garcinia* species using HPLC system.

4.2.1 Plant material, extract preparation and extraction yield

During collection trips; matured, healthy fruits of ten different *Garcinia* species namely *G. gummi-gutta*, *G. hombroniana*, *G. indica*, *G. livingstonei*, *G. mangostana*, *G. morella*, *G. pushpangadaniana*, *G. spicata*, *G. talbotii* and *G. xanthochymus* were collected. Along with above species, six different developmental stages of fruits of *G. indica* were also collected based on number of days after fertilisation. Dried rinds of matured fruits of different *Garcinia* species and various developmental stages of *G. indica* fruits were extracted with methanol solvent, concentrated and used for the analysis. After complete extract preparation, extraction yield for all samples was calculated. Dried fruit rinds of different developmental stages of *G. indica* yielded semi-solid extracts which are ranged from 4.77 to 6.48 g per 10 g of fruit (Table 4.3). Among different *Garcinia* species dry fruit rind of *G. livingstonei* yielded highest quantity i.e. 8.1 g and *G. morella* yielded lowest quantity i.e. 1.45 g of extract per 10 g of fruit (Table 4.4).

4.2.2 Thin-layer chromatography (TLC) for confirmation of hydroxycitric acid

TLC was carried out with different methanolic extracts to confirm the presence of hydroxycitric acid (HCA) in samples. Formation of yellowish orange coloured spots after applying 1% sodium metavanadate spray was observed in all samples which confirmed the presence of HCA. Also, presence of HCA was confirmed by Rf values at yellowish orange coloured spots which was found in range of 0.26 to 0.35.

4.2.3 High performance liquid chromatography (HPLC) analysis

To quantify both the bioactive compounds HCA and garcinol reversed phase HPLC analysis was carried out. Initially to quantify both compounds simultaneous method was tried. Since, this method was not suitable for both the compounds; two separate methods were developed, validated and used for quantification.

4.2.3.1 Method development, validation and quantification of hydroxycitric acid (HCA)

4.2.3.1.1 Method development and equipment conditions

The chromatographic conditions were developed by changing different mobile phases such as 0.1% orthophosphoric acid in water and acetonitrile mixture; acetonitrile and water mixed with methanol and acetic acid. Also, to develop the method two different elution patterns were checked as gradient as well as isocratic mode by changing flow rates. Finally suitable and easy method was developed and used for further analysis with 8 mM sulfuric acid as mobile phase using isocratic elution pattern with 1 ml/min flow rate at 210 nm wavelength. After injecting standard HCA solution two separate peaks of hydroxycitric acid and hydroxycitric acid lactone were obtained with two different retention times 3.2 and 1.9 mins respectively (Fig. 4.8a; Table 4.5).

Table 4.5: Peak details for standard HCA detected at 210 nm wavelength.

Peak	Name	R. T. (min)	Area	Height
1	HCA-lactone	1.958	74281	10192
2	HCA	3.220	325212	30555
Total			399493	40748

4.2.3.1.2 Method validation

After development of method it must be validated. Hence for method validation; carry over test, system suitability test and linearity were performed. These all tests were found to occur in acceptable limit (Fig. 4.8).

4.2.3.1.2.1 Carry over test

Carry over test was performed to check the appearance of an analyte in a run when a blank containing no analyte is injected. In this methanol as a blank was injected and it showed absence of any peak of an analyte in it (limit < 0.0025 %) (Fig. 4.8a).

4.2.3.1.2.2 System suitability

The system suitability test was checked using reproducibility test (% RSD) with six injections of standard solutions of HCA. Percent RSD for standard HCA was 0.126% (limit < 1%) (Fig. 4.8b).

4.2.3.1.2.3 Linearity and calibration curve

To check the linearity, calibration curve was constructed using different concentrations of standard HCA verses area. Calibration curve was linear in the concentration range 5-50 µg/ml and showed good linear regression with correlation coefficient (R^2 = 0.9999, limit > 0.999) and regression equation y = 6525.92x + 325.611 (Fig. 4.8c).

4.2.3.1.3 Quantification of hydroxycitric acid (HCA) from samples

Based on the HPLC method developed and validated, quantification of HCA from extracts of different developmental stages of *G. indica* and matured stage of other *Garcinia* fruit rinds was carried out. Presence or absence of HCA in samples was confirmed by comparing their retention times (R.T.) with standard HCA solution R.T and quantity was determined from the peak area of HPLC chromatograms. Representative HPLC chromatograms showing HCA peak area for fruit extracts of different *Garcinia* species and developmental stages of *G. indica* are provided in Figures 4.9 and 4.10. Since, HCA is one of the major organic acid present in genus *Garcinia*; all the different developmental stages of dry fruit rinds of *G. indica* showed the presence of HCA which is varied from 776.94 mg to 2713 mg per 10 g of fruit rind (Table 4.3). Highest amount of HCA was found in fruit rind of developmental Stage-I (Fig. 4.10b); however, 1880.54 mg of HCA quantity was obtained in matured fruit rind i.e. developmental Stage-VI (Fig. 4.10d). Along with *G. indica* fruit rinds, the extract obtained from mature fruit rinds of different *Garcinia* species showed presence of HCA. Extract of dried fruit rind of *G. indica* showed highest amount of HCA i.e. 1880.54 mg and *G. mangostana* showed lowest amount of HCA i.e. 15.39 mg per 10 g of fruit rind (Table 4.4).

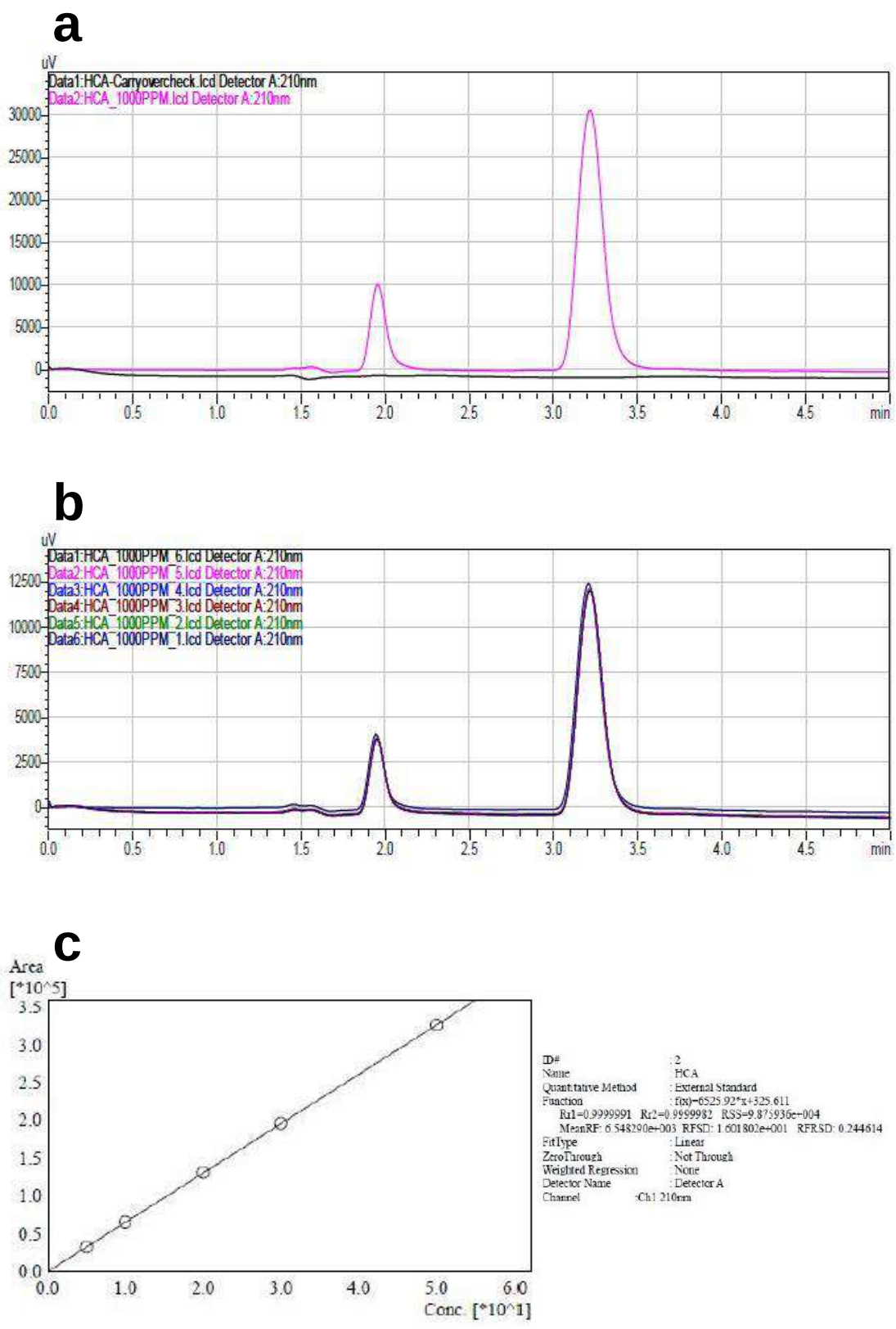


Figure 4.8: a. HPLC chromatogram of standard HCA (pink colour) and carry over test (black colour); b. reproducibility test; c. calibration curve showing linearity.

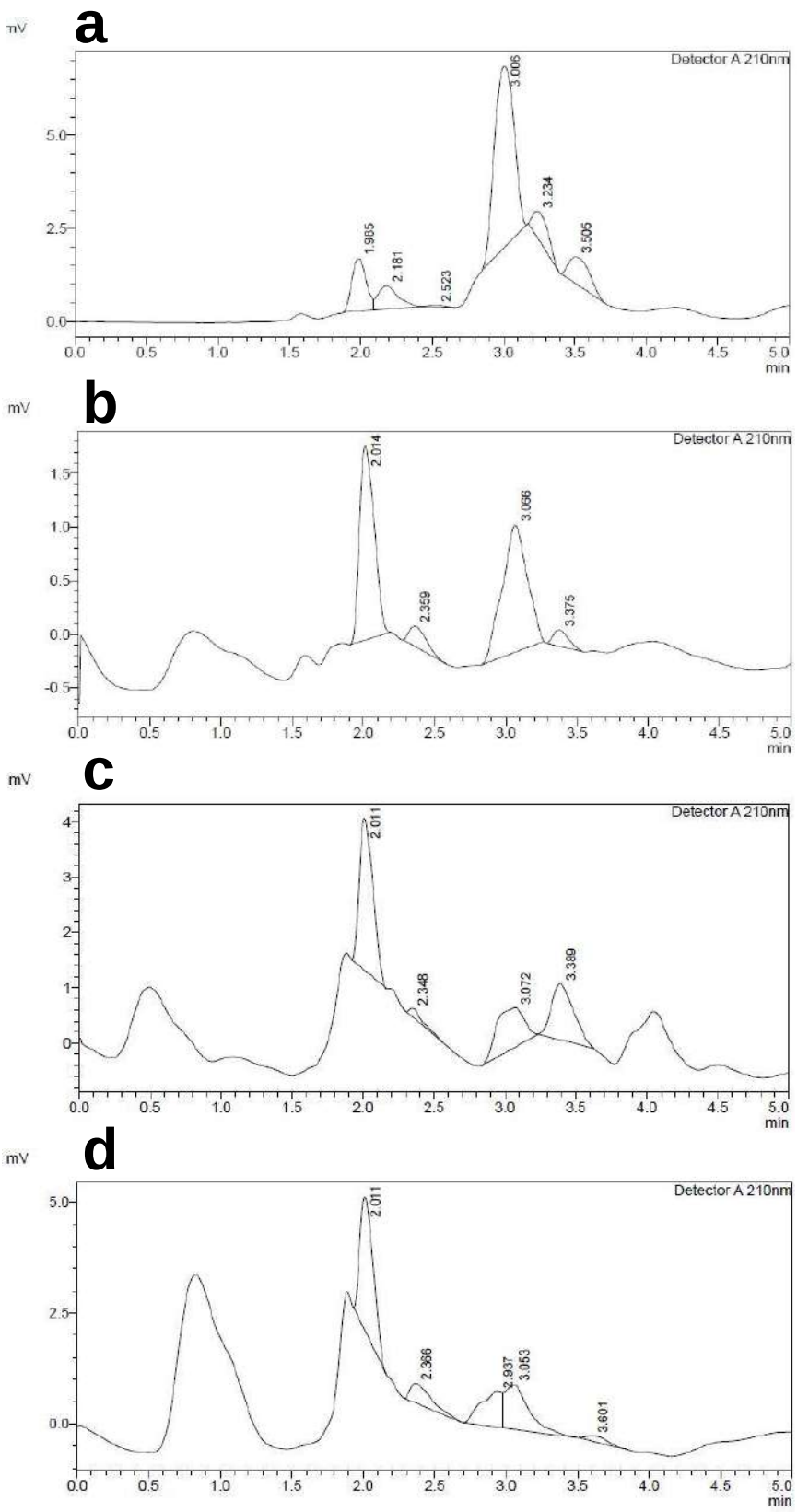


Figure 4.9: Representative HPLC chromatograms showing HCA peak area for fruit extracts of different *Garcinia* species. a. *G. gummi-gutta*; b. *G. pushpangadaniana*; c. *G. spicata*; d. *G. talbotii*.

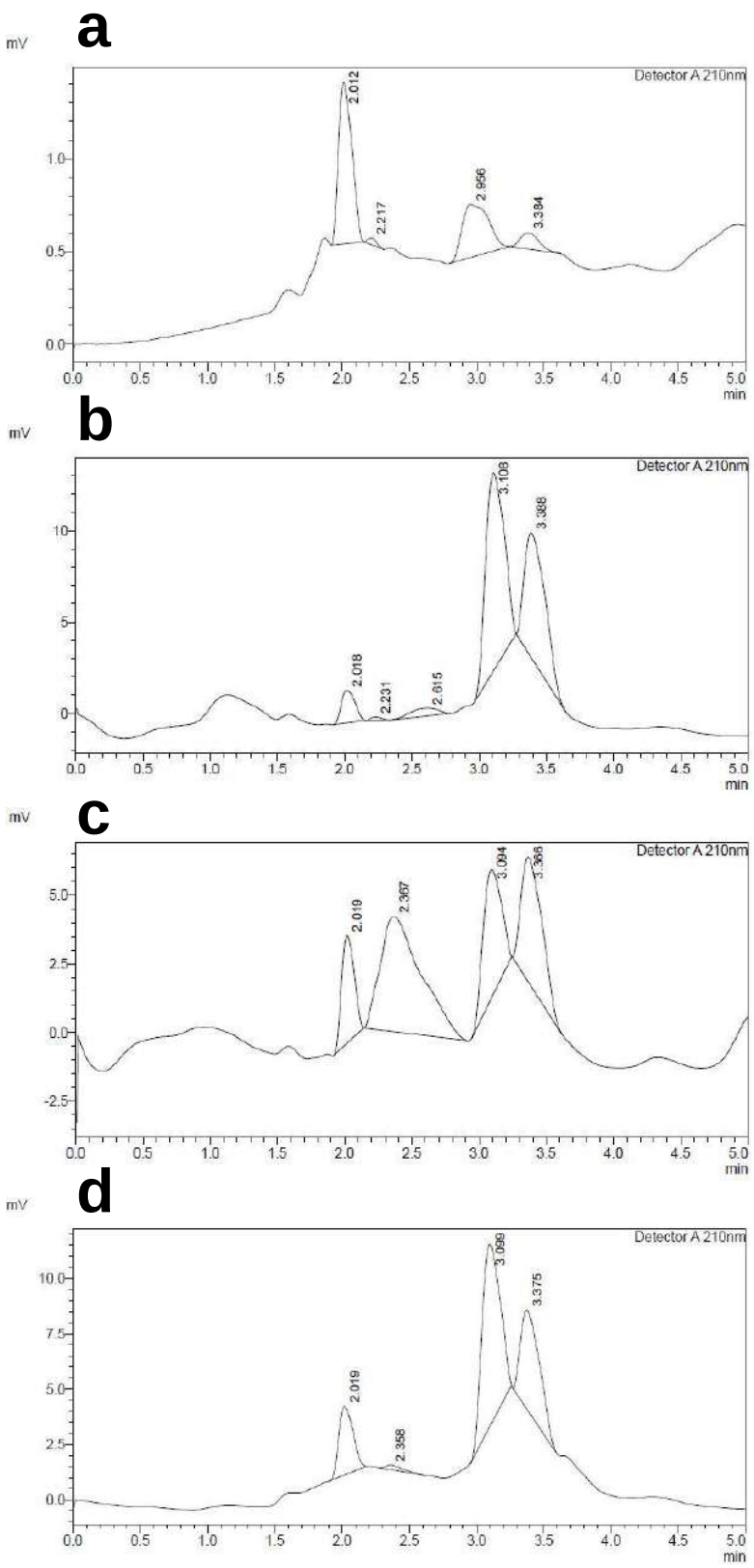


Figure 4.10: Representative HPLC chromatograms showing HCA peak area for fruit extracts of different *Garcinia* species and developmental stages of *G. indica*. a. *G. xanthochymus*; b. *G. indica* Stage-I; c. *G. indica* Stage-V; d. *G. indica* Stage-VI.

Table 4.3: Extract yield and concentration of hydroxycitric acid and garcinol in fruit rind of different developmental stages of *Garcinia indica*.

Sample No.	Developmental stage of fruit	Extract yield (g/10g of fruit rind)	HCA (mg/10g of fruit rind)	Garcinol (mg/10g of fruit rind)
1	Stage-I (15 DAF)	4.96	2713.00	-
2	Stage-II (30 DAF)	4.86	1936.82	105.13
3	Stage-III (45 DAF)	6.00	2227.37	676.02
4	Stage-IV (60 DAF)	4.77	776.94	42.49
5	Stage-V (75 DAF)	6.13	2314.21	490.07
6	Stage-VI (90 DAF)	6.48	1880.54	88.48

Note: DAF- number of days after fertilisation

Table 4.4: Extract yield and concentration of hydroxycitric acid and garcinol in matured fruit rind of different *Garcinia* species.

Sample No.	Species	Extract yield (g/10g of fruit rind)	HCA (mg/10g of fruit rind)	Garcinol (mg/10g of fruit rind)
1	*G. gummi-gutta*	5.55	1874.20	-
2	*G. hombroniana*	2.03	16.83	0.71
3	*G. livingstonei*	8.1	-	531.36
4	*G. mangostana*	2.59	15.39	-
5	*G. morella*	1.45	-	28.68
6	*G. pushpangadaniana*	6.44	47.96	1279.49
7	*G. spicata*	5.77	484.90	240.00
8	*G. talbotii*	7.26	75.12	37.38
9	*G. xanthochymus*	5.61	599.22	308.45

4.2.3.2 Method development, validation and quantification of garcinol

4.2.3.2.1 Method development and equipment conditions

To develop the method, different chromatographic conditions were tried such as elution pattern and wavelength. Two different elution patterns were checked gradient as well as isocratic by changing flow rates. Also, two different wavelengths as 215 nm and 276 nm were checked with UV detector. Finally easy and reliable

method was developed which was used for further analysis with isocratic elution pattern and detection wavelength of 215 nm. Two separate peaks of garcinol and iso-garcinol were found after injecting the standard garcinol solution. Garcinol and iso-garcinol peaks were observed at two different retention times as 5.6 and 4.6 mins respectively (Fig. 4.11a; Table 4.6).

Table 4.6: Peak details for standard garcinol detected at 215 nm wavelength.

Peak	Name	R. T. (min)	Area	Height
1	Iso-garcinol	4.644	1484066	87500
2	Garcinol	5.621	11217734	497957
Total			12701800	585457

4.2.3.2.2 Method validation

For method validation following parameters were checked such as- carry over test, system suitability test and linearity. These all tests were found to occur in acceptable limit (Fig. 4.11).

4.2.3.2.2.1 Carry over test

This test was performed to check the appearance of an analyte in a run when a blank containing no analyte is injected. In this methanol as a blank has been injected in which no peak was detected (limit < 0.0025 %) (Fig. 4.11a).

4.2.3.2.2.2 System suitability

To check system suitability, reproducibility (% RSD) test was carried out. In this test six injections of standard garcinol solution showed consistent results. Percent RSD for standard garcinol was 0.474 % (limit < 1%) (Fig. 4.11b).

4.2.3.2.2.3. Linearity and calibration curve

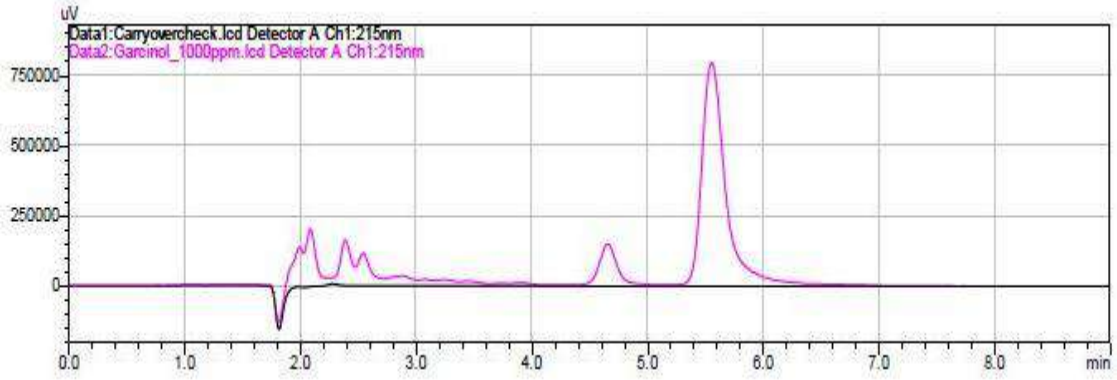
To assess the linearity, calibration curve was plotted using concentration range of 0.5-10 µg/ml of standard garcinol based on detector responses. It was found that

calibration curve was linear in the concentration range and showed accuracy. The correlation coefficient (R^2) of the calibration curve was 0.9967 (limit > 0.999) and regression equation obtained was y = 883954x + 4252.42 (Fig. 4.11c).

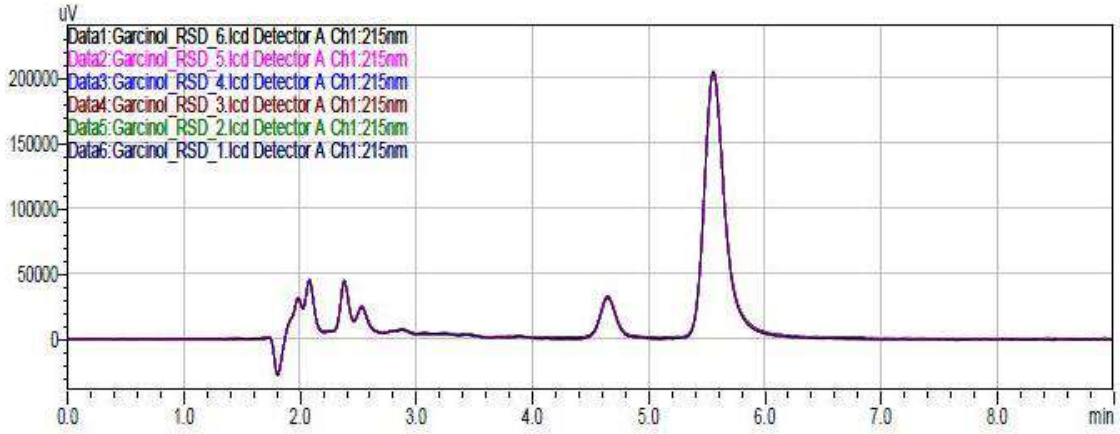
4.2.3.2.3. Quantification of garcinol from samples

To quantify the garcinol from extracts of different developmental stages of *G. indica* and matured stage of other *Garcinia* fruit rinds above developed and validated HPLC method was used. Based on the retention time (R.T.) of standard garcinol solution, presence of garcinol in samples was confirmed and quantity was estimated from the peak area of HPLC chromatograms. Representative HPLC chromatograms showing garcinol peak area for fruit extracts of different *Garcinia* species and developmental stages of *G. indica* are provided in Figures 4.12 and 4.13. Garcinol was found to occur in all the different developmental stages of fruit rinds of *G. indica* except developmental Stage-I which possess youngest fruits and the quantity is varied from 42.49 mg to 676.02 mg per 10 g of fruit rind (Table 4.3). The fruit rind of developmental Stage-III possesses highest amount of garcinol (Fig. 4.13c); however, matured fruit rind i.e. developmental Stage-VI showed 88.48 mg quantity of garcinol. Also, the extracts prepared from mature fruit rinds of different *Garcinia* species showed presence of garcinol. Among them *G. pushpangadaniana* extract showed highest amount of garcinol i.e. 1279.49 mg and *G. hombroniana* showed lowest amount of garcinol i.e. 0.71 mg per 10 g of fruit rind (Table 4.4).

a



b



c

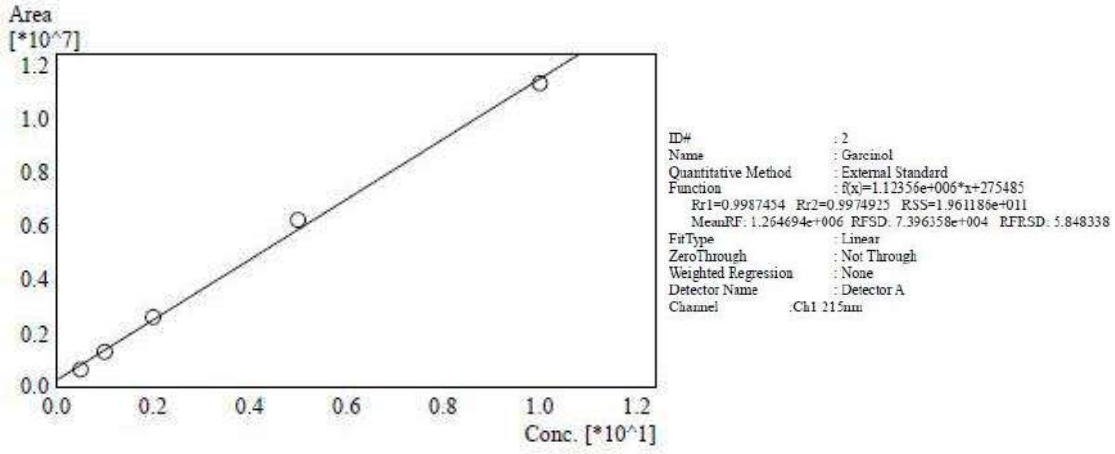


Figure 4.11: a. HPLC chromatogram of standard garcinol (pink colour) and carry over test (black colour); b. reproducibility test; c. calibration curve showing linearity.

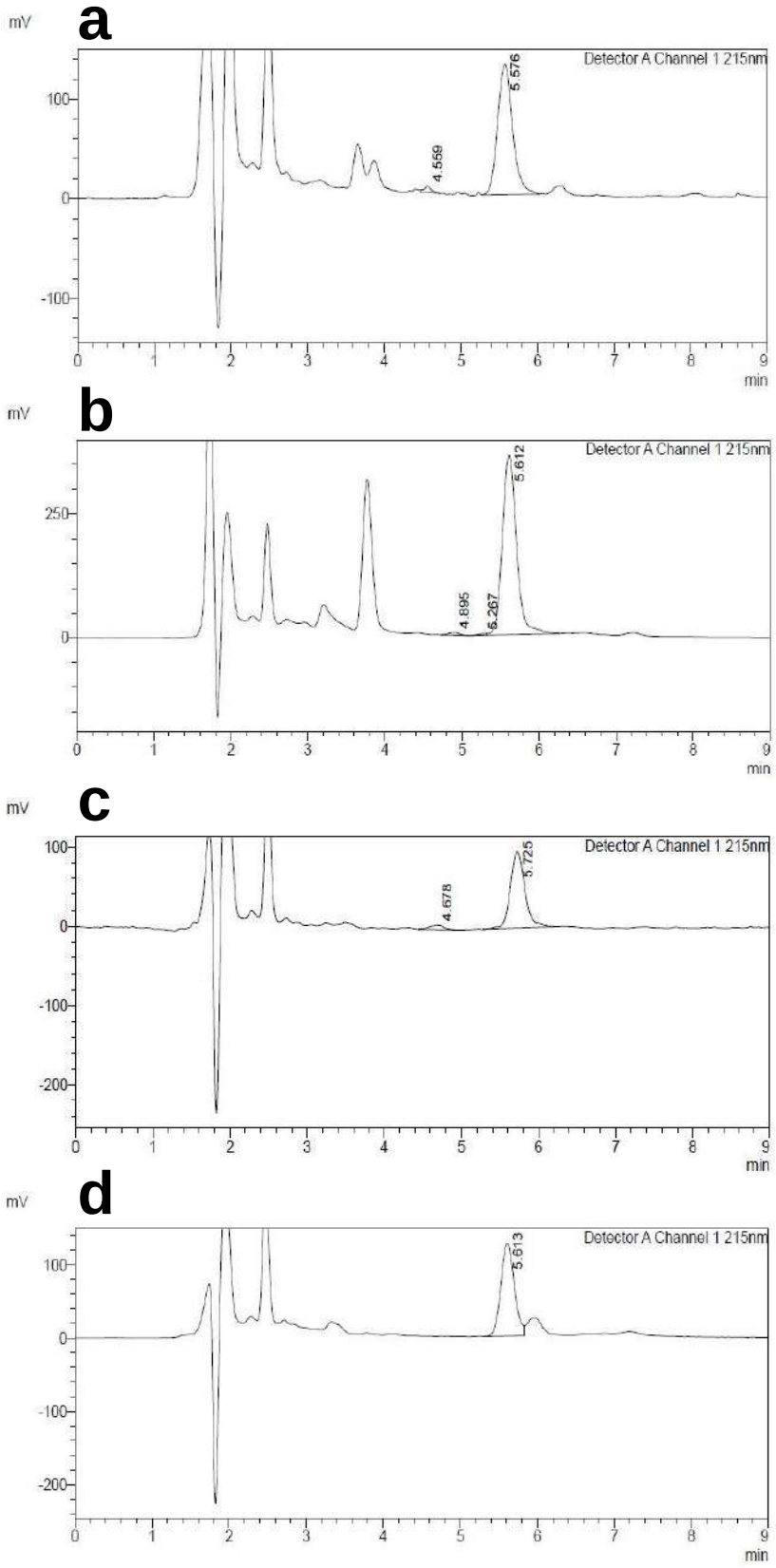


Figure 4.12: Representative HPLC chromatograms showing garcinol peak area for fruit extracts of different *Garcinia* species. a. *G. livingstonei*; b. *G. pushpangadaniana*; c. *G. spicata*; d. *G. talbotii*.

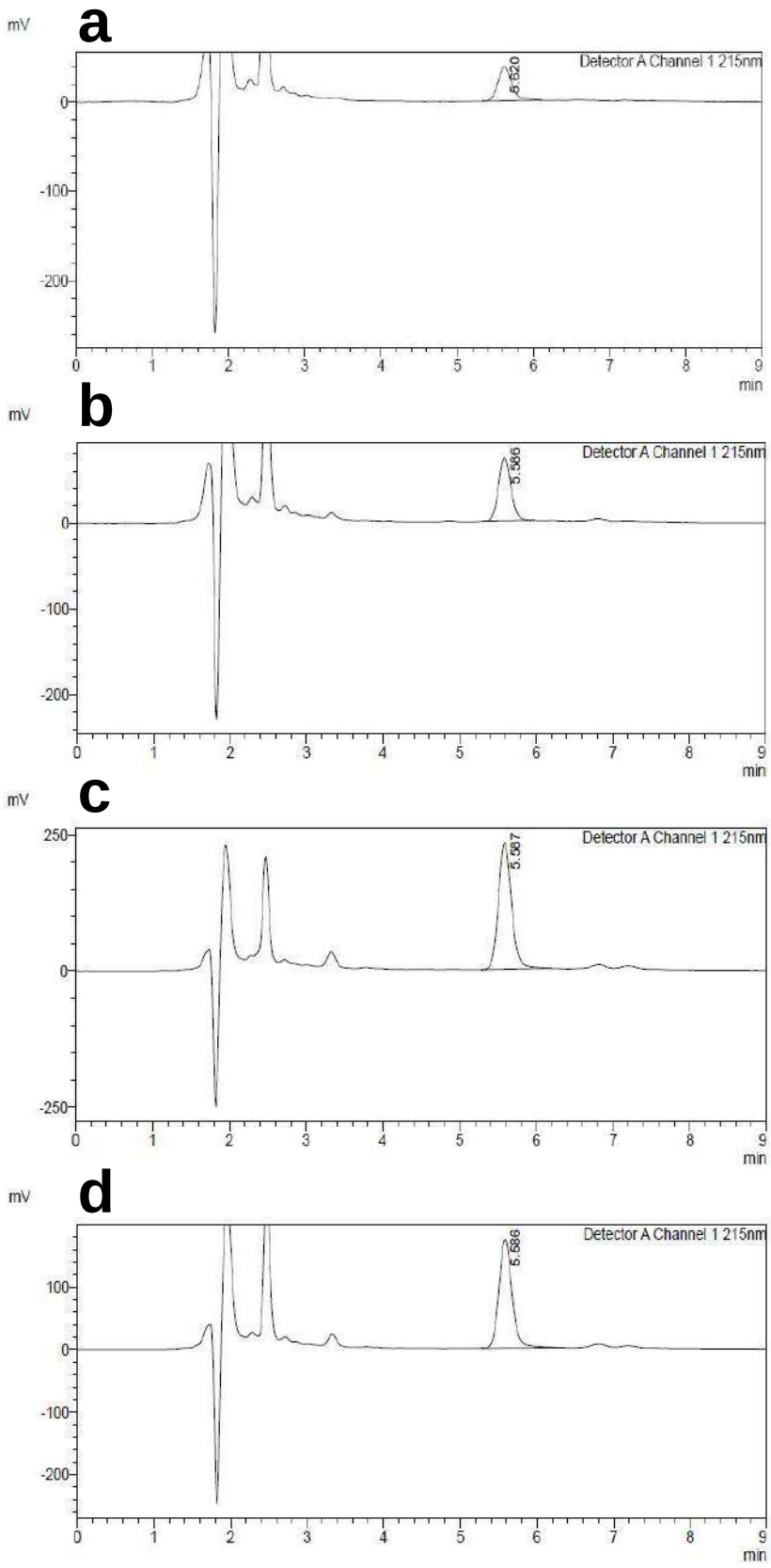


Figure 4.13: Representative HPLC chromatograms showing garcinol peak area for fruit extracts of different *Garcinia* species and developmental stages of *G. indica*. a. *G. xanthochymus*; b. *G. indica* Stage-II; c. *G. indica* Stage-III; d. *G. indica* Stage-V.

4.3 Phytochemical profiling of fruits of selected *Garcinia* species from the Western Ghats using GC-MS.

Phytochemical profiling was carried out using GC-MS which revealed the presence of several compounds. Methanolic extracts of dried mature fruit rinds of all 16 *Garcinia* species with 3 varieties from the Western Ghats were used for the analysis. After the analysis, results were represented in the form of GC-MS chromatograms and tables showing identity of the compounds at different retention times. At different retention times, different peaks were found which corresponds to presence of different constituents from the extracts and their percent composition was expressed as peak area percentage. In the present study, for each *Garcinia* species, the major chemical compounds have been listed along with the percent composition. Some of these major compounds are unique to the particular species and similarly some of the major compounds are also common among all the species. The both common as well as unique chemical compounds in major and minor quantities are provided in the result section for each *Garcinia* species studied.

4.3.1 Phytochemicals from the extract of *G. cowa* fruit rinds

The phytochemicals detected in extract of *G. cowa* fruit rinds are represented in the chromatogram (Fig. 4.14) and their respective details are provided in Table 4.7. It revealed the presence of major compounds such as Propanoic acid, 2-chloro- (22.67%); 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (18.46%); Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl (16.06%); Gluconic acid (11.72%); 3-Hexenoic acid, 3-methyl-, methyl ester (10.25%); Phthalic acid, di-(1-hexen-5-yl) ester (7.20%); 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene (3.44%); Heptasiloxane hexadecamethyl- (1.54%); Cyclononasiloxane, octadecamethyl- (1.17%) and Silane, cyclohexyldimethoxymethyl- (1.14%). The unique phytochemical compounds are Dimethylisopropylsilyloxycyclobutane (0.63%); 1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disiloxanyl tris (0.39%); 9-Octadecenoic acid (Z)-, methyl ester (0.36%) and Silane, dimethyldimethyl((dodec-9-ynyloxy)silyloxy)ethoxy- (0.08%). These constituents are not present in any other species.

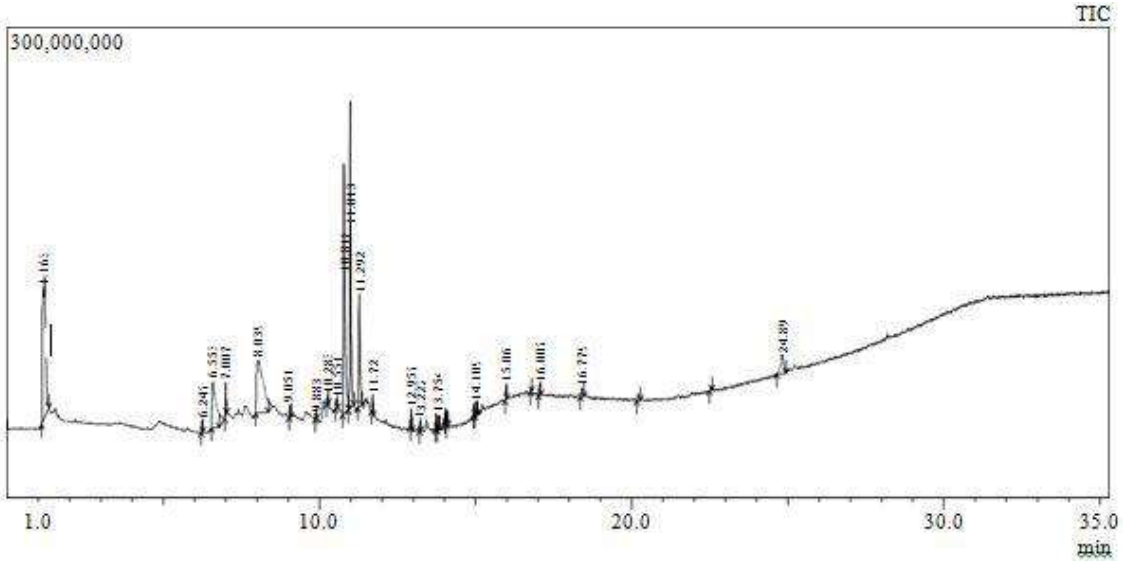


Figure 4.14: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. cowa* fruit rind.

Table 4.7: Compounds identified at each peak and respective peak details for *G. cowa* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.163	605985831	22.67	84472243	Propanoic acid, 2-chloro-
2	6.247	9519369	0.36	7495925	Undecane
3	6.553	274002288	10.25	30047109	3-Hexenoic acid, 3-methyl-, methyl ester
4	7.007	30393504	1.14	20939657	Silane, cyclohexyldimethoxymethyl-
5	8.039	493495251	18.46	33541324	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
6	9.051	16768349	0.63	7624406	Dimethylisopropylsilyloxycyclobutane
7	9.883	9211023	0.34	3616845	2-Butenedioic acid, 2,3-dimethyl-, dimethyl ester
8	10.283	9367272	0.35	6437288	Cycloheptasiloxane, tetradecamethyl-
9	10.551	11077909	0.41	6391879	Phenol, 2,4-bis(1,1-dimethylethyl)-
10	10.818	429171651	16.06	80080158	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl
11	11.013	313300164	11.72	76843277	Gluconic acid
12	11.292	192510464	7.20	71894147	Phthalic acid, di-(1-hexen-5-yl) ester
13	11.72	16211069	0.61	12114108	Cyclooctasiloxane, hexadecamethyl-
14	12.957	15848406	1.17	12790168	Cyclononasiloxane, octadecamethyl-
15	13.222	8912686	0.33	6637174	Perhydro-3-(alpha-methoxy-4,5-methylenedioxy-2-nitrobenzylidene)-2-oxofuran
16	13.754	14352440	0.54	9878288	Hexadecanoic acid, methyl ester
17	13.852	15190188	0.57	9833686	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
18	14.034	10477549	0.39	9261045	1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disiloxanyl tris
19	14.109	15508691	0.58	9729518	Dibutyl phthalate
20	14.947	9696363	0.36	6849089	9-Octadecenoic acid (Z)-, methyl ester
21	15.06	15770790	1.54	10523164	Heptasiloxane hexadecamethyl-
22	16.007	15409274	0.80	4946947	Tetracosamethyl-cyclododecasiloxane
23	16.779	1853592	0.08	1962943	Silane, dimethyldimethyl((dodec-9-ynyloxy)silyloxy)ethoxy-
24	24.89	91885103	3.44	12500100	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene
		657242463	100.0	556553363	

4.3.2 Phytochemicals from the extract of *G. gamblei* fruit rinds

An endemic species *G. gamblei* which is recently described was checked for the presence of phytochemicals showed chemical profile as provided in the chromatogram (Fig. 4.15) and their respective details in Table 4.8. It showed the presence of major compounds as- Ethene, (methylsulfonyl)- (33.93%); Tetracosamethyl-cyclododecasiloxane (10.34%); Phthalic acid, di-(1-hexen-5-yl) ester (10%); Heptasiloxane hexadecamethyl- (9.6%); Tetradecanoic acid (5.64%); Cyclodecasiloxane, eicosamethyl (4.64%); Cyclooctasiloxane, hexadecamethyl- (3.78%); Cyclononasiloxane, octadecamethyl- (3.69%); 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaenene (2.92%); n-Hexadecanoic acid (2.88%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.06%); Tridecanoic acid, 4,8,12-trimethyl-, methyl ester (1.47%); Cycloheptasiloxane, tetradecamethyl- (1.46%) and Dibutyl phthalate (1.44%).

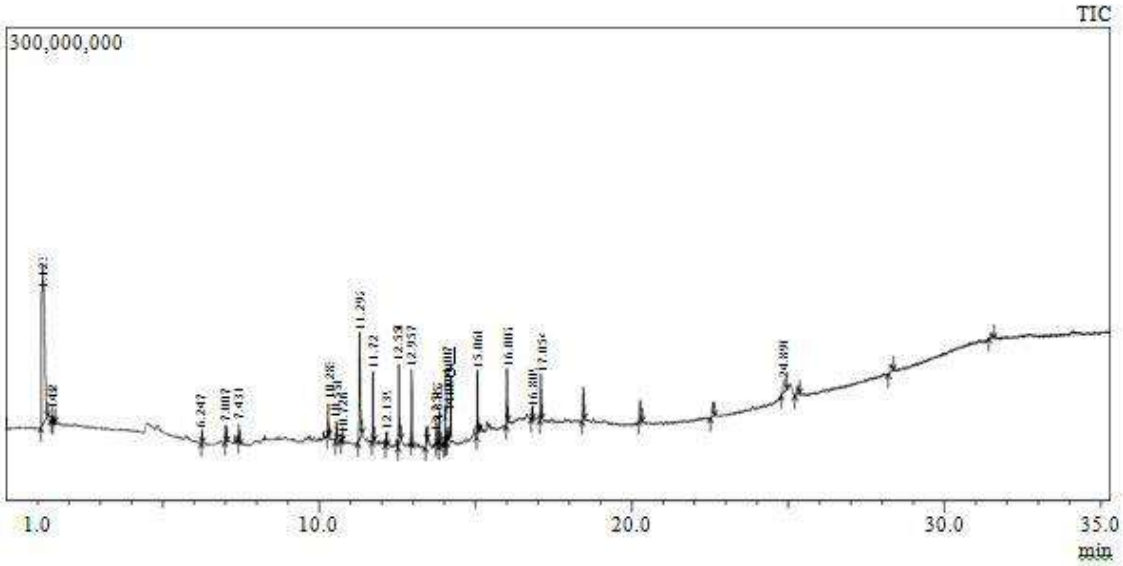


Figure 4.15: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. gamblei* fruit rinds.

Table 4.8: Compounds identified at each peak and respective peak details for *G. gamblei* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	587026934	33.93	86040175	Ethene, (methylsulfonyl)-
2	1.432	4056588	0.23	2869683	Trichloromethane
3	1.515	3879023	0.22	1975268	Silane, dimethoxydimethyl-
4	6.247	10104625	0.58	7846563	Undecane
5	7.007	14493269	0.84	10840992	Silane, cyclohexyldimethoxymethyl-
6	7.431	13953611	0.81	10994185	Silanol, trimethyl-, formate
7	10.283	25337098	1.46	20143571	Cycloheptasiloxane, tetradecamethyl-
8	10.551	16418842	0.95	11996441	Phenol, 2,4-bis(1,1-dimethylethyl)-
9	10.726	5254980	0.30	4304344	Dodecane, 2,6,11-trimethyl-
10	11.292	173078975	10.00	68374094	Phthalic acid, di-(1-hexen-5-yl) ester
11	11.72	65365570	3.78	44773028	Cyclooctasiloxane, hexadecamethyl-
12	12.139	11497493	0.66	8057672	Eicosane
13	12.550	97497235	5.64	50672119	Tetradecanoic acid
14	12.957	63860136	3.69	47572408	Cyclononasiloxane, octadecamethyl-
15	13.751	25454047	1.47	14914026	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester
16	13.852	35591525	2.06	22204149	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
17	14.017	49808993	2.88	28103910	n-Hexadecanoic acid
18	14.109	24877865	1.44	14086007	Dibutyl phthalate
19	14.166	15264320	0.88	10486910	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
20	15.060	54711560	9.6	40418542	Heptasiloxane hexadecamethyl-
21	16.007	54603862	10.34	28517244	Tetracosamethyl-cyclododecasiloxane
22	16.809	11755576	0.68	7214524	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
23	17.054	80153258	4.64	52436535	Cyclodecasiloxane, eicosamethyl
24	24.890	50561904	2.92	7822415	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaenene
		494607289	100.0	602664805	

4.3.3 Phytochemicals from the extract of *G. gummi-gutta* var. *gummi-gutta* fruit rinds

The variety *G. gummi-gutta* var. *gummi-gutta* commonly used, indicated the presence of constituents as provided in the chromatogram (Fig. 4.16) and their respective details in Table 4.9. The major constituents found were Ethene, (methylsulfonyl)- (22.81%); Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl (19.85%); Gluconic acid (11.85%); Phthalic acid, di-(1-hexen-5-yl) ester (7.49%); 3-Heptenoic acid, methyl ester (6.50%); Hexadecanoic acid, methyl ester (3.70%); Tetracosamethyl-cyclododecasiloxane (3.29%); Citric acid, trimethyl ester (2.95%); n-Hexadecanoic acid (2.12%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane (1.75%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (1.66%); Cyclooctasiloxane, hexadecamethyl- (1.40%); Tetradecanoic acid (1.20%); 9-Octadecenoic acid, methyl ester, (E)- (1.17%); Cyclononasiloxane, octadecamethyl- (1.14%); Octadecanoic acid, methyl ester (1.12%); Silane, cyclohexyldimethoxymethyl- (1.12%) and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.02%). Some of the unique compounds also found such as Hexadecanoic acid, 14-methyl-, methyl ester (0.12%); 7-Hexadecenoic acid, methyl ester, (Z)- (0.52%); Bromacil (0.26%) and 4a,7,7,10a-Tetramethyldodecahydrobenzo[f]chromen-3-ol (0.56%).

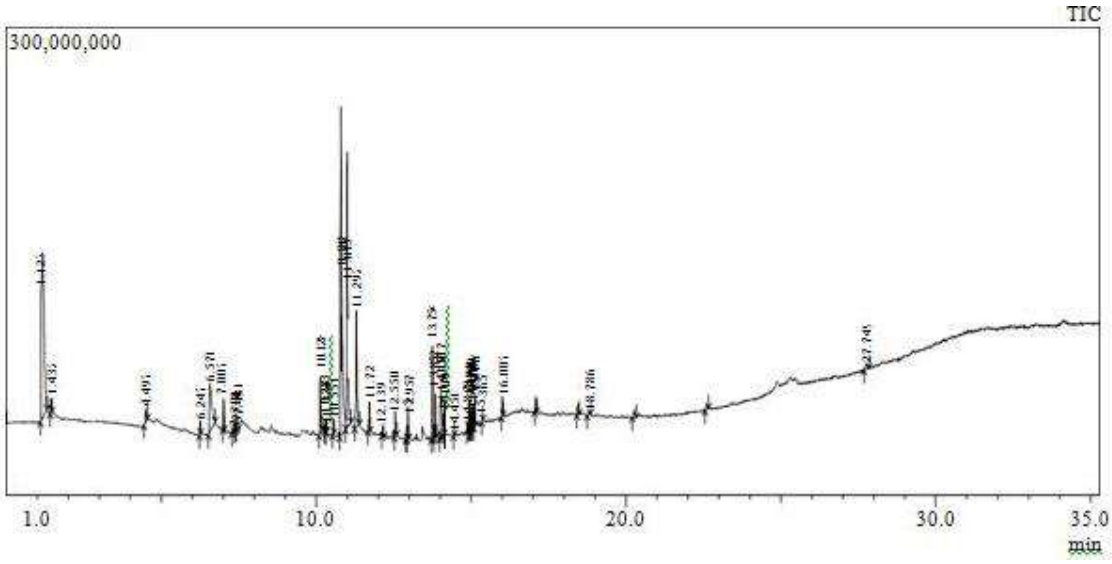


Figure 4.16: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. gummi-gutta* var. *gummi-gutta* fruit rind.

Table 4.9: Compounds identified at each peak and respective peak details for *G. gummi-gutta* var. *gummi-gutta* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	591072316	22.81	84971079	Ethene, (methylsulfonyl)-
2	1.432	15460252	0.60	9446529	Trichloromethane
3	4.497	20067794	0.77	8981668	3-Aminopyrazine 1-oxide
4	6.247	10856406	0.42	8324107	Undecane
5	6.570	168449584	6.50	31000657	3-Heptenoic acid, methyl ester
6	7.007	29050263	1.12	20437544	Silane, cyclohexyldimethoxymethyl-
7	7.302	3279501	0.13	2210121	Propanoyl chloride, 3-chloro-
8	7.384	3826394	0.15	2624104	Naphthalene
9	7.431	9086293	0.35	7739980	Silanol, trimethyl-, formate
10	10.128	76368493	2.95	36501988	Citric acid, trimethyl ester
11	10.283	8853967	0.34	7464739	Cycloheptasiloxane, tetradecamethyl-
12	10.551	14034740	0.54	10501282	Phenol, 2,4-bis(1,1-dimethylethyl)-
13	10.818	514318510	19.85	95295117	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl
14	11.013	307060226	11.85	90270071	Gluconic acid
15	11.292	194037188	7.49	74525141	Phthalic acid, di-(1-hexen-5-yl) ester
16	11.72	36178099	1.40	19253501	Cyclooctasiloxane, hexadecamethyl-
17	12.139	7944713	0.31	6131551	Eicosane
18	12.550	31076227	1.20	16352299	Tetradecanoic acid
19	12.895	10658677	0.41	6588072	Geranylgeraniol
20	12.957	29572628	1.14	21539476	Cyclononasiloxane, octadecamethyl-
21	13.194	3979850	0.15	3482167	Heneicosane
22	13.754	95770427	3.70	59655174	Hexadecanoic acid, methyl ester
23	13.852	43234334	1.66	28005101	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
24	14.017	55010685	2.12	32008383	n-Hexadecanoic acid
25	14.055	45374939	1.75	25832026	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane

26	14.109	24741089	0.95	13747130	Dibutyl phthalate
27	14.450	3104931	0.12	2537422	Hexadecanoic acid, 14-methyl-, methyl ester
28	14.878	8643314	0.33	4219013	1-Octadecanol
29	14.941	26339419	1.02	17821758	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
30	14.970	30306619	1.17	18705829	9-Octadecenoic acid, methyl ester, (E)-
31	15.000	13476563	0.52	11126692	7-Hexadecenoic acid, methyl ester, (Z)-
32	15.060	19939517	0.77	14873414	Heptasiloxane hexadecamethyl-
33	15.116	28904674	1.12	20055434	Octadecanoic acid, methyl ester
34	15.365	4614925	0.18	3382305	Octadecanoic acid
35	16.007	19973248	3.29	12865092	Tetracosamethyl-cyclododecasiloxane
36	18.786	6649350	0.26	2477867	Bromacil
37	27.749	14586470	0.56	3261279	4a,7,7,10a-Tetramethyldodecahydrobenzo[f]chromen-3-ol
		525902625	100.0	834215112	

4.3.4 Phytochemicals from the extract of *G. gummi-gutta* var. *conicarpa* fruit rinds

An endemic variety *G. gummi-gutta* var. *conicarpa* revealed the presence of compounds as provided in the chromatogram (Fig. 4.17) and their respective details in Table 4.10. The major compounds detected were Ethene, (methylsulfonyl)- (27.52%); Citric acid, trimethyl ester (18.36%); Tetradecanoic acid (10.50%); Phthalic acid, di-(1-hexen-5-yl) ester (9.48%); 3-Heptenoic acid, methyl ester (8.89%); Tetracosamethyl-cyclododecasiloxane (3.48%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.74%); n-Hexadecanoic acid (2.60%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane (1.92%); Cycloheptasiloxane, tetradecamethyl- (1.44%); Heptasiloxane, hexadecamethyl- (1.42%); Silane, cyclohexyldimethoxymethyl- (1.33%); Cyclononasiloxane, octadecamethyl- (1.31%); Cyclooctasiloxane, hexadecamethyl- (1.20%) and Nonacosane (1.09%). Some of the unique compounds were also detected such as Citric acid, triethyl ester (0.33%); 6-Ethyl-2,3-dimethyl-tetrahydro-thiopyran-4-ol (0.35%) and Silane, dimethyldi(but-3-enyloxy)- (0.51%).

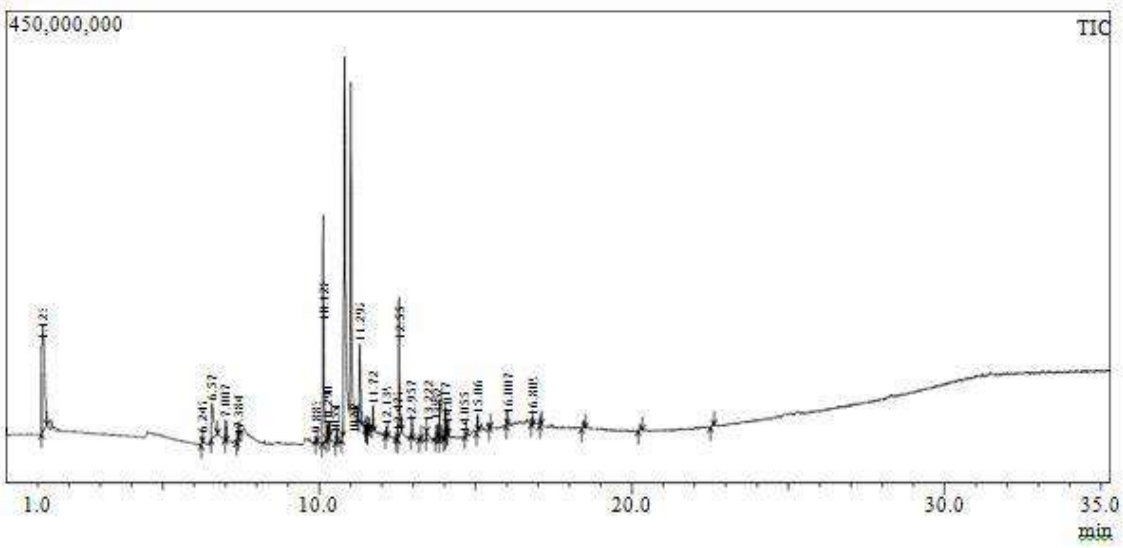


Figure 4.17: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. gummi-gutta* var. *conicarpa* fruit rinds.

Table 4.10: Compounds identified at each peak and respective peak details for *G. gummi-gutta* var. *conicarpa* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	587407940	27.52	85965700	Ethene, (methylsulfonyl)-
2	6.247	8254121	0.39	6652805	Undecane
3	6.57	189668178	8.89	33820323	3-Heptenoic acid, methyl ester
4	7.007	28355525	1.33	19906203	Silane, cyclohexyldimethoxymethyl-
5	7.384	10396305	0.49	6390760	Naphthalene
6	7.431	9625579	0.45	8818470	Silanol, trimethyl-, formate
7	9.883	9291059	0.44	4522829	2-Butenedioic acid, 2,3-dimethyl-, dimethyl ester
8	10.128	391885638	18.36	95244041	Citric acid, trimethyl ester
9	10.283	30791021	1.44	11175062	Cycloheptasiloxane, tetradecamethyl-
10	10.333	12904690	0.60	9109362	Heptadecane, 8-methyl-
11	10.551	19095398	0.89	14003317	Phenol, 2,4-bis(1,1-dimethylethyl)-
12	10.747	7063617	0.33	5679543	Citric acid, triethyl ester
13	11.292	202466783	9.48	73149828	Phthalic acid, di-(1-hexen-5-yl) ester
14	11.478	3957481	0.19	3585580	Methyl (4S,5R)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylate
15	11.527	7554312	0.35	5912971	6-Ethyl-2,3-dimethyl-tetrahydro-thiopyran-4-ol
16	11.586	10826164	0.51	7761938	Silane, dimethyldi(but-3-enyloxy)-
17	11.72	25623766	1.20	21086279	Cyclooctasiloxane, hexadecamethyl-
18	12.139	3948106	0.33	3940530	Eicosane
19	12.477	23196681	1.09	12970314	Nonacosane
20	12.479	4615948	0.22	3870938	Hexadecane
21	12.55	224113506	10.50	88359348	Tetradecanoic acid
22	12.554	4364623	0.20	3591663	Tetracosane
23	12.957	28004310	1.31	21672921	Cyclononasiloxane, octadecamethyl-
24	13.222	2170444	0.10	2759517	Perhydro-3-(alpha-methoxy-4,5-methylenedioxy-2-nitrobenzylidene)-2-oxofuran
25	13.852	58480976	2.74	38083209	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d

26	14.017	55411243	2.60	33248757	n-Hexadecanoic acid
27	14.055	41002446	1.92	21844810	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
28	14.109	11180463	0.52	8338771	Dibutyl phthalate
29	14.166	4919194	0.23	4721979	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
30	15.06	22542736	1.42	16214765	Heptasiloxane, hexadecamethyl-
31	16.007	20192785	3.48	13242726	Tetracosamethyl-cyclododecasiloxane
32	16.809	10255677	0.48	6541046	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
		77340747	100.0	694647493	

4.3.5 Phytochemicals from the extract of *G. gummi-gutta* var. *papilla* fruit rinds

Figure 4.18 and Table 4.11 shows the chromatogram for detected phytochemicals and their respective details for the fruit extract from rare variety *G. gummi-gutta* var. *papilla*. D-galactitol, 3,6-anhydro-1,2,4,5-tetra-o-methyl- (20.65%); Cyclohexanecarboxylic acid, methyl ester (7.90%); Butanedioic acid, 3-hydroxy-2,2-dimethyl-, diethyl ester (1.35%); D-Galactitol, 2,3,4-tri-O-methyl-, triacetate (1.16%) and Pseudourea, 2-(2-aminoethyl)-2-thio- (1.04%) were observed as unique phytochemicals with major quantity. Some other phytochemicals were also found in major quantity such as Ethene, (methylsulfonyl)- (21.34%); Citric acid, trimethyl ester (14.34%); Tetradecanoic acid (3.68%); n-Hexadecanoic acid (2.15%); Hexadecanoic acid, methyl ester (1.97%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (1.91%) and Cyclooctasiloxane, hexadecamethyl- (1.60%). Along with major phytochemicals, unique phytochemicals with minor quantities were also found viz. Succinic acid, 2-decyl propyl ester (0.56%); s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino- (0.07%); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (0.33%) and 4-Hydroxyphenyllactic acid, ethyl ester, di-TMS (0.18%).

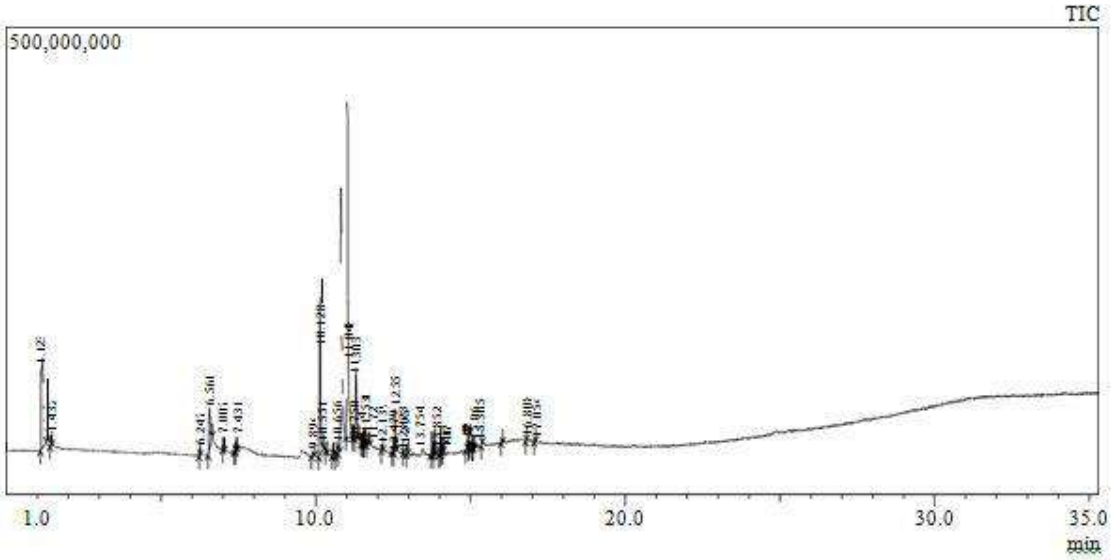


Figure 4.18: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. gummi-gutta* var. *papilla* fruit rinds.

Table 4.11: Compounds identified at each peak and respective peak details for *G. gummi-gutta* var. *papilla* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	489435367	21.34	85710428	Ethene, (methylsulfonyl)-
2	1.432	19498392	0.85	11576859	Trichloromethane
3	6.247	11420556	0.50	8582256	Undecane
4	6.560	181227156	7.90	45466827	Cyclohexanecarboxylic acid, methyl ester
5	7.007	19193543	0.84	14821445	Silane, cyclohexyldimethoxymethyl-
6	7.384	19937770	0.87	8074275	Naphthalene
7	7.431	13406647	0.58	10455116	Silanol, trimethyl-, formate
8	9.894	9073051	0.40	4156741	2-Pentenedioic acid, 4-methyl-, dimethyl ester
9	10.128	324157371	14.13	90811481	Citric acid, trimethyl ester
10	10.551	17458231	0.76	12268706	Phenol, 2,4-bis(1,1-dimethylethyl)-
11	10.656	5698645	0.26	3343828	Oxalic acid, 2-isopropylphenyl pentyl ester
12	10.750	12803083	0.56	9949657	Succinic acid, 2-decyl propyl ester
13	11.048	473627393	20.65	72827154	D-galactitol, 3,6-anhydro-1,2,4,5-tetra-o-methyl-
14	11.238	30930372	1.35	14262427	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, diethyl ester
15	11.303	224813052	9.80	66625111	Diethyl Phthalate
16	11.478	15064684	0.66	9737798	Methyl (4S,5R)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylate
17	11.530	23937160	1.04	15241497	Pseudourea, 2-(2-aminoethyl)-2-thio-
18	11.587	26657546	1.16	15776067	D-Galactitol, 2,3,4-tri-O-methyl-, triacetate
19	11.72	36595561	1.60	13140581	Cyclooctasiloxane, hexadecamethyl-
20	12.139	8993690	0.39	6600191	Eicosane
21	12.479	5318996	0.23	3591873	Hexadecane
22	12.55	84384947	3.68	46042447	Tetradecanoic acid
23	12.823	1648878	0.07	1657457	s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino-
24	12.957	10553290	0.46	8230435	Cyclononasiloxane, octadecamethyl-
25	13.194	6178590	0.27	4982354	Heneicosane

26	13.749	6410350	0.28	4076204	Triacontanoic acid, methyl ester
27	13.754	45067941	1.97	25995304	Hexadecanoic acid, methyl ester
28	13.852	43892228	1.91	28908086	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
29	14.017	49342791	2.15	28499645	n-Hexadecanoic acid
30	14.109	9629103	0.42	6858528	Dibutyl phthalate
31	14.872	11626707	0.51	5641653	Behenic alcohol
32	14.941	5637002	0.25	4845664	9,12-Octadecadienoic acid (Z,Z)-, methyl ester/ Linoleic acid, methyl ester
33	14.995	7467753	0.33	5577530	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
34	15.06	17075723	0.97	11538626	Heptasiloxane, hexadecamethyl-
35	15.365	5615569	0.24	3898172	Octadecanoic acid
36	16.808	4226033	0.18	2759565	4-Hydroxyphenyllactic acid, ethyl ester, di-TMS
37	17.054	5098093	0.44	3995787	Cyclodecasiloxane, eicosamethyl
		288305163	100.0	721482985	

4.3.6 Phytochemicals from the extract of *G. hombroniana* fruit rinds

*G*arcinia *hombroniana* fruit rind extract showed the presence of major phytoconstituents such as Ethene, (methylsulfonyl)- (29.48%); Gluconic acid (11.85%); Phthalic acid, di-(1-hexen-5-yl) ester (10.43%); Tetracosamethyl-cyclododecasiloxane (6.79%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane (3.77%); n-Hexadecanoic acid (3%); Tetracosane (2.72%); Cyclononasiloxane, octadecamethyl- (2.51%); Cyclooctasiloxane, hexadecamethyl- (2.46%); Heptasiloxane, hexadecamethyl- (2.14%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.12%); Cyclodecasiloxane, eicosamethyl (1.85%); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (1.59%); Dibutyl phthalate (1.55%); Decane, 1-iodo- (1.54%); Eicosane (1.27%) and Phenol, 2,4-bis(1,1-dimethylethyl)- (1.20%). Some unique phytoconstituents were also found in the extract such as Nonane, 1-iodo- (0.27%); Dodecane, 2,6,10-trimethyl- (0.14%); Valeric acid, dodecyl ester (0.09%); Hexadecane, 1-chloro- (0.35%); Nonadecane (0.24%); 2-Bromotetradecane (0.23%); Dodecahydrosqualene (0.13%) and Heptadecane, 2-methyl- (0.48%). Pentadecanoic acid (11.03%); Hexatriacontane (1.30%); 2H-Pyran-2-one, 6-heptyltetrahydro- (1.24%); 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- (1.03%) and Tridecanol, 2-ethyl-2-methyl- (1.02%) these were the phytoconstituents found to occur as an unique and in major quantities (Fig. 4.19; Table 4.12).

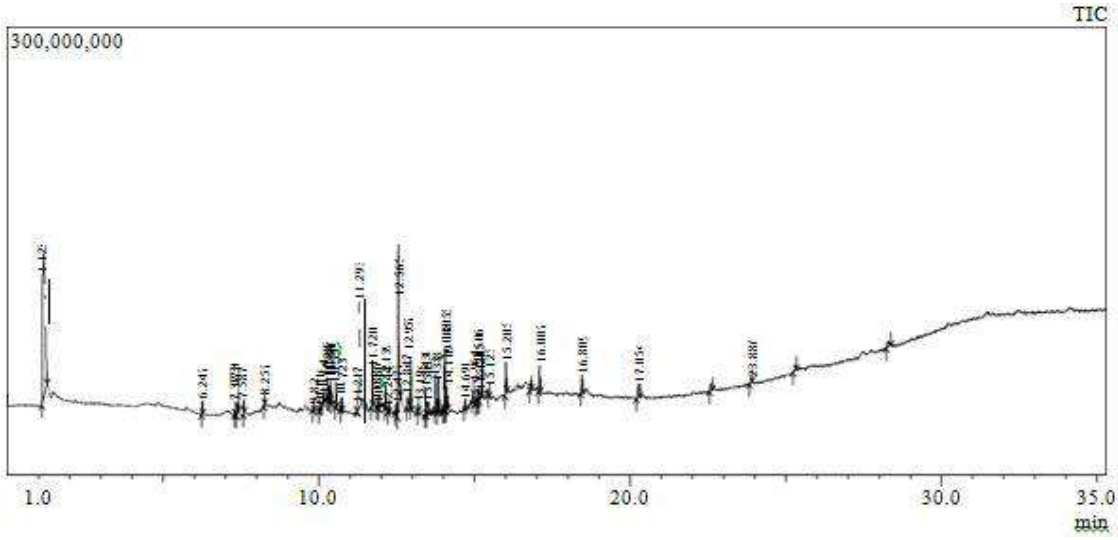


Figure 4.19: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. hombroniana* fruit rind.

Table 4.12: Compounds identified at each peak and respective peak details for *G. hombroniana* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	526733867	29.48	84131942	Ethene, (methylsulfonyl)-
2	6.247	10901674	0.61	8349221	Undecane
3	7.302	4282130	0.24	3372107	Propanoyl chloride, 3-chloro-
4	7.384	13886422	0.78	7396235	Naphthalene
5	7.587	11511745	0.64	8291050	1,3,5-Trioxane
6	8.257	4743430	0.27	4164267	Nonane, 1-iodo-
7	9.821	2595885	0.15	2321469	Caryophyllene
8	10.014	2445494	0.14	2437539	Dodecane, 2,6,10-trimethyl-
9	10.142	7071124	0.40	4178468	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
10	10.283	14619164	0.82	11440344	Cycloheptasiloxane, tetradecamethyl-
11	10.333	12947360	0.72	9218904	Heptadecane, 8-methyl-
12	10.387	18354830	1.03	14776220	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-/ Germacrene-D
13	10.551	21462323	1.20	16631451	Phenol, 2,4-bis(1,1-dimethylethyl)-
14	10.723	12682834	0.71	8133375	Octadecane, 1-chloro-
15	11.217	1568009	0.09	1682240	Valeric acid, dodecyl ester
16	11.292	186897635	10.43	71974473	Phthalic acid, di-(1-hexen-5-yl) ester
17	11.720	43962342	2.46	30874930	Cyclooctasiloxane, hexadecamethyl-
18	11.886	6331137	0.35	4677825	Hexadecane, 1-chloro-
19	11.945	4245853	0.24	2931250	Nonadecane
20	12.139	22772787	1.27	16317521	Eicosane
21	12.244	4084881	0.23	3034720	2-Bromotetradecane
22	12.477	8460585	0.47	7119064	Nonacosane
23	12.554	48645774	2.72	24862634	Tetracosane
24	12.565	197096655	11.03	73678072	Pentadecanoic acid
25	12.847	22115728	1.24	7051549	2H-Pyran-2-one, 6-heptyltetrahydro-/ .delta.-Dodecalactone

26	12.957	44814709	2.51	34056243	Cyclononasiloxane, octadecamethyl-
27	13.194	7309280	0.41	5262549	Heneicosane
28	13.505	23183224	1.30	8409420	Hexatriacontane
29	13.852	37795121	2.12	23885973	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
30	14.017	53533817	3.00	30356157	n-Hexadecanoic acid
31	14.055	67404785	3.77	41168385	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
32	14.109	27655608	1.55	15227871	Dibutyl phthalate
33	14.166	28388444	1.59	17154303	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
34	14.690	7955690	0.79	6294170	Dotriacontane
35	14.964	2293815	0.13	1766278	Dodecahydrosqualene/ Cosbiol/ Squalane
36	15.06	38218636	2.14	26265942	Heptasiloxane, hexadecamethyl-
37	15.125	18248323	1.02	6422796	Tridecanol, 2-ethyl-2-methyl-
38	15.205	27457558	1.54	7860529	Decane, 1-iodo-
39	16.005	12411282	0.69	3241359	1,3,3,3-Tetramethyldisiloxanyl tris (trimethylsilyl) orthosilicate
40	16.007	32326255	6.79	21249239	Tetracosamethyl-cyclododecasiloxane
41	16.809	10783686	0.60	6142931	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
42	17.054	33044659	1.85	17524221	Cyclodecasiloxane, eicosamethyl
43	23.886	8666019	0.48	2860527	Heptadecane, 2-methyl-
		691910579	100.0	704195763	

4.3.7 Phytochemicals from the extract of *G. indica* fruit rinds

An endemic and common species *G. indica* revealed the presence of several compounds as provided in the chromatogram (Fig. 4.20) and their respective details in Table 4.13. The major compounds identified were Phthalic acid, di-(1-hexen-5-yl) ester (17.83%); Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl (17.71%); Gluconic acid (13.31%); 3-Hexenoic acid, 3-methyl-, methyl ester (12.68%); Ethene, (methylsulfonyl)- (12.54%); Citric acid, trimethyl ester (3.05%); Heptasiloxane, hexadecamethyl- (1.09%) and Cyclooctasiloxane, hexadecamethyl- (1.01%). Some of the distinctive compounds were also identified such as Acetonitrile, trichloro- (0.15%); Benzene, 1,2-dimethyl- (0.67%); Benzene, (1-methylethyl)- (0.30%); 1-Hexanol, 2-ethyl- (0.33%); Benzaldehyde dimethyl acetal (2.12%); Pentanedioic acid, 2,4-dimethyl-, dimethyl ester (0.14%); 3-Tetradecene, (Z)- (0.21%); Decane, 5-propyl- (0.19%); Tetradecane (0.47%); 2-Methyl-4-phenyl-butyric acid, methyl ester (0.13%); Pentadecane (0.82%); 2-Propenoic acid, tridecyl ester (0.52%) and Sulfurous acid, 2-ethylhexyl hexyl ester (0.22%). Also, some compounds were obtained as an unique and major in quantity during the analysis such as 3,5-Octadiyne (3.89%); Benzene, 1,3-dimethyl- (1.74%); Phthalic anhydride (1.58%) and Azulene (1.40%).

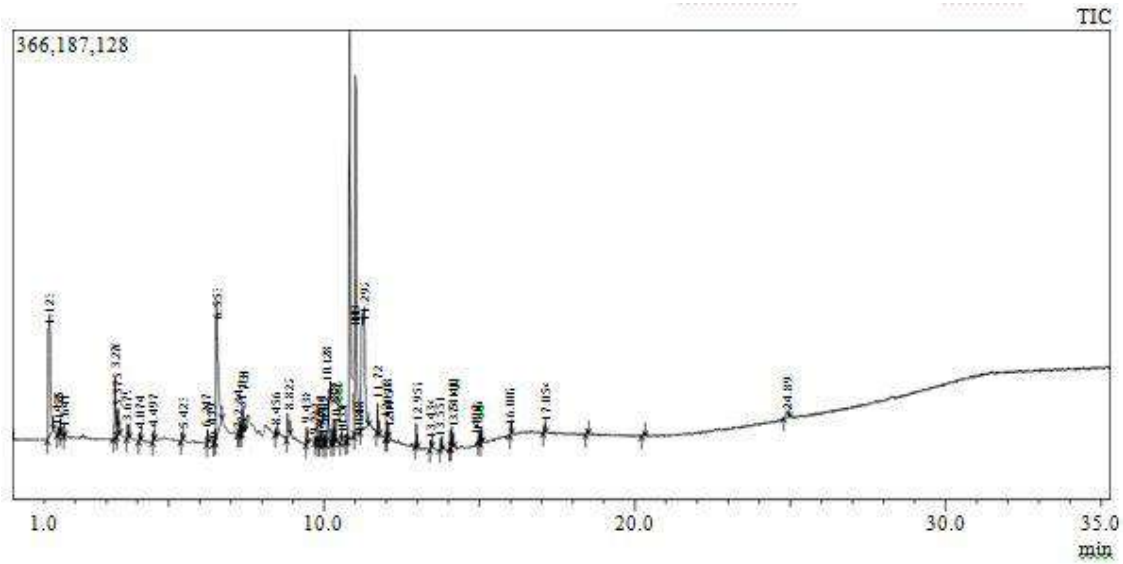


Figure 4.20: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. indica* fruit rind.

Table 4.13: Compounds identified at each peak and respective peak details for *G. indica* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	477806112	12.54	86249096	Ethene, (methylsulfonyl)-
2	1.432	6690787	0.18	3298497	Trichloromethane
3	1.515	5749763	0.15	5087722	Silane, dimethoxydimethyl-
4	1.641	5731349	0.15	3334777	Acetonitrile, trichloro-
5	3.276	148326610	3.89	47643847	3,5-Octadiyne
6	3.375	66134356	1.74	21366293	Benzene, 1,3-dimethyl-
7	3.679	25630465	0.67	11037622	Benzene, 1,2-dimethyl-
8	4.074	11500385	0.30	6789206	Benzene, (1-methylethyl)-/ Cumene
9	4.497	12945386	0.34	7877309	3-Aminopyrazine 1-oxide
10	5.423	12733571	0.33	6410895	1-Hexanol, 2-ethyl-
11	6.247	13159682	0.35	9841585	Undecane
12	6.459	3773325	0.10	2667017	Benzaldehyde dimethyl acetal
13	6.553	483010185	12.68	85228488	3-Hexenoic acid, 3-methyl-, methyl ester
14	7.234	5200652	0.14	2741885	Pentanedioic acid, 2,4-dimethyl-, dimethyl ester
15	7.310	7910580	0.21	6410895	3-Tetradecene, (Z)-
16	7.389	53282153	1.40	21496096	Azulene
17	8.456	7176063	0.19	5356597	Decane, 5-propyl-
18	8.822	60229117	1.58	19094273	Phthalic anhydride
19	9.438	18047888	0.47	13388385	Tetradecane
20	9.722	4962459	0.13	3839358	2-Methyl-4-phenyl-butyric acid, methyl ester
21	9.821	2825829	0.07	2039948	Caryophyllene
22	9.894	32905726	0.86	10745147	2-Pentenedioic acid, 4-methyl-, dimethyl ester
23	10.025	13959882	0.37	6930673	D-Alanine, N-neopentyloxycarbonyl-, octadecyl ester
24	10.128	116224306	3.05	46001631	Citric acid, trimethyl ester
25	10.283	27130711	0.71	16711003	Cycloheptasiloxane, tetradecamethyl-

26	10.357	31338353	0.82	17440943	Pentadecane
27	10.551	10375718	0.27	7113432	Phenol, 2,4-bis(1,1-dimethylethyl)-
28	10.818	674552531	17.71	86059983	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl
29	11.013	506958187	13.31	83003429	Gluconic acid
30	11.292	679226498	17.83	78549576	Phthalic acid, di-(1-hexen-5-yl) ester
31	11.72	38511954	1.01	23618017	Cyclooctasiloxane, hexadecamethyl-
32	12.008	19793062	0.52	13795579	2-Propenoic acid, tridecyl ester
33	12.045	8236320	0.22	5360217	Sulfurous acid, 2-ethylhexyl hexyl ester
34	12.957	25617483	0.67	18664944	Cyclononasiloxane, octadecamethyl-
35	13.434	11897320	0.31	6989946	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
36	13.551	13134985	0.34	4795333	Methoxyacetic acid, 4-tridecyl ester
37	13.754	14226014	0.37	8631499	Hexadecanoic acid, methyl ester
38	14.109	30571335	0.80	16374338	Dibutyl phthalate
39	14.97	14031025	0.37	8661299	9-Octadecenoic acid, methyl ester, (E)-/ Elaidic acid, methyl ester
40	15.06	18522291	1.09	13421392	Heptasiloxane, hexadecamethyl-
41	16.007	11994054	0.51	8564646	Tetracosamethyl-cyclododecasiloxane
42	17.054	13540931	0.36	7010083	Cyclodecasiloxane, eicosamethyl-
43	24.89	32833498	0.89	4955310	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaenene
		778408901	100.0	864598211	

4.3.8. Phytochemicals from the extract of *G. imbertii* fruit rinds

Fruit rind extract of southern Western Ghats endemic species *G. imbertii* showed the presence of major as well as unique compounds as provided in the chromatogram (Fig. 4.21) and their respective details in Table 4.14. The unique compounds detected in major quantities were alpha.-Caryophyllene (4.51%); Tricyclo[20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy- (1.84%); 10,13-Octadecadienoic acid, methyl ester (1.22%); 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde (1.20%) and 1H-Cycloprop[e]azulen (1.12%). Some other major compounds detected were Ethene, (methylsulfonyl)- (35.57%); Phthalic acid, di-(1-hexen-5-yl) ester (10.64%); .gamma.-Sitosterol (5.93%); n-Hexadecanoic acid (3.40%); Stigmasterol (3.38%); Tetradecanoic acid (3.14%); Trichloromethane (2.31%); Hexadecanoic acid, methyl ester (2.28%); Caryophyllene (2.23%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.23%); 1,1,3,3,5,5,7,7,9,9,11,11,13, 13,15,15-Hexadecamethyloctasiloxane (1.83%); Dibutyl phthalate (1.81%); 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (1.62%); Cyclooctasiloxane, hexadecamethyl- (1.45%); Phenol, 2,4-bis(1,1-dimethylethyl)- (1.25%); Tetracosamethyl-cyclododecasiloxane (1.14%); Cyclodecasiloxane, eicosamethyl- (1.05%) and unique compounds with minor quantities detected were Dodecane, 2-methyl- (0.14%); 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (0.12%); 1-Octadecanesulphonyl chloride (0.13%); 9-Octadecyne (0.13%); Tetratriacontane (0.18%).

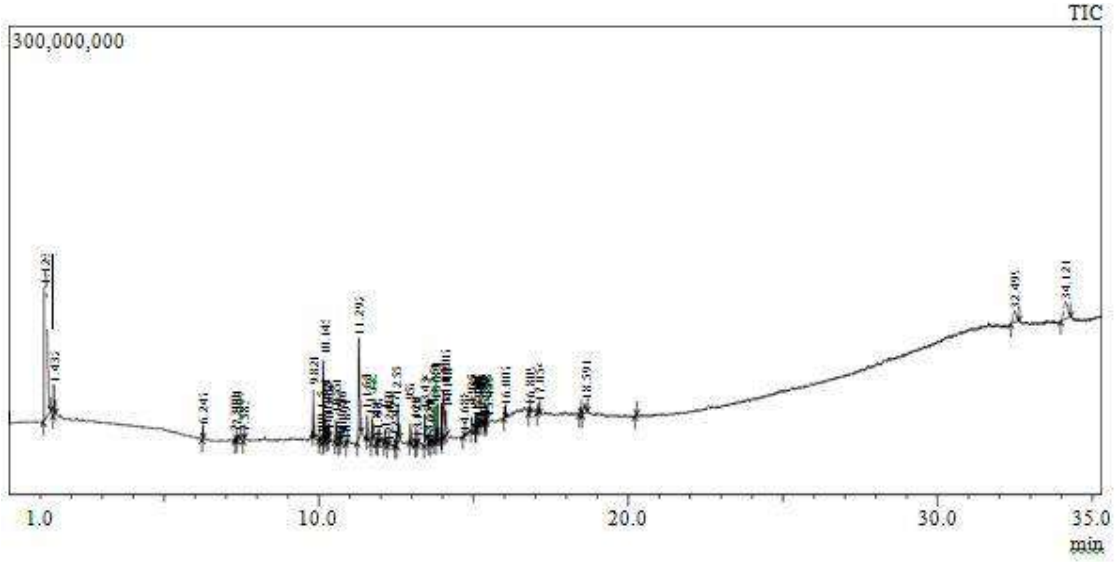


Figure 4.21: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. imbertii* fruit rind.

Table 4.14: Compounds identified at each peak and respective peak details for *G. imbertii* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	603029762	35.57	86217831	Ethene, (methylsulfonyl)-
2	1.432	39251315	2.31	19314339	Trichloromethane
3	6.247	10479935	0.62	7920556	Undecane
4	7.300	3609361	0.21	2139059	Phenyl methylphosphonofluoridate
5	7.384	10536430	0.62	3260457	Naphthalene
6	7.585	3770500	0.22	2787535	1-Diisopropylsilyloxycyclohexane
7	9.821	37776881	2.23	28826926	Caryophyllene
8	10.015	2416748	0.14	1998021	Dodecane, 2-methyl-
9	10.145	76450428	4.51	51700988	.alpha.-Caryophyllene
10	10.217	18958213	1.12	13794749	1H-Cycloprop[e]azulen
11	10.283	8984298	0.53	7262236	Cycloheptasiloxane, tetradecamethyl-
12	10.333	3972964	0.23	3821794	Heptadecane, 8-methyl-
13	10.551	21215624	1.25	13181299	Phenol, 2,4-bis(1,1-dimethylethyl)-
14	10.656	5474058	0.32	2232794	Oxalic acid, 2-isopropylphenyl pentyl ester
15	10.723	4174740	0.46	2754996	Octadecane, 1-chloro-
16	10.891	2110343	0.12	1556453	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl/ Actinidiolide
17	11.292	180423930	10.64	64554846	Phthalic acid, di-(1-hexen-5-yl) ester
18	11.558	20354529	1.20	14993271	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde
19	11.72	24637467	1.45	14347697	Cyclooctasiloxane, hexadecamethyl-
20	11.887	2923436	0.17	2161691	Pentadecane, 2,6,10,14-tetramethyl-
21	12.139	10579857	0.62	7425462	Eicosane
22	12.245	2286600	0.13	2034012	1-Octadecanesulphonyl chloride
23	12.477	5152217	0.37	4347019	Nonacosane
24	12.55	53248803	3.14	25330664	Tetradecanoic acid
25	12.957	15724120	0.93	12503164	Cyclononasiloxane, octadecamethyl-

26	13.120	2273948	0.13	2019330	9-Octadecyne
27	13.434	27407500	1.62	17608681	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
28	13.551	1860669	0.11	1442922	Methoxyacetic acid, 4-tridecyl ester
29	13.620	879585	0.05	833688	Dodecane, 2-methyl-
30	13.749	14664611	0.86	6056461	Triacontanoic acid, methyl ester
31	13.754	38687268	2.28	22226382	Hexadecanoic acid, methyl ester
32	13.852	37775878	2.23	24394479	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
33	14.017	57602605	3.40	30931471	n-Hexadecanoic acid
34	14.055	30958704	1.83	19538003	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
35	14.109	30697023	1.81	16190379	Dibutyl phthalate
36	14.688	3114220	0.18	2190683	Tetratriacontane
37	14.935	20740332	1.22	6509967	10,13-Octadecadienoic acid, methyl ester
38	15.06	12651903	0.75	8864836	Heptasiloxane, hexadecamethyl-
39	15.203	3908469	0.23	2555550	Decane, 5-ethyl-5-methyl-
40	15.205	3372026	0.20	2232857	Decane-1-iodo-
41	15.365	2600753	0.15	1671023	Octadecanoic acid
42	15.392	245664	0.04	185471	Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5.alpha.,12.beta.)-
43	15.459	129209	0.01	681520	Fumaric acid, 10-chlorodecyl nonyl ester
44	16.007	11536317	1.14	7515497	Tetracosamethyl-cyclododecasiloxane
45	16.809	7561093	0.45	4062476	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
46	17.054	13368884	1.05	6132067	Cyclodecasiloxane, eicosamethyl-
47	18.591	31247402	1.84	6261874	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
48	32.499	57311037	3.38	7590642	Stigmasterol
49	34.121	100512645	5.93	10832268	.gamma.-Sitosterol
		691985260	100.0	611480224	

4.3.9 Phytochemicals from the extract of *G. livingstonei* fruit rinds

In *G. livingstonei* fruit extract Propanoic acid, 2-chloro- (30.71%); Phthalic acid, di-(1-hexen-5-yl) ester (12.43%); n-Hexadecanoic acid (10.15%); Tetradecanoic acid (9.43%); .gamma.-Sitosterol (4.71%); Hexadecanoic acid, methyl ester (4.33%); Trichloromethane (3.53%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.56%); Octadecanoic acid (2.12%); Dibutyl phthalate (1.74%); 9-Octadecenoic acid, methyl ester, (E)- (1.70%); Oleic Acid (1.54); 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (1.34%); Cholesta-4,6-dien-3-ol, (3.beta.)- (1.34%); Octadecanoic acid, methyl ester (1.30%) and Phenol, 2,4-bis(1,1-dimethylethyl)- (1.27%) were found as major phytochemicals. Methoxyacetic acid, 4-hexadecyl ester (0.30%); Bis (dodecanamido)methane (0.26%); Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.beta., 8.alpha.(1E,5E)] (0.84%) and Succinimide, 3-(3-cyclohexen-1-yl)-N-phenyl- (0.30%) were detected as an unique phytochemicals. Along with these phytochemicals 1-Triacontanol (2.82%) and 9-Octadecene, 1-[2-(octadecyloxy)ethoxy]-, (Z)- (1.46%) were found as an unique compounds and in major quantities (Fig. 4.22; Table 4.15).

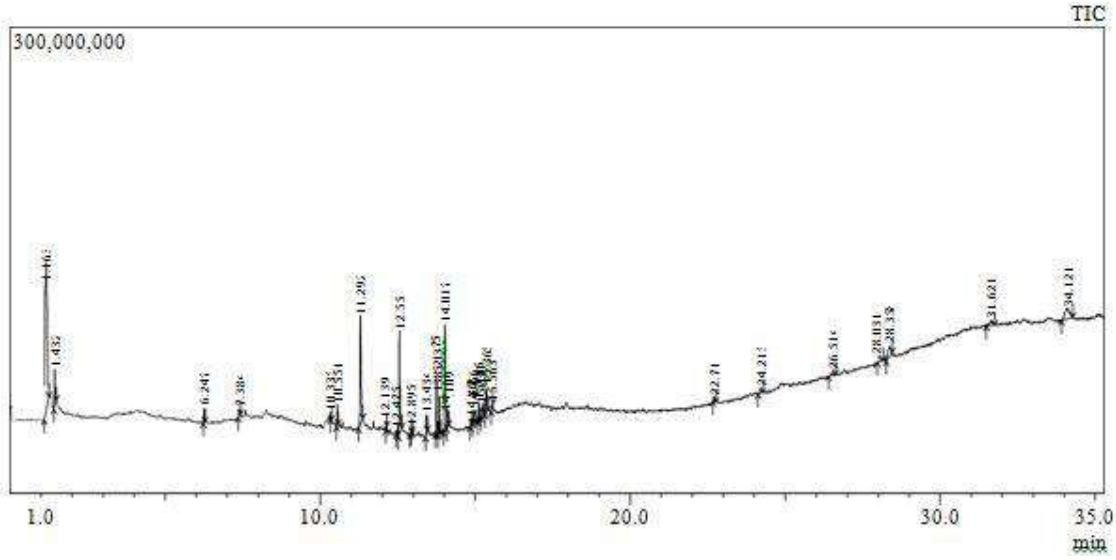


Figure 4.22: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. livingstonei* fruit rinds.

Table 4.15: Compounds identified at each peak and respective peak details for *G. livingstonei* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.163	450515999	30.71	79855732	Propanoic acid, 2-chloro-
2	1.432	51767243	3.53	23770045	Trichloromethane
3	6.247	12739038	0.87	9532071	Undecane
4	7.384	8889967	0.61	4626914	Naphthalene
5	10.335	5586714	0.38	4942312	Nonane, 5-(1-methylpropyl)-
6	10.551	18688614	1.27	13342531	Phenol, 2,4-bis(1,1-dimethylethyl)-
7	11.292	182311503	12.43	70481882	Phthalic acid, di-(1-hexen-5-yl) ester
8	12.139	4980825	0.34	4551913	Eicosane
9	12.475	4370856	0.30	3661873	Methoxyacetic acid, 4-hexadecyl ester
10	12.55	138373722	9.43	66402530	Tetradecanoic acid
11	12.895	3011437	0.21	2947789	Geranylgeraniol
12	12.957	2162678	0.15	2494188	Cyclononasiloxane, octadecamethyl-
13	13.434	19584001	1.34	12708336	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
14	13.754	63442704	4.33	37622996	Hexadecanoic acid, methyl ester
15	13.852	37624177	2.56	24874017	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
16	14.017	148957931	10.15	68878903	n-Hexadecanoic acid
17	14.109	25552711	1.74	9383221	Dibutyl phthalate
18	14.872	9240422	0.63	4460267	Behenic alcohol
19	14.97	24962287	1.70	8238213	9-Octadecenoic acid, methyl ester, (E)-/ Elaidic acid, methyl ester
20	15.116	19097251	1.30	13012162	Octadecanoic acid, methyl ester
21	15.230	22585080	1.54	7406175	Oleic Acid
22	15.365	31087206	2.12	16181392	Octadecanoic acid
23	15.563	3868367	0.26	1255848	Bis(dodecanamido)methane
24	22.71	9255202	0.63	3218376	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-/ trans-

					squalene
25	24.215	12361145	0.84	3176386	Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.beta.,8.alpha.(1E,5E)]
26	26.514	4390994	0.30	2437326	Succinimide, 3-(3-cyclohexen-1-yl)-N-phenyl-
27	28.031	19599657	1.34	3726870	Cholesta-4,6-dien-3-ol, (3.beta.)-
28	28.358	41345079	2.82	7202313	1-Triacontanol/ Myricyl alcohol
29	31.621	21391973	1.46	3585166	9-Octadecene, 1-[2-(octadecyloxy)ethoxy]-, (Z)-
30	34.121	69126494	4.71	7322107	.gamma.-Sitosterol/ Clionasterol
		466871277	100.0	521299854	

4.3.10 Phytochemicals from the extract of *G. mangostana* fruit rinds

The separation of compounds and their details from *G. mangostana* fruit extract are provided in chromatogram (Fig. 4.23) and Table 4.16. The major compounds detected were Ethene, (methylsulfonyl)- (33.22%); Phthalic acid, di-(1-hexen-5-yl) ester (11.48%); Hexadecanoic acid, methyl ester (3.95%); Tetracosane (2.70%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.31%); Dibutyl phthalate (1.86%); Trichloromethane (1.80%); Cyclodecasiloxane, eicosamethyl- (1.77%); Tetracosamethyl-cyclododecasiloxane (1.64%); Octadecanoic acid, methyl ester (1.38%); n-Hexadecanoic acid (1.37%); 9-Octadecenoic acid, methyl ester, (E)- (1.20%); Cyclononasiloxane, octadecamethyl- (1.16%); Cyclooctasiloxane, hexadecamethyl- (1.09%) and n-Nonadecanol-1 (1.02%). The distinctive compounds identified were 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl- (0.14%); Tridecane (0.17%); 1,2-Benzenedicarboxylic acid, butyl decyl ester (0.53%) and 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (0.37%). Apart from these some distinctive compounds with major quantity were also found such as Disiloxane, hexabutoxy- (12.88%); Triacontane (4.37%); 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12,16,20,24,28,32-octamethyl-3,7,11,15,19,23,27,31-tritriacontaoctaenyl)-, [r-(all-E)]- (2.06%); Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- (1.79%) and Copaene (1.54%).

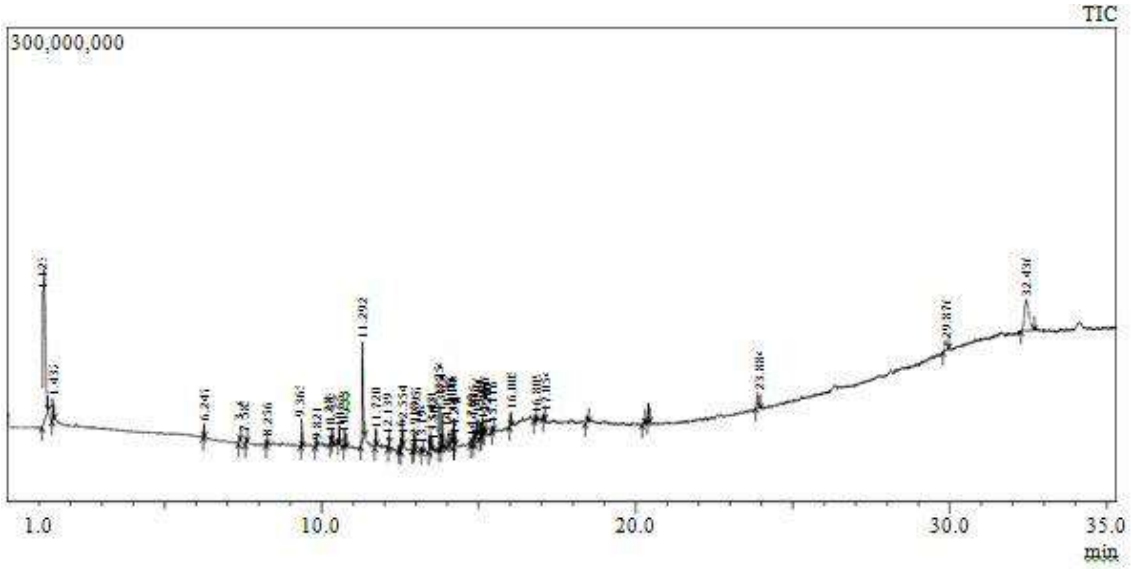


Figure 4.23: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. mangostana* fruit rinds.

Table 4.16: Compounds identified at each peak and respective peak details for *G. mangostana* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	499329331	33.22	84838430	Ethene, (methylsulfonyl)-
2	1.432	27125163	1.80	14265641	Trichloromethane
3	6.247	10658198	0.71	8163169	Undecane
4	7.384	3768448	0.25	2438265	Naphthalene
5	7.585	5694882	0.38	3642815	1-Diisopropylsilyloxycyclohexane
6	8.256	2614010	0.17	2613266	Dodecane, 4,6-dimethyl-
7	9.365	23212882	1.54	17140604	Copaene
8	9.821	5063801	0.34	3443872	Caryophyllene
9	10.283	5607395	0.37	4626042	Cycloheptasiloxane, tetradecamethyl-
10	10.333	7048053	0.81	4085686	Heptadecane, 8-methyl-
11	10.551	13450591	0.89	11062167	Phenol, 2,4-bis(1,1-dimethylethyl)-
12	10.733	26873142	1.79	11171644	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-/ Eudesma-3,7(11)-diene
13	11.292	172564211	11.48	63956924	Phthalic acid, di-(1-hexen-5-yl) ester
14	11.720	16398148	1.09	11523104	Cyclooctasiloxane, hexadecamethyl-
15	12.139	6052770	0.72	5250870	Eicosane
16	12.477	4938341	0.33	4147206	Nonacosane
17	12.554	40520792	2.70	19806225	Tetracosane
18	12.918	2142694	0.14	1912648	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-
19	12.957	17377787	1.16	13601509	Cyclononasiloxane, octadecamethyl-
20	13.194	4522181	0.30	3037466	Heneicosane
21	13.434	12475865	0.83	8454705	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
22	13.502	2551024	0.17	2692445	Tridecane
23	13.754	59308788	3.95	28759718	Hexadecanoic acid, methyl ester

24	13.852	34656435	2.31	21560804	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
25	14.017	20629026	1.37	11370966	n-Hexadecanoic acid
26	14.109	27961631	1.86	15998781	Dibutyl phthalate
27	14.248	7898768	0.53	5339951	1,2-Benzenedicarboxylic acid, butyl decyl ester
28	14.765	5576463	0.37	3196214	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester
29	14.875	15384923	1.02	6087248	n-Nonadecanol-1
30	14.97	18094225	1.20	9604544	9-Octadecenoic acid, methyl ester, (E)-/ Elaidic acid, methyl ester
31	15.06	13704954	0.90	9500242	Heptasiloxane, hexadecamethyl-
32	15.116	20729139	1.38	12005740	Octadecanoic acid, methyl ester
33	16.005	12301994	0.82	8234565	1,3,3,3-Tetramethyldisiloxanyl tris (trimethylsilyl) orthosilicate
34	16.007	12193097	1.64	4695308	Tetracosamethyl-cyclododecasiloxane
35	16.809	5711385	0.38	3390220	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
36	17.054	26630298	1.77	17601584	Cyclodecasiloxane, eicosamethyl-
37	23.884	36907513	4.37	11357215	Triacontane
38	29.876	30920328	2.06	6208250	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12,16,20,24,28,32-octamethyl-3,7,11,15,19,23,27,31-tritriacontaoctaenyl)-, [r-(all-E)]-/ gamma-tocotrienol
39	32.436	193700687	12.88	19734534	Disiloxane, hexabutoxy-
		452299363	100.0	496520587	

4.3.11 Phytochemicals from the extract of *G. morella* fruit rinds

In the methanolic extract of *G. morella* fruits Caryophyllene (6.99%); 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (2.35%); n-Hexadecanoic acid (1.86%); Ethyl .alpha.-d-glucopyranoside (1.27%); 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (1.05%) and Stigmasterol (1.02%) were found as major phytochemicals. N-dl-Alanylglycine (0.26%); 3-Piperidinol, 1-acetyl-6-propyl-, (3R-trans)- (0.87%); 2-Pentanone (0.25%); 2,3-Butanediol, [R-(R*,R*)]- (0.24%); 1,3-Cyclopentadiene, 5-(1-methylethylidene)- (0.41%); 1,2-Benzenediol (0.39%); Methyl 4-O-acetyl-2,3,6-tri-O-ethyl-.alpha.-d-galactopyranoside (0.36%); 3-Hydroxy-2-(2-methylcyclohex-1-enyl)propionaldehyde (0.47%); 1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)- (0.27%); Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)- (0.40%); Caffeine (0.33%); 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (0.39%); 3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid (0.31%); 9-Octadecenoic acid, (E)- (0.53%) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (0.47%) were found as unique phytochemicals. Also, some phytochemicals were observed as an unique with major quantities such as Ethane, 1-chloro-1-fluoro- (32.13%); 4-Penten-2-ol (17.23%); Sucrose (6.78%); (1R,3R,4R,5R)-(-)-Quinic acid (6.68%); Isopropyl Alcohol (3.71%); Caryophyllene oxide (2.58%); Acetic acid (1.92%); 3-Deoxy-d-mannoic lactone (1.33%); 2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)- (1.25%) and Propane, 2-fluoro-2-methyl- (1.07%) (Fig. 4.24; Table 4.17).

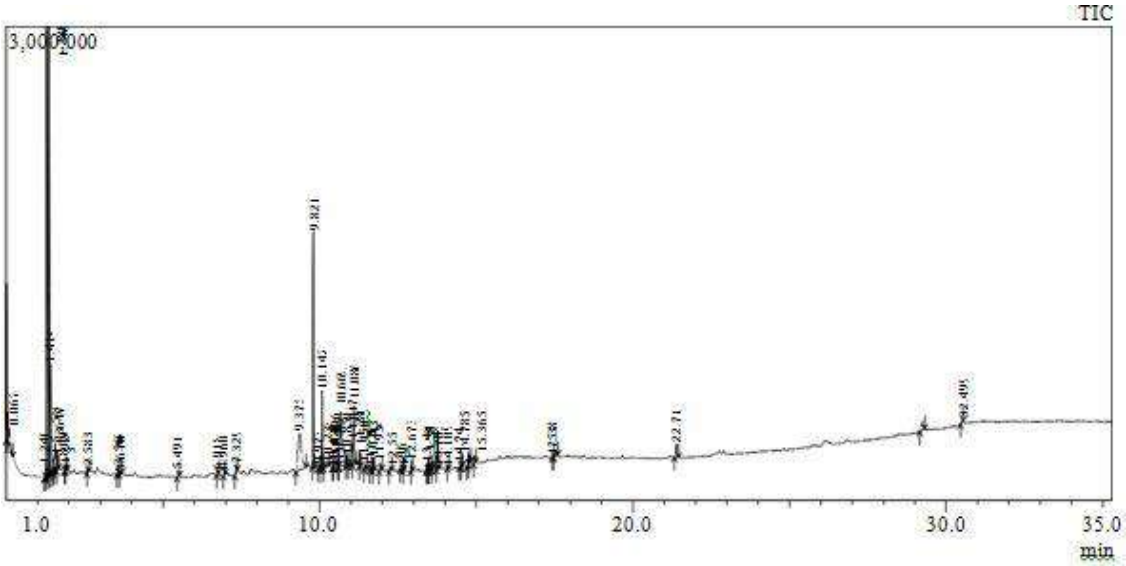


Figure 4.24: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. morella* fruit rinds.

Table 4.17: Compounds identified at each peak and respective peak details for *G. morella* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	0.067	346776	1.25	98818	2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)-
2	1.240	73087	0.26	76802	N-dl-Alanylglycine
3	1.288	8930683	32.13	3062561	Ethane, 1-chloro-1-fluoro-
4	1.369	4790138	17.23	3742669	4-Penten-2-ol
5	1.414	1032429	3.71	697065	Isopropyl Alcohol
6	1.535	297982	1.07	80260	Propane, 2-fluoro-2-methyl-
7	1.553	533473	1.92	177364	Acetic acid
8	1.65	242034	0.87	116252	3-Piperidinol, 1-acetyl-6-propyl-, (3R-trans)-
9	1.869	15013	0.05	15473	Hydrazinecarbothioamide
10	1.924	70668	0.25	36301	2-Pentanone
11	2.583	66731	0.24	37675	2,3-Butanediol, [R-(R*,R*)]-
12	3.562	114592	0.41	47206	1,3-Cyclopentadiene, 5-(1-methylethylidene)-
13	3.670	37148	0.13	12434	p-Xylene/ Benzene, 1,4-dimethyl-
14	5.491	44807	0.16	31811	Benzyl Alcohol
15	6.918	35918	0.13	24628	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
16	6.960	51112	0.18	31094	Acetic acid, phenylmethyl ester
17	7.329	108977	0.39	52667	1,2-Benzenediol
18	9.375	1885623	6.78	234985	Sucrose
19	9.821	1941947	6.99	1489362	Caryophyllene
20	9.973	43196	0.16	23266	Silane, dimethyldi(but-2-enyloxy)-
21	10.142	654630	2.35	494140	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
22	10.233	100381	0.36	29575	Methyl 4-O-acetyl-2,3,6-tri-O-ethyl-.alpha.-d-galactopyranoside
23	10.420	19745	0.07	10519	1-Heptatriacotanol
24	10.496	368742	1.33	61623	3-Deoxy-d-mannoic lactone
25	10.669	1855631	6.68	344138	(1R,3R,4R,5R)-(-)-Quinic acid
26	10.718	47763	0.17	29730	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-/

					.delta.-Cadinene, (+)-
27	11.017	352247	1.27	137392	Ethyl .alpha.-d-glucopyranoside
28	11.086	717596	2.58	373117	Caryophyllene oxide
29	11.280	131690	0.47	104449	3-Hydroxy-2-(2-methylcyclohex-1-enyl)propionaldehyde
30	11.303	161872	0.58	63036	Diethyl Phthalate
31	11.450	75686	0.27	31011	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-
32	11.620	23265	0.08	16503	Andrographolide
33	11.713	110270	0.40	81745	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-
34	11.934	108698	0.39	48574	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol/ Coniferyl alcohol
35	12.55	66885	0.24	20937	Tetradecanoic acid
36	12.596	92176	0.33	37005	Caffeine
37	12.673	35479	0.13	29302	2-Naphthalenol, 2,3,4,4a,5,6,7-octahydro-1,4a-dimethyl-7-(2-hydroxy-1-methylethyl)
38	13.434	86525	0.55	66576	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
39	13.754	49712	0.18	34562	Hexadecanoic acid, methyl ester
40	13.785	85035	0.31	18556	3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid
41	14.017	516119	1.86	268743	n-Hexadecanoic acid
42	14.094	21909	0.08	18060	Pentadecanal-
43	14.109	185684	0.67	100804	Dibutyl phthalate
44	14.740	124568	0.50	69988	9,12-Octadecadienoic acid (Z,Z)-/ Linoleic acid
45	14.785	146350	0.53	55553	9-Octadecenoic acid, (E)-/ trans-oleic acid
46	14.875	21124	0.08	18008	n-Nonadecanol-1
47	15.365	93919	0.34	50966	Octadecanoic acid
48	16.681	28562	0.10	14566	Tetracontane
49	17.538	131987	0.47	43790	1,2-Benzenedicarboxylic acid, diisooctyl ester/ Corflex 880
50	22.71	290945	1.05	86108	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-/ trans-squalene
51	32.499	282429	1.02	60921	Stigmasterol
52	34.121	68279	0.25	20641	.gamma.-Sitosterol
		27798747	100.0	12986339	

4.3.12 Phytochemicals from the extract of *G. pushpangadaniana* fruit rinds

An endemic and newly described species *G. pushpangadaniana* revealed the presence of unique phytoconstituents with major quantities which are provided in the chromatogram (Fig. 4.25) and their respective details in Table 4.18. It includes Lupan-3-ol, acetate (13.74%); Lanosterol (8.19%); Handianol (7.46%); 8,11,14-Eicosatrienoic acid, methyl ester (6.88%); Decanoic acid, 2-oxo-, methyl ester (6.27%); 2,5,5,8a-Tetramethyl-3-oxo-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester (5.20%); Isozonarol (4.33%) and Heptadecanoic acid, 14-methyl-, methyl ester (2.86%). Along with these the common phytoconstituents with major quantities were also identified such as Propanoic acid, 2-chloro- (13.67%); Citric acid, trimethyl ester (4.44%); Phthalic acid, di-(1-hexen-5-yl) ester (3.50%); Eicosanoic acid, methyl ester (3.29%); Heptasiloxane, hexadecamethyl- (2.56%); Hexasiloxane,tetradecamethyl (1.67%); Oleic Acid (1.63%); Tetracosamethyl-cyclododecasiloxane (1.45%); Cyclodecasiloxane, eicosamethyl- (1.31%) and 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane (1.10%). Also, the unique phytoconstituents with minor quantities detected such as 2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl- (2.alpha.,4a.alpha.,8a.alpha.)- (0.29%); 1-Phenanthrenecarboxylic acid, 7-ethyltetradecahydro (0.93%); 4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone (0.40%) and 1,16-Dibromohexadecane (0.87%).

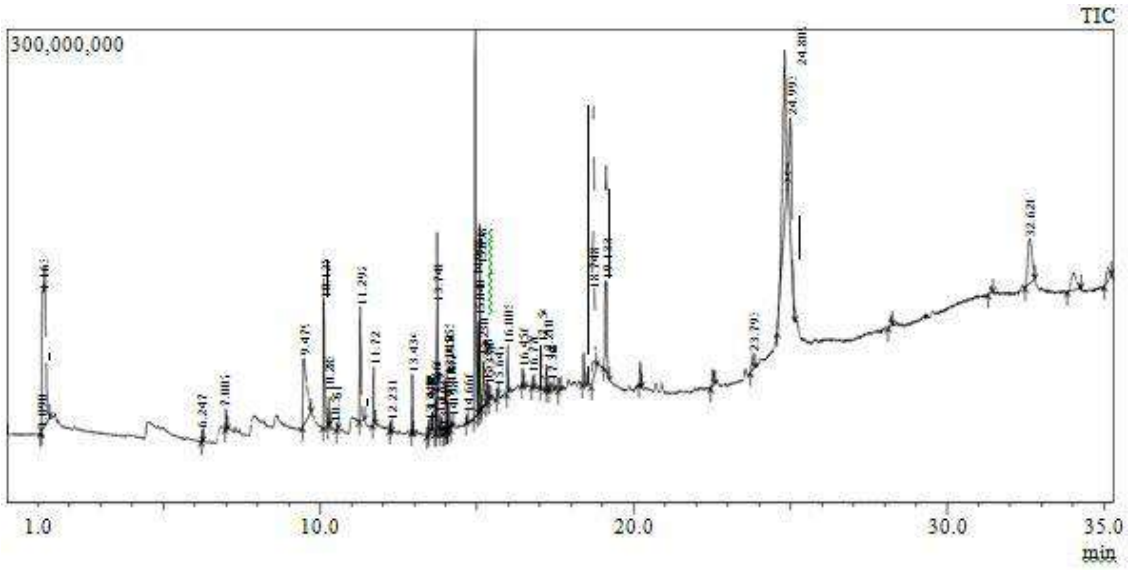


Figure 4.25: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. pushpangadaniana* fruit rinds.

Table 4.18: Compounds identified at each peak and respective peak details for *G. pushpangadaniana* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.090	3535127	0.06	2554146	5,7-Dioxa-1-octene, 1-chloro-4-isopropyl-3-methyl-
2	1.163	758598720	13.67	86146196	Propanoic acid, 2-chloro-
3	6.247	9934573	0.18	7434843	Undecane
4	7.007	18405043	0.33	12328848	Silane, cyclohexyldimethoxymethyl-
5	9.479	348114301	6.27	42486827	Decanoic acid, 2-oxo-, methyl ester
6	10.128	246425669	4.44	80960663	Citric acid, trimethyl ester
7	10.283	27842443	0.50	18304727	Cycloheptasiloxane, tetradecamethyl-
8	10.551	5116986	0.11	4012441	Phenol, 2,4-bis(1,1-dimethylethyl)-
9	11.292	194406389	3.50	72688342	Phthalic acid, di-(1-hexen-5-yl) ester
10	11.72	54609909	0.98	36176643	Cyclooctasiloxane, hexadecamethyl-
11	12.231	9260022	0.17	6444173	Methyl tetradecanoate/ Methyl myristate
12	13.434	22024214	0.40	12624766	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
13	13.440	6569042	0.12	5762918	Silane, dimethyl(dimethyl(2-hexyloxy)silyloxy)hexyloxy-
14	13.666	30063921	0.54	20723885	9-Hexadecenoic acid, methyl ester, (Z)-
15	13.740	182501973	3.29	81721496	Eicosanoic acid, methyl ester/ Arachidic acid
16	13.852	15284337	0.28	9999679	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
17	13.940	5594789	0.10	3191970	8-Heptadecene, 1-chloro-
18	14.017	53529977	0.96	29858794	n-Hexadecanoic acid
19	14.055	60777358	1.10	39504647	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
20	14.109	37380371	0.67	18055758	Dibutyl phthalate
21	14.198	7546776	0.14	3762703	Hexadecanoic acid, ethyl ester
22	14.666	7529711	0.14	5482997	2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid
23	14.968	381944496	6.88	78834778	8,11,14-Eicosatrienoic acid, methyl ester
24	15.040	92830769	1.67	58132102	Hexasiloxane,tetradecamethyl

25	15.06	50322179	2.56	30322269	Heptasiloxane, hexadecamethyl-
26	15.098	158500517	2.86	75773782	Heptadecanoic acid, 14-methyl-, methyl ester
27	15.230	90505309	1.63	28837011	Oleic Acid
28	15.365	31968074	0.58	13137442	Octadecanoic acid
29	15.370	16996812	0.31	9904948	Ethyl Oleate
30	15.647	7625431	0.14	5568070	2,7,11-Trimethyl-4-phenylthiododeca-2,6,10-triene
31	16.005	48810456	0.88	37447559	1,3,3,3-tetramethyldisiloxanyl tris (trimethylsilyl) orthosilicate
32	16.007	38759915	1.45	10437372	Tetracosamethyl-cyclododecasiloxane
33	16.456	20445822	0.37	11464796	Methyl 18-methylnonadecanoate
34	16.776	15841471	0.29	7738901	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)-
35	17.054	72887948	1.31	25024213	Cyclodecasiloxane, eicosamethyl-
36	17.210	33579554	0.93	16290276	1-Phenanthrenecarboxylic acid, 7-ethyltetradecahydro
37	17.368	22306304	0.40	5506350	4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone
38	18.748	288449013	5.20	52496511	2,5,5,8a-Tetramethyl-3-oxo-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester
39	19.133	240404297	4.33	52478223	Isozonarol
40	23.793	48497028	0.87	10566053	1,16-Dibromohexadecane
41	24.809	762453893	13.74	107714027	Lupan-3-ol, acetate
42	24.993	413998841	7.46	74166575	Handianol
43	32.620	266179618	8.19	28931453	Lanosterol
		208359398	100.0	341000173	

4.3.13. Phytochemicals from the extract of *G. rubro-echinata* fruit rinds

The chromatogram (Fig. 4.26) and Table 4.19 depicted presence of phytochemicals from fruit extract of the southern Western Ghats endemic species *G. rubro-echinata*. It revealed the presence of major phytochemicals as Ethene, (methylsulfonyl)- (12.85%); Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- (6.31%); Phthalic acid, di-(1-hexen-5-yl) ester (4.47%); Tetradecanoic acid (3.83%); Cyclooctasiloxane, hexadecamethyl- (3.56%); Heptasiloxane, hexadecamethyl- (1.28%); n-Hexadecanoic acid (1.10%); Hexadecanoic acid, methyl ester (1%) and unique phytochemicals as Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.beta.) (0.12%); .alpha.-Cadinol (0.21%); Naphthalene, 1,6-dimethyl-4-(1-methylethyl)- (0.11%); 7-Hexadecenal, (Z)- (0.23%); 1-Octadecanol (0.09%); Chrom-6-ol-4-one, 2,3-dihydroxy-2,2,7,8-tetramethyl- (0.22%); 1,3,5-Cycloheptatriene, 3,4,7,7-tetramethyl-2,5-bis(trimethylsilyl)- (0.11%); Methyloctadecyldichlorosilane (0.15%). Along with the above phytochemicals it showed presence of major phytochemicals which are distinctive such as 3-Acetyl-1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-3H-2,3-benzodiazepine (47.20%); 1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo (7.2.0)undec-3-ene (4.58%); 3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl- (2.07%); 1,5-Heptadien-4-ol, 3,3,6-trimethyl- (1.40%); Caryophyllenyl alcohol (1.16%) and D-Glycero-D-gulo-Heptonic acid, 2,3,5,6,7-pentakis-O-(trimethylsilyl)-, .gamma.-lactone (1.16%).

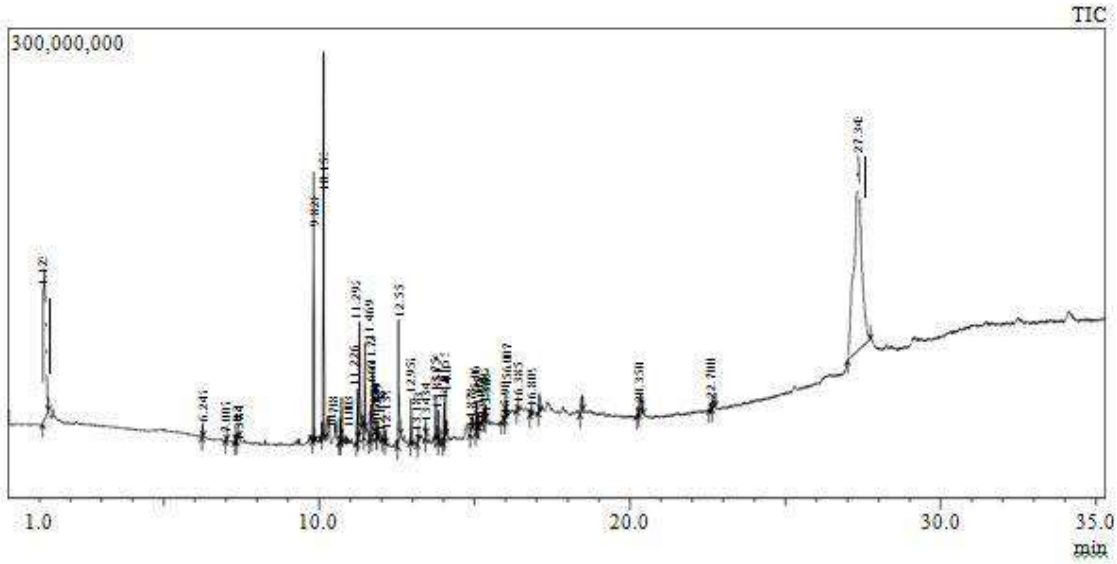


Figure 4.26: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. rubro-echinata* fruit rind.

Table 4.19: Compounds identified at each peak and respective peak details for *G. rubro-echinata* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	540503981	12.85	84902515	Ethene, (methylsulfonyl)-
2	6.247	9411302	0.22	7471634	Undecane
3	7.007	2176304	0.05	2091905	Silane, cyclohexyldimethoxymethyl-
4	7.3	4372280	0.10	3201238	Phenyl methylphosphonofluoridate
5	7.384	4075546	0.10	2243915	Naphthalene
6	9.828	192600222	4.58	97784716	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undec-3-ene/ Humulene(v1)
7	10.153	265688102	6.31	94885439	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-/ .alpha.-Selinene
8	10.718	53120891	1.26	28576218	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-/ .delta.-Cadinene, (+)-
9	11.003	21617351	0.51	15271781	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-/ .gamma.-Cadinene
10	11.226	48792999	1.16	32764539	Caryophyllenyl alcohol
11	11.292	188039108	4.47	71604341	Phthalic acid, di-(1-hexen-5-yl) ester
12	11.469	87162535	2.07	57037130	3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl-/ Humulol
13	11.666	58856166	1.40	21753393	1,5-Heptadien-4-ol, 3,3,6-trimethyl-/ Artemisia alcohol
14	11.72	123284465	3.56	41779280	Cyclooctasiloxane, hexadecamethyl-
15	11.850	4890817	0.12	5120966	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.beta.)
16	11.889	8680592	0.21	7599538	.alpha.-Cadinol
17	12.058	4662899	0.11	4379085	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-/ Cadalin
18	12.139	8520192	0.20	7091701	Eicosane
19	12.55	161185003	3.83	78368485	Tetradecanoic acid
20	12.957	37496431	0.89	28581984	Cyclononasiloxane, octadecamethyl-
21	13.183	9569684	0.23	5359597	7-Hexadecenal, (Z)-

22	13.434	9291850	0.22	7639897	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
23	13.749	6214417	0.15	4693213	Triacontanoic acid, methyl ester
24	13.754	42115911	1.00	26868779	Hexadecanoic acid, methyl ester
25	13.852	29499862	0.70	20297623	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
26	14.017	46320245	1.10	23893194	n-Hexadecanoic acid
27	14.055	35082053	0.83	27187400	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
28	14.878	3346076	0.09	2157380	1-Octadecanol
29	15.06	22407554	1.28	11514446	Heptasiloxane, hexadecamethyl-
30	15.205	6168395	0.15	2996580	Decane, 1-iodo-
31	15.302	9289562	0.22	6219825	Chrom-6-ol-4-one, 2,3-dihydroxy-2,2,7,8-tetramethyl-
32	15.365	3077406	0.07	2474829	Octadecanoic acid
33	15.905	4500853	0.11	2911665	1,3,5-Cycloheptatriene, 3,4,7,7-tetramethyl-2,5-bis(trimethylsilyl)-
34	16.007	25094596	0.87	17026297	Tetracosametthyl-cyclododecasiloxane
35	16.385	6321232	0.15	3212530	Methyloctadecyldichlorosilane
36	16.809	6506105	0.15	3681465	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
37	20.350	48784983	1.16	12925340	D-Glycero-D-gulo-Heptonic acid, 2,3,5,6,7-pentakis-O-(trimethylsilyl)-, .gamma.-lactone
38	22.700	13626274	0.32	5170032	Squalene
39	27.343	986011434	47.20	124204862	3-Acetyl-1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-3H-2,3-benzodiazepine
		176094498	100.0	1002944757	

4.3.14 Phytochemicals from the extract of *G. spicata* fruit rinds

In the methanolic extract of *G. spicata* fruit rind Ethene, (methylsulfonyl)- (39.96%); Phthalic acid, di-(1-hexen-5-yl) ester (14.86%); Tetradecanoic acid (14.13%); n-Hexadecanoic acid (4.44%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (3.19%); Triacontanoic acid, methyl ester (2.99%); Octadecanoic acid, methyl ester (2.29%); Phenol, 2,4-bis(1,1-dimethylethyl)- (1.82%); Decane, 1-iodo- (1.35%) and 1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene (1.15%) were detected as major compounds. Ether, bis[2-(ethylthio)ethyl] (0.44%); Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- (0.28%) and Heptadecanoic acid, ethyl ester (0.10%) were detected as unique compounds. Phospholane (2.72%); 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol (1.27%) and Nonane, 1,9-dibromo- (1.20%) were found as unique compounds with major quantities (Fig. 4.27; Table 4.20).

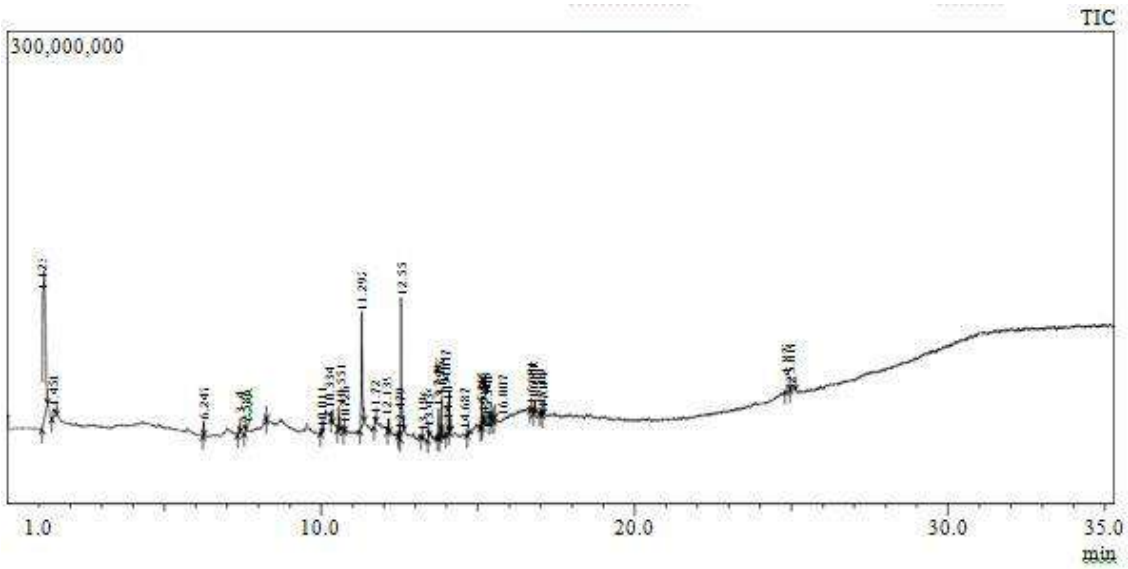


Figure 4.27: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. spicata* fruit rind.

Table 4.20: Compounds identified at each peak and respective peak details for *G. spicata* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	504053438	39.96	84730368	Ethene, (methylsulfonyl)-
2	1.450	34345743	2.72	5631939	Phospholane
3	6.247	11857758	0.94	9279835	Undecane
4	7.384	7870218	0.62	4644132	Naphthalene
5	7.586	5593598	0.44	3982783	Ether, bis[2-(ethylthio)ethyl]
6	10.011	3533210	0.28	2834610	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-/ beta-sesquiphellandrene
7	10.333	2406837	0.19	2267087	Heptadecane, 8-methyl-
8	10.334	8301672	0.66	5958918	Hexadecane, 1-iodo-
9	10.551	22974404	1.82	14506104	Phenol, 2,4-bis(1,1-dimethylethyl)-
10	10.726	3489349	0.28	2804244	Dodecane, 2,6,11-trimethyl-
11	11.292	187408787	14.86	73795846	Phthalic acid, di-(1-hexen-5-yl) ester
12	11.72	8527484	0.68	4516814	Cyclooctasiloxane, hexadecamethyl-
13	12.139	11931087	0.95	8467237	Eicosane
14	12.479	2924887	0.23	2945606	Hexadecane
15	12.55	178180637	14.13	87930017	Tetradecanoic acid
16	13.194	2266182	0.18	2461804	Heneicosane
17	13.434	6749604	0.54	5014341	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
18	13.749	37668534	2.99	18775004	Triacontanoic acid, methyl ester
19	13.852	40250606	3.19	25410334	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
20	14.017	55969609	4.44	22935841	n-Hexadecanoic acid
21	14.109	9203549	0.73	6575425	Dibutyl phthalate
22	14.687	3592925	0.28	2716583	Octadecane, 1-iodo-
23	15.116	28927166	2.29	18333857	Octadecanoic acid, methyl ester

24	15.205	13816586	1.35	5149888	Decane, 1-iodo-
25	15.365	5804492	0.46	3780127	Octadecanoic acid
26	15.541	1287312	0.10	1323628	Heptadecanoic acid, ethyl ester
27	16.007	3470047	0.28	1904538	Tetracosamethyl-cyclododecasiloxane
28	16.681	3365409	0.27	2590788	Tetracontane
29	16.809	14465818	1.15	7326007	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
30	17.009	6552511	0.52	3400257	Di-n-decylsulfone
31	24.872	15177367	1.20	3284362	Nonane, 1,9-dibromo-
32	25.017	15996449	1.27	3305352	4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol/ Oleanan-3-ol
		257963275	100.0	448583676	

4.3.15 Phytochemicals from the extract of *G. talbotii* fruit rinds

The phytochemical profile of *G. talbotii* fruit extract is shown in Figure 4.28. The major phytochemicals detected were Phthalic acid, di-(1-hexen-5-yl) ester (12.57%); Cyclodecasiloxane, eicosamethyl- (7.37%); Tetracosamethyl-cyclododecasiloxane (5.18%); Cyclooctasiloxane, hexadecamethyl- (4.15%); Tetradecanoic acid (4.08%); Cyclononasiloxane, octadecamethyl- (2.71%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (2.70%); n-Hexadecanoic acid (2.54%); Heptasiloxane, hexadecamethyl- (2.36%); Cycloheptasiloxane, tetradecamethyl- (1.56%); Dibutyl phthalate (1.28%) and Phenol, 2,4-bis(1,1-dimethylethyl)- (1.13%). The unique compounds found were Butane, 1,1'-oxybis[4-chloro- (0.21%) and Sulfurous acid, hexyl octyl ester (0.18%). Propane, 2-methyl-1-(methylthio)- (42.75%) and 1,2-Cyclopentanedione (4.12%) were found as unique compounds with major quantities (Table 4.21).

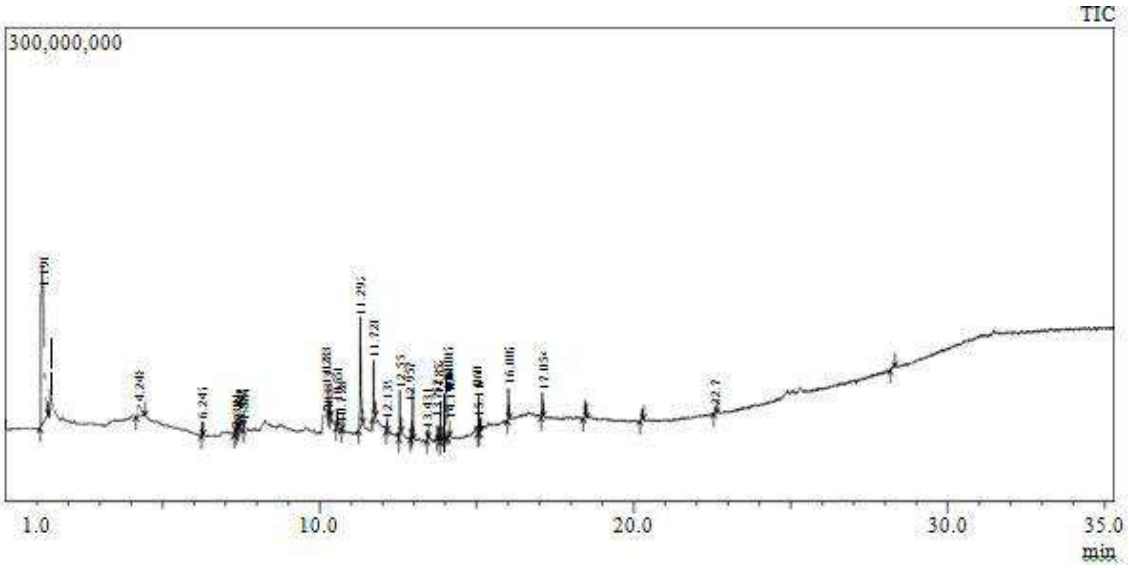


Figure 4.28: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. talbotii* fruit rind.

Table 4.21: Compounds identified at each peak and respective peak details for *G. talbotii* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.190	615728739	42.75	84393089	Propane, 2-methyl-1-(methylthio)-
2	4.248	59377336	4.12	7230317	1,2-Cyclopentanedione
3	6.247	11783457	0.82	8486242	Undecane
4	7.301	3021747	0.21	2305478	Butane, 1,1'-oxybis-4-chloro-
5	7.384	7479008	0.52	3842294	Naphthalene
6	7.431	1060923	0.08	1222845	Silanol, trimethyl-, formate
7	7.587	1981994	0.14	1540102	1,3,5-Trioxane
8	10.283	22447869	1.56	17894871	Cycloheptasiloxane, tetradecamethyl-
9	10.333	3320265	0.23	3004799	Heptadecane, 8-methyl-
10	10.551	16307939	1.13	11684037	Phenol, 2,4-bis(1,1-dimethylethyl)-
11	10.728	2644111	0.18	2290836	Sulfurous acid, hexyl octyl ester
12	11.292	181078602	12.57	70712398	Phthalic acid, di-(1-hexen-5-yl) ester
13	11.720	59830320	4.15	35976813	Cyclooctasiloxane, hexadecamethyl-
14	12.139	8238011	0.57	6037040	Eicosane
15	12.55	58813183	4.08	28920114	Tetradecanoic acid
16	12.957	38996304	2.71	29956036	Cyclononasiloxane, octadecamethyl-
17	13.434	7042651	0.49	5530523	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
18	13.751	13686765	0.95	8540282	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester
19	13.852	38906162	2.70	25164545	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
20	14.017	36586849	2.54	21724447	n-Hexadecanoic acid
21	14.109	18502961	1.28	10526930	Dibutyl phthalate
22	15.060	33951334	2.36	23341676	Heptasiloxane, hexadecamethyl-
23	15.116	12611861	0.88	7100583	Octadecanoic acid, methyl ester
24	16.007	31477086	5.18	19477114	Tetracosamethyl-cyclododecasiloxane
25	17.054	51539772	7.37	31977245	Cyclodecasiloxane, eicosamethyl-
26	22.7	6135369	0.43	5237276	Squalene
		342550618	100.0	474117932	

4.3.16 Phytochemicals from the extract of *G. travancorica* fruit rinds

Fruit extract of the southern Western Ghats species *G. travancorica* have shown large group of compounds and the separation of the compounds is provided in the chromatogram (Fig. 4.29) and their respective details in Table 4.22. The major compounds detected were 1,4-Benzenedicarboxylic acid, diethyl ester (8.48%); Diethyl 2-(p-tolyl) malonate (7.57%); Diethyl Phthalate (3.19%); 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentae (2.66%); n-Hexadecanoic acid (2.54%); Squalene (2.43%); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (2.11%); .gamma.-Sitosterol (1.75%); Cyclononasiloxane, octadecamethyl- (1.62%); Alanine, N-neopentyloxycarbonyl-, octadecyl ester (1.46%); .gamma.-Cadinene (1.27%); Ethyl .alpha.-d-glucopyranoside (1.22%) and Tetracosamethyl-cyclododecasiloxane (1.05%). This particular species revealed presence of many unique compounds such as Undecane, 5-ethyl- (0.88%); Silane, [3-(2,3-epoxypropoxy)propyl]ethoxydimethyl- (0.27%); 1,2,3-Propanetriol, 1-acetate (0.99%); Octanal (0.11%); Methyl 3,4-O-isopropylidene-L-threonate (0.44%); Dimethyl{bis[(2Z)-pent-2-en-1-yloxy]}silane (0.20%); .alpha.-D-Mannofuranoside, 1-O-heptyl- (0.98%); Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer (0.44%); 2(3H)-Benzofuranone, hexahydro-3a,7a-dimethyl-3-methylene-, cis- (0.10%); n-Butyric acid 2-ethylhexyl ester (0.17%); Cubedol (0.18%); Phytol, acetate (0.28%); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.43%); 1-Tetradecen-3-yne (0.79%); Bis(2-ethylhexyl) maleate (0.25%); 1,1'-Biphenyl, 5-hydroxy-3,4'-dimethoxy- (0.38%); Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate (0.24%); 1,1'-Biphenyl, 6-hydroxy-2',3',4'-trimethoxy- (0.20%); Octadecanamide (0.13%); 2,2'-Bis(bromomethyl)-1,1'-biphenyl (0.11%); 17-Oxo-4-propyl-3,4-seco-5.alpha.-androstan-3-oic acid methyl ester (0.97%); Ambrosiol (0.17%); 13,14-Epoxyursan-3-ol, acetate (0.15%); Vinyl stearate (0.95%); But-3-en-2-one, 4-amino-4-(4,6-dimethyl-2-pyrimidylamino)- (0.18%); Pentacosane (0.15%); Phenol, 3-methoxy-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-, acetate, (E,E)- (0.31%); Benahorin (0.26%); Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy] (0.10%); 9-Octadecene, 1-[3-(octadecyloxy)propoxy]-, (Z)- (0.12%); Bis(cis-13-docosenamido)methane (0.16%); 1,3-Dicyclohexylbutane (0.46%); 1-Hydroxymethyl-3,3,7,11 tetramethyltricyclo [5.4.0.0(4,11)] undecane (0.17%); Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]

benzoate (0.15%); Butanoic acid, 4-(4,5,6,7-tetrahydro-3-acetyl-4-oxo-2-benzothiophenyl)- (0.16%); 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol (0.12%); Pentasiloxane, dodecamethyl- (0.17%); Tetrapentacontane, 1,54-dibromo- (0.20%); Pyrimido[5,4-E]1,2,4-triazine-5,7(6H,8H)-dione, 6,8-dimethyl-, 1-oxide (0.19%); 1,2-Bis(trimethylsilyl)benzene (0.12%); Cyclotetrasiloxane, octamethyl- (0.39%); 1-Heptacosanol (0.90%); 1,3,5,7-Tetraethyl-1,7-dibutoxytetrasiloxane (0.11%); 1-Bromoeicosane (0.21%); 2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether (0.23%); 3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane (0.5%) and Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester (0.12%). Some unique compounds with major quantities were also observed such as Quinic acid (9.68%); 2,4-Hexadiyne-1,6-diol (9.08%); 5-Aminomethylene-6-hydroxy-4-methyl-2-oxo-2,5-dihydro-3-pyridinecarbonitrile (7.51%); Galactopyranoside, 1-octylthio-1-deoxy- (2.62%); Benzene, 1-ethoxymethyl-4-tert-butyl- (2.52%); 2,4-Hexadien-1-one, 1,3,5-triphenyl- (1.97%); dl-Glyceraldehyde dimer (1.91%); Ylangene (1.33%) and Bis(2-ethylhexyl) phthalate (1.06%).

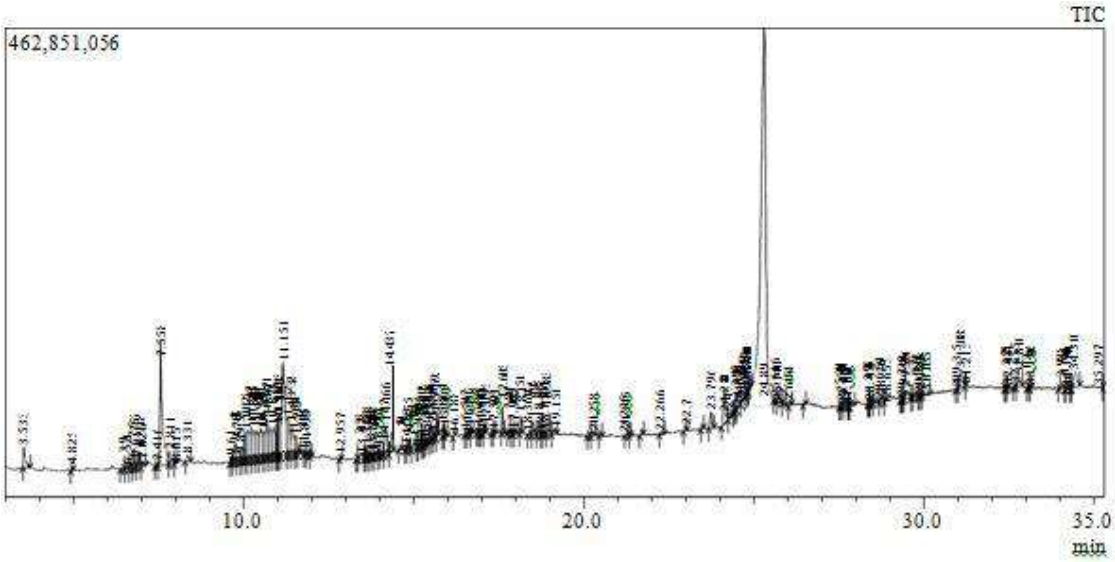


Figure 4.29: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. travancorica* fruit rinds.

Table 4.22: Compounds identified at each peak and respective peak details for *G. travancorica* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	3.535	76935235	1.91	19804480	dl-Glyceraldehyde dimer
2	4.825	5299696	0.08	2745256	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
3	6.390	4052707	0.06	1889622	Benzoic acid, methyl ester
4	6.520	5652072	0.09	2325545	2-Propanamine, N-methyl-N-nitroso-
5	6.757	56026534	0.88	19670308	Undecane, 5-ethyl-
6	6.918	47908700	0.75	16032765	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
7	7.026	17549831	0.27	4667326	Silane, [3-(2,3-epoxypropoxy)propyl]ethoxydimethyl-
8	7.416	5891096	0.09	2621637	2-Hexanol, 2,5-dimethyl-, (S)-
9	7.558	579903386	9.08	103183291	2,4-Hexadiyne-1,6-diol
10	7.811	63012817	0.99	12931395	1,2,3-Propanetriol, 1-acetate
11	8.015	6894419	0.11	1308986	Octanal
12	8.331	27811408	0.44	6095352	Methyl 3,4-O-isopropylidene-L-threonate
13	9.617	12759364	0.20	6171637	Dimethyl{bis[(2Z)-pent-2-en-1-yloxy]}silane
14	9.740	62533818	0.98	18029297	.alpha.-D-Mannofuranoside, 1-O-heptyl-
15	9.782	84833138	1.33	17932345	Ylangene
16	10.025	93521276	1.46	27709077	D-Alanine, N-neopentyloxycarbonyl-, octadecyl ester
17	10.205	179045283	7.51	33077156	5-Aminomethylene-6-hydroxy-4-methyl-2-oxo-2,5-dihydro-3-pyridinecarbonitrile
18	10.334	9427916	0.15	3646821	Hexadecane, 1-iodo-
19	10.671	161028931	2.52	39099004	Benzene, 1-ethoxymethyl-4-tert-butyl-
20	10.965	151304763	8.48	32097297	1,4-Benzenedicarboxylic acid, diethyl ester
21	11.003	81140733	1.27	40976220	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-/ .gamma.-Cadinene
22	11.017	78229910	1.22	19498334	Ethyl .alpha.-d-glucopyranoside
23	11.151	618147492	9.68	92971639	Quinic acid
24	11.229	169301327	7.57	42164949	Diethyl 2-(p-tolyl) malonate

25	11.303	203813438	3.19	39251383	Diethyl Phthalate
26	11.433	167290801	2.62	27476439	Galactopyranoside, 1-octylthio-1-deoxy-
27	11.595	28082834	0.44	9392637	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer
28	11.72	18196909	0.28	9815406	Cyclooctasiloxane, hexadecamethyl-
29	11.794	6664005	0.10	3667508	2(3H)-Benzofuranone, hexahydro-3a,7a-dimethyl-3-methylene-, cis-
30	11.855	10656865	0.17	2478816	n-Butyric acid 2-ethylhexyl ester
31	11.951	11251344	0.18	7051232	Cubedol
32	12.55	4161268	0.07	3122706	Tetradecanoic acid
33	12.957	24385339	1.62	10705251	Cyclononasiloxane, octadecamethyl-
34	13.332	5268076	0.08	3737935	6,11-Dimethyl-2,6,10-dodecatrien-1-ol
35	13.434	34828800	0.88	11437860	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
36	13.603	17833829	0.28	10070334	Phytol, acetate
37	13.754	23105277	0.36	12884740	Hexadecanoic acid, methyl ester
38	13.852	19715286	0.31	8433874	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
39	13.913	27623355	0.43	5366014	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
40	14.017	162059911	2.54	80331325	n-Hexadecanoic acid
41	14.094	12970835	0.35	4680545	Pentadecanal-
42	14.166	134562539	2.11	27592542	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
43	14.437	6468961	0.10	2968275	Octadecanal
44	14.69	18433142	0.49	5616253	Dotriacontane
45	14.74	38419987	0.60	16103995	9,12-Octadecadienoic acid (Z,Z)-/ Linoleic acid
46	14.875	43187235	0.68	21394706	n-Nonadecanol-1
47	14.941	10482971	0.16	6446749	9,12-Octadecadienoic acid (Z,Z)-, methyl ester/ Linoleic acid, methyl ester
48	14.97	10047560	0.16	3061701	9-Octadecenoic acid, methyl ester, (E)-/ Elaidic acid, methyl ester
49	15.040	2361167	0.04	2381278	Hexasiloxane, tetradecamethyl-
50	15.06	13362105	0.21	3063228	Heptasiloxane, hexadecamethyl-
51	15.365	52445406	0.82	23766788	Octadecanoic acid
52	15.501	50398721	0.79	16325261	1-Tetradecen-3-yne

53	15.586	16080383	0.25	7646837	Bis(2-ethylhexyl) maleate
54	15.864	24096338	0.38	11851590	1,1'-Biphenyl, 5-hydroxy-3,4'-dimethoxy-
55	16.007	41352697	1.05	10285156	Tetracosamethyl-cyclododecasiloxane
56	16.188	4795547	0.08	2412554	1-Monolinoleoylglycerol trimethylsilyl ether/ Linoleic acid 2,3-bis-(o-TMS)-propyl ester
57	16.517	9619775	0.24	4640344	Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate
58	16.590	2901992	0.05	588776	N-Methyl-1-adamantaneacetamide
59	16.702	12998704	0.20	6135991	1,1'-Biphenyl, 6-hydroxy-2',3',4'-trimethoxy-
60	16.915	872197	0.01	1172786	3-Bromo-2-([[(3-bromothien-2-yl)thio]methyl]thio)thiophene
61	16.983	5172359	0.08	2333383	10-Methyl-E-11-tridecen-1-ol propionate
62	17.009	3460583	0.05	1744556	Di-n-decylsulfone
63	17.301	8371684	0.13	4082055	Octadecanamide
64	17.365	7183532	0.11	3217203	2,2'-Bis(bromomethyl)-1,1'-biphenyl
65	17.605	61913497	0.97	27560247	17-Oxo-4-propyl-3,4-seco-5.alpha.-androstan-3-oic a
66	17.982	10987623	0.17	3921783	Azuleno[4,5-b]furan-2(3H)-one, decahydro-8,9-dihydroxy-6,9a-dimethyl-3-methylene, [3aS-(3a.alpha.,6.beta.,6a.alpha.,8.alpha.,9.alpha.,9a.beta/ Ambrosiol
67	18.360	9347691	0.15	1170740	13,14-Epoxyursan-3-ol, acetate
68	18.499	60521372	0.95	13145977	Octadecanoic acid, ethenyl ester/ Vinyl stearate
69	18.775	11501424	0.18	2524950	But-3-en-2-one, 4-amino-4-(4,6-dimethyl-2-pyrimidylamino)-
70	18.850	9343910	0.15	3177587	Pentacosane
71	18.983	67604113	1.06	20231584	Bis(2-ethylhexyl) phthalate
72	19.150	20106743	0.31	6407793	Phenol, 3-methoxy-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-, acetate, (E,E)-
73	20.258	16663940	0.26	4848315	4-(1,1-Dimethylallyl)-9-methoxy-7H-furo[3,2 g][1]benzopyran-7-one/ Benahorin
74	21.225	6602496	0.10	1354138	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]/ Thymol-TMS
75	21.345	7481436	0.12	3079632	9-Octadecene, 1-[3-(octadecyloxy)propoxy]-, (Z)-
76	22.266	9962161	0.16	1829995	Bis(cis-13-docosenamido)methane/ (13Z,13′Z)-N,N'-Methylenebis(13-docosenamide)

77	22.7	65269514	2.43	17248519	Squalene
78	23.796	24174287	0.38	7514047	6,10-Dodecadien-3-ol, 3,7,11-trimethyl-
79	24.280	29564660	0.46	4659765	Cyclohexane, 1,1'-(1-methyl-1,3-propanediyl)bis-/ 1,3-Dicyclohexylbutane
80	24.450	2504652	0.04	2095462	Cinnamic acid, 3,4-dimethoxy-, trimethylsilyl ester
81	24.580	5591756	0.09	843438	Oxalic acid, monoamide, N-(4-methoxybenzyl)-, undecyl ester
82	24.690	10792200	0.17	1870824	1-Hydroxymethyl-3,3,7,11 tetramethyltricyclo[5.4.0.0(4,11)] undecane
83	24.755	1500943	0.02	1525771	3-Hydroxyphenylpropionic acid, ethyl ester, TMS
84	24.840	2430658	0.04	1484537	7,9-Dimethyl-8-nitrobicyclo[4.3.1]decan-10-one
85	24.880	6659211	0.15	2322208	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate/ 4-(Trimethylsilyl)oxy-3-methylbenzoic acid, trimethylsilyl ester
86	24.89	170194522	2.66	35579282	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentae
87	25.616	9911923	0.16	3507202	Butanoic acid, 4-(4,5,6,7-tetrahydro-3-acetyl-4-oxo-2-benzothiophenyl)-
88	26.064	7700502	0.12	2284475	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol
89	27.520	4539556	0.17	1229990	Pentasiloxane, dodecamethyl-
90	27.605	12643757	0.20	3965554	Tetrapentacontane, 1,54-dibromo-
91	27.800	1779500	0.03	1276440	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-
92	27.830	12163702	0.19	2041201	Pyrimido[5,4-E]1,2,4-triazine-5,7(6H,8H)-dione, 6,8-dimethyl-, 1-oxide
93	28.031	25264085	0.40	4497598	Cholesta-4,6-dien-3-ol, (3.beta.)-
94	28.370	3661843	0.08	2259539	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-trimethylsilo
95	28.395	1699587	0.05	1798137	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-
96	28.430	7538971	0.12	1739307	1,2-Bis(trimethylsilyl)benzene
97	28.655	14430446	0.39	3227664	Cyclotetrasiloxane, octamethyl-
98	29.320	1947141	0.03	1185865	3-(2-Benzyl-benzoimidazol-1-yl)-propane-1,2-diol
99	29.350	2456010	0.04	1980683	Octadecanoic acid, 16-oxo-, methyl ester
100	29.396	6053206	0.09	3054496	1,3-Dithiane, 2-methyl-
101	29.520	57600928	0.90	8112387	1-Heptacosanol
102	29.670	6818210	0.11	2128289	1,3,5,7-Tetraethyl-1,7-dibutoxytetrasiloxane
103	29.840	6014021	0.09	2346822	1-(2-Isopropyl-phenyl)-3,6,6-trimethyl-1,5,6,7-tetrahydro-indazol-4-one
104	29.894	13147174	0.21	3655732	1-Bromoeicosane

105	30.035	14546444	0.23	1884664	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether
106	30.935	3056014	0.05	1410402	1,4-Benzenedicarboxylic acid, bis(4-butylphenyl) ester/ Terephthalic acid bis(4-butylphenyl) ester
107	31.085	126848378	1.97	17507547	2,4-Hexadien-1-one, 1,3,5-triphenyl-
108	31.215	2115833	0.03	1960917	1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane
109	32.380	17720460	0.5	2208904	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
110	32.425	3059800	0.05	1981896	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane/ Butyl tris (trimethylsilyl) orthosilicate
111	32.499	61060985	0.96	9414738	Stigmasterol
112	33.135	1687705	0.03	1501603	2-Pyrrolidinethione, 4,4,5-trimethyl-5-(4,4-dimethyl-
113	33.988	2379983	0.04	1481259	Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl ester
114	34.121	111789295	1.75	14919408	.gamma.-Sitosterol
115	34.170	7827798	0.12	1449984	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester
116	34.310	1747180	0.05	1349127	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol
117	35.297	794853	0.01	847795	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester
		386409919	100.00	586547289	

4.3.17 Phytochemicals from the extract of *G. wightii* fruit rinds

The chromatogram (Fig. 4.30) and Table 4.23 depicted presence of phytochemicals from fruit extract of southern Western Ghats endemic species *G. wightii*. It revealed the presence of major phytochemicals such as Ethene, (methylsulfonyl)- (21.39%); Phthalic acid, di-(1-hexen-5-yl) ester (17.56%); 1,4-benzenedicarboxylic acid, diethyl ester (13.15%); Diethyl 2-(p-tolyl)malonate (9.98%); Hexadecanoic acid, methyl ester (3.93%); Stigmasterol (2.12%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d (1.89%); Dibutyl phthalate (1.65%); n-Hexadecanoic acid (1.61%); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.55%); Caryophyllene (1.08%) and Tetracosane (1.07%). Also, it revealed the presence of unique phytochemicals such as Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- (0.22%); 1H-Cyclopropa[a] naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha., 7a.beta.,7b.alpha.)]- (0.73%); Docosane (0.73%); Heptacosanoic acid, methyl ester (0.20%); Z-2-Octadecen-1-ol (0.73%); n-Heptadecanol-1 (0.57%); Heneicosane, 3-methyl- (0.42%); E,E,Z-1,3,12-Nonadecatriene-5,14-diol (0.57%) and l-Phenylalanine, n-pentafluoropropionyl-, octadecyl ester (0.84%). Along with the above some unique compounds with major quantities were also found such as l-Phenylalanine, n-heptafluorobutyryl-, hexadecyl ester (2.67%); Z,Z-5,16-Octadecadien-1-ol acetate (2.31%); Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- (2.07%) and 10s,11s-Himachala-3(12),4-diene (1.06%).

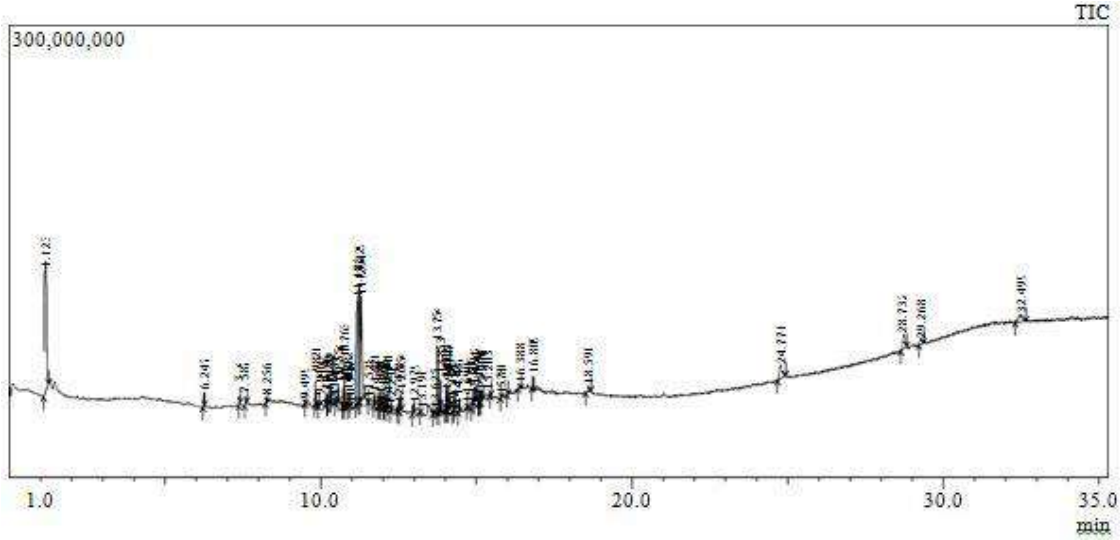


Figure 4.30: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. wightii* fruit rinds.

Table 4.23: Compounds identified at each peak and respective peak details for *G. wightii* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.123	406611118	21.39	76459525	Ethene, (methylsulfonyl)-
2	6.247	12580334	0.66	9464807	Undecane
3	7.384	10502604	0.55	5728465	Naphthalene
4	7.587	8050269	0.42	5332156	1,3,5-Trioxane
5	8.256	4094175	0.22	3502269	Dodecane, 4,6-dimethyl-
6	9.499	2238044	0.12	2431866	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-/ beta-emelene
7	9.821	20557571	1.08	15545243	Caryophyllene
8	9.946	3335946	0.18	2298974	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-/ alpha-Gurjunene
9	10.142	6873503	0.36	4947784	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
10	10.283	2581044	0.14	2869075	Cycloheptasiloxane, tetradecamethyl-
11	10.333	14659603	0.77	5234315	Heptadecane, 8-methyl-
12	10.335	4129548	0.22	4330456	Nonane, 5-(1-methylpropyl)-
13	10.451	4098630	0.22	3461125	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-/ Eudesma-4(14),11-diene
14	10.551	11518232	0.61	10451654	Phenol, 2,4-bis(1,1-dimethylethyl)-
15	10.727	20163858	1.06	12146929	10s,11s-Himachala-3(12),4-diene
16	10.763	39342602	2.07	28548353	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-/ alpha-Bulnesene
17	10.882	13788042	0.73	9266870	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-
18	10.946	864020	0.05	1228164	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]-/ 1.beta.-Cadin-4-en-10-ol/ alpha-Muurolol
19	10.965	249959518	13.15	69391295	1,4-benzenedicarboxylic acid, diethyl ester

20	11.229	189682285	9.98	78385114	Diethyl 2-(p-tolyl)malonate
21	11.292	333698846	17.56	69380515	Phthalic acid, di-(1-hexen-5-yl) ester
22	11.528	1897652	0.10	1816824	Tetradecane, 2,6,10-trimethyl-
23	11.72	10123104	0.53	6938960	Cyclooctasiloxane, hexadecamethyl-
24	11.853	1653111	0.09	1330544	Decane, 1-bromo-2-methyl-
25	11.887	869673	0.05	994304	Pentadecane, 2,6,10,14-tetramethyl-
26	11.939	2628458	0.14	1955397	Methoxyacetic acid, 2-octyl ester
27	12.025	811801	0.04	593380	Batilol
28	12.061	1771086	0.09	1755511	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
29	12.141	13829394	0.73	9619775	Docosane
30	12.250	3843788	0.20	2896667	Heptacosanoic acid, methyl ester
31	12.55	5179597	0.27	4593804	Tetradecanoic acid
32	12.554	20404227	1.07	11455736	Tetracosane
33	12.979	13806019	0.73	6024117	Z-2-Octadecen-1-ol
34	13.196	2502356	0.13	2476301	Octadecane, 5-methyl-
35	13.625	3127094	0.16	2551664	Sulfurous acid, octadecyl 2-propyl ester
36	13.749	12456389	0.66	7440533	Triacontanoic acid, methyl ester
37	13.754	74775769	3.93	43310786	Hexadecanoic acid, methyl ester
38	13.852	35940657	1.89	21628946	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
39	14.017	30648632	1.61	17694536	n-Hexadecanoic acid
40	14.055	16861874	0.89	10236153	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
41	14.109	31355898	1.65	15631270	Dibutyl phthalate
42	14.259	1744570	0.09	1308312	[1,1’-Bicyclopropyl]-2-octanoic acid, 2’-hexyl-, methyl ester
43	14.292	2110372	0.11	1802728	Z-11-Pentadecenol
44	14.437	12282893	0.65	8623758	Octadecanal
45	14.687	1170475	0.06	1493630	Octadecane, 1-iodo-
46	14.873	10862700	0.57	4991559	n-Heptadecanol-1
47	14.941	29536480	1.55	9786950	9,12-Octadecadienoic acid (Z,Z)-, methyl ester/ Linoleic acid, methyl ester
48	15.06	4691847	0.25	3984367	Heptasiloxane, hexadecamethyl-

49	15.203	1783871	0.09	2037711	Decane, 5-ethyl-5-methyl-
50	15.780	2884751	0.15	2160789	Oxirane, tetradecyl-/ 1,2-epoxyhexadecane
51	16.388	8006218	0.42	3269647	Heneicosane, 3-methyl-
52	16.809	15874545	0.84	8989024	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
53	17.054	4010534	0.21	3006876	Cyclodecasiloxane, eicosamethyl-
54	18.590	10807295	0.57	3737904	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
55	24.771	50746890	2.67	9267958	l-Phenylalanine, n-heptafluorobutyryl-, hexadecyl ester
56	28.732	43898346	2.31	9662422	Z,Z-5,16-Octadecadien-1-ol acetate
57	29.268	16041935	0.84	3347471	l-Phenylalanine, n-pentafluoropropionyl-, octadecyl ester
58	32.499	40336476	2.12	4905694	Stigmasterol
		900606569	100.0	673726962	

4.3.18 Phytochemicals from the extract of *G. xanthochymus* fruit rinds

Garcinia xanthochymus fruit extract showed the presence of major compounds such as Propanoic acid, 2-chloro- (15.53%); Tetracosamethyl-cyclododecasiloxane (12.54%); Citric acid, trimethyl ester (7.57%); Cyclononasiloxane, octadecamethyl- (5.75%); Phthalic acid, di-(1-hexen-5-yl) ester (4.71%); Cyclodecasiloxane, eicosamethyl- (4.69%); 9-Octadecenoic acid, methyl ester, (E)- (4.68%); Eicosanoic acid, methyl ester (4.07%); Cyclooctasiloxane, hexadecamethyl- (2.33%); Cycloheptasiloxane, tetradecamethyl- (2.02%); Octadecanoic acid (2%); n-Hexadecanoic acid (1.91%); Heptasiloxane, hexadecamethyl- (1.52%) and unique compounds such as Propanoic acid, 2-hydroxy-, methyl ester, (.+/-.)- (0.08%); Pentanoic acid, 4-oxo- (0.54%); Cyclohexasiloxane, dodecamethyl- (0.09%); 2,3-Dimethyldodecane (0.44%); Cyclopentanetridecanoic acid, methyl ester (0.12%); Ethyl 9-hexadecenoate (0.13%); Octadecanoic acid, ethyl ester (0.77%). Some unique compounds with major quantities were also found viz. 13,27-Cycloursan-3-one (7.19%); 4,22-Stigmastadiene-3-one (6.88%); Pregnane-3,11,20-trione, (5.alpha.)- (4.56%) and cis-Vaccenic acid (1.48%) (Fig. 4.31; Table 4.24).

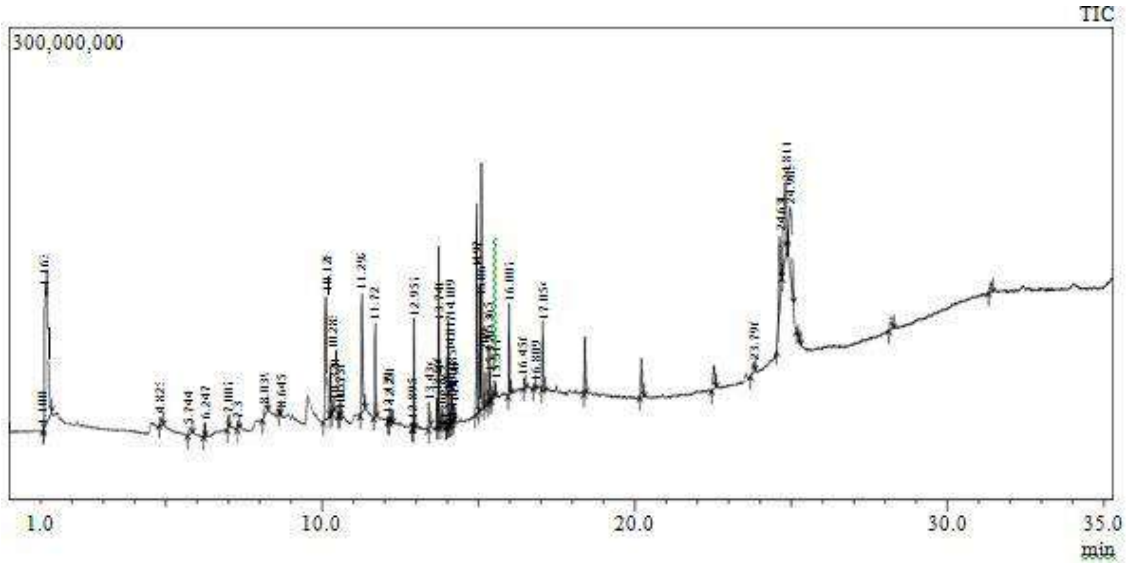


Figure 4.31: GC-MS chromatogram showing presence of phytochemicals in methanolic extract of *G. xanthochymus* fruit rinds.

Table 4.24: Compounds identified at each peak and respective peak details for *G. xanthochymus* fruit extract.

Peak	R.T. (min)	Area	Area (%)	Height	Name
1	1.100	3289365	0.08	2368721	Propanoic acid, 2-hydroxy-, methyl ester, (.+/-.)-
2	1.163	639156797	15.53	87404451	Propanoic acid, 2-chloro-
3	4.825	25154735	0.61	5283802	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	5.744	22124639	0.54	4309934	Pentanoic acid, 4-oxo-/ Levulinic acid
5	6.247	11633805	0.28	8263031	Undecane
6	7.007	10481222	0.25	7803111	Silane, cyclohexyldimethoxymethyl-
7	7.3	9205381	0.22	5150504	Phenyl methylphosphonofluoridate
8	8.039	35643302	0.87	6231505	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
9	8.645	3658169	0.09	3072811	Cyclohexasiloxane, dodecamethyl-
10	10.128	310237410	7.57	78255113	Citric acid, trimethyl ester
11	10.283	83197778	2.02	37336122	Cycloheptasiloxane, tetradecamethyl-
12	10.320	18082354	0.44	7039700	2,3-Dimethyldodecane
13	10.551	8720661	0.21	6631234	Phenol, 2,4-bis(1,1-dimethylethyl)-
14	10.575	4961686	0.12	2927194	Cyclopentanetridecanoic acid, methyl ester
15	11.292	193990289	4.71	75567855	Phthalic acid, di-(1-hexen-5-yl) ester
16	11.72	96068253	2.33	59524441	Cyclooctasiloxane, hexadecamethyl-
17	12.139	4306646	0.10	2832927	Eicosane
18	12.231	12388490	0.30	8187259	Methyl tetradecanoate
19	12.895	5407798	0.13	4239989	Geranylgeraniol
20	12.957	95815964	5.75	69709797	Cyclononasiloxane, octadecamethyl-
21	13.434	34276515	0.83	16838226	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester/ Phthalic acid, butyl isobutyl ester
22	13.666	26917158	0.65	18760588	9-Hexadecenoic acid, methyl ester, (Z)-
23	13.740	167579463	4.07	67067471	Eicosanoic acid, methyl ester/ Arachidic acid
24	13.852	17732281	0.43	11655918	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d

25	14.017	78794610	1.91	43586643	n-Hexadecanoic acid
26	14.109	39391875	0.96	19753348	Dibutyl phthalate
27	14.145	5352934	0.13	2664883	Ethyl 9-hexadecenoate
28	14.198	15063778	0.37	10391108	Hexadecanoic acid, ethyl ester
29	14.97	192697883	4.68	79032929	9-Octadecenoic acid, methyl ester, (E)-/ Elaidic acid, methyl ester
30	15.06	37729403	1.52	7927868	Heptasiloxane, hexadecamethyl-
31	15.209	60719311	1.48	21911106	cis-Vaccenic acid
32	15.230	7585508	0.18	4080108	Oleic Acid
33	15.365	82372199	2.00	36719578	Octadecanoic acid
34	15.370	36713584	0.89	18988160	Ethyl Oleate
35	15.517	31525549	0.77	11354649	Octadecanoic acid, ethyl ester
36	16.007	304053948	12.54	69111002	Tetracosamethyl-cyclododecasiloxane
37	16.456	10938661	0.27	6689075	Methyl 18-methylnonadecanoate
38	16.809	6310314	0.15	3347524	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene
39	17.054	102615540	4.69	66946034	Cyclodecasiloxane, eicosamethyl-
40	23.796	28696693	0.70	6369006	6,10-Dodecadien-3-ol, 3,7,11-trimethyl-
41	24.630	187766595	4.56	46804757	Pregnane-3,11,20-trione, (5.alpha.)-
42	24.811	283238336	6.88	48581627	4,22-Stigmastadiene-3-one
43	24.963	295745660	7.19	39791832	13,27-Cycloursan-3-one
		115181859	100.0	333061129	

4.4 Evaluation of anti-oxidant and anti-cancer (cell line screening) activities of fruit extracts of selected *Garcinia* species.

4.4.1 Anti-oxidant activity

Anti-oxidant potential of fruits of different *Garcinia* species was assessed using two different assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)]. To check the activity methanolic extracts of dried mature fruit rinds were used.

4.4.1.1 DPPH assay

DPPH assay method was used to evaluate antioxidant potential of standard antioxidant L-ascorbic acid and fruit samples of different *Garcinia* species by taking absorbance at 517 nm using spectrophotometer. Initially after addition of methanolic solution of DPPH the colour obtained was purple to dark purple. Scavenging of DPPH radical is the basis of antioxidant assay. As standard antioxidant L-ascorbic acid scavenges the DPPH radical it reduces the colour of the reaction i.e. purple colour turns into yellowish colour or clear solution. Figure 4.32 represents the calibration curve for L-ascorbic acid concentration verses inhibition percentage. As ascorbic acid concentration is increasing, the percentage inhibition is also increasing. Regression analysis was used to access the linearity of the method using equation, $y = bx + c$ (y- peak area, b- slope of line, x- concentration, c- intercept). Calibration curve was linear in this concentration range and showed good regression ($y = 6.9638x$, $R^2 = 0.9764$) (Fig. 4.32). Similarly, calibration curves for different *Garcinia* plant extracts were linear in specified concentration range and showed good regression (Figs. 4.32 & 4.33). These equations were used to calculate the half maximal inhibitory concentration i.e. IC_{50} values for standard and different *Garcinia* extracts. Results were expressed in terms of ascorbic acid equivalents.

The standard L-ascorbic acid showed free radical scavenging against DPPH with 7.18 µg/ml IC_{50} value. All *Garcinia* species extracts evaluated also showed potential antioxidant activity (Table 4.25). Among them recently published an endemic species *G. pushpangadaniana* showed highest antioxidant potential with

15.01 µg/ml IC_{50} value however, it is found lowest in another endemic species *G. wightii* with 49.52 µg/ml IC_{50} value.

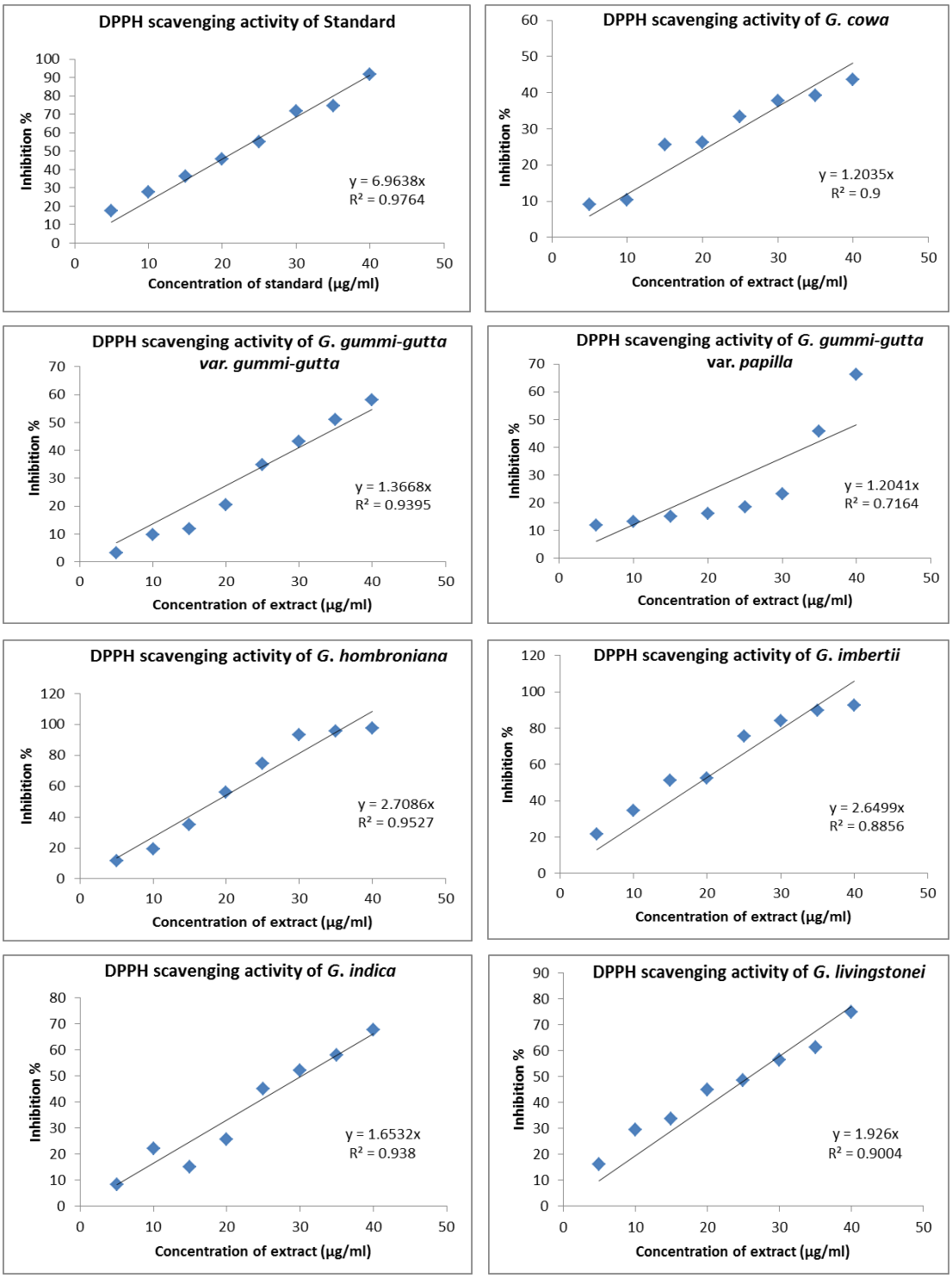


Figure 4.32: DPPH radical scavenging activity of standard (L-ascorbic acid) and different *Garcinia* species fruit rind extracts.

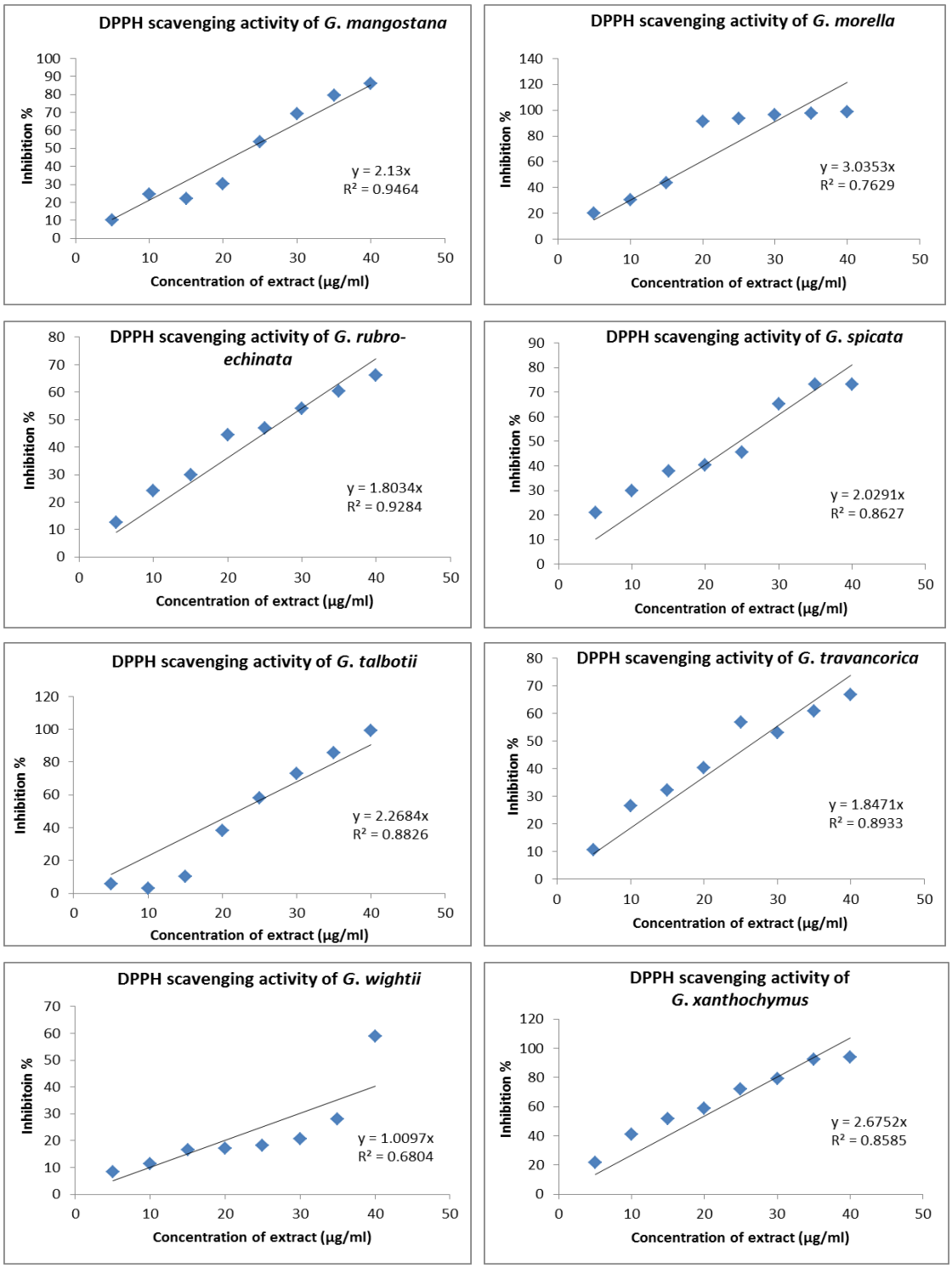


Figure 4.33: DPPH radical scavenging activity of different *Garcinia* species fruit rind extracts.

Table 4.25: IC_{50} values showing antioxidant potential of standard (L-ascorbic acid) and different *Garcinia* species fruit rind extracts.

Sample	IC_{50} value (µg/ml)
L-ascorbic acid	7.18
G. cowa Roxb. ex DC.	41.55
G. gamblei Shameer *et al*.	25.38
G. gummi-gutta var. *conicarpa* (Wight) N. P. Singh	44.48
G. gummi-gutta var. *gummi-gutta* Roxb.	36.58
G. gummi-gutta var. *papilla* (Wight) N. P. Singh	41.52
G. hombroniana Pierre	18.46
G. imbertii Bourd.	18.87
G. indica Choisy	30.24
G. livingstonei T. Anderson	25.96
G. mangostana L.	23.47
G. morella Desr.	16.47
G. pushpangadaniana T. Sabu *et al*.	15.01
G. rubro-echinata Kosterm.	27.73
G. spicata Hook. f.	24.64
G. talbotii Raiz.ex Sant	22.04
G. travancorica Bedd.	27.07
G. wightii T. Anderson	49.52
G. xanthochymus Hook. f.	18.69

4.4.1.2 ABTS assay

An antioxidant assay ABTS is based on decolourisation technique in which radical is generated directly in stable form prior to reaction with putative antioxidants. The pre-formed blue/ green radical monocation of ABTS ($ABTS^{\cdot+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. The extent of decolourisation as percentage inhibition of the $ABTS^{\cdot+}$ radical cation is determined as a function of concentration of antioxidant and duration of the reaction. An inhibition of ABTS monocation ($ABTS^{\cdot+}$) was checked using fruit rind extracts of different *Garcinia* species and compared with standard antioxidant trolox by taking absorbance at 734 nm using spectrophotometer. Figure 4.34 shows the calibration curve for concentration of trolox against inhibition percentage. As concentration of trolox is increasing, the percentage inhibition is also increasing. Regression analysis was used to access the linearity of the method using

equation, y = bx + c (y- peak area, b- slope of line, x- concentration, c- intercept). Calibration curve was linear in this concentration range and showed good regression (y = 2.3837x, R^2 = 0.7458) (Fig. 4.34). Similarly, calibration curves for different *Garcinia* species fruit rind extracts were linear in specified concentration range and showed good regression (Figs. 4.34 & 4.35). These equations were used to calculate the half maximal inhibitory concentration i.e. IC_{50} values for standard trolox and different *Garcinia* fruit rind extracts. Results were expressed in terms of trolox equivalents.

Standard trolox showed antioxidant potential with 20.98 µg/ml IC_{50} value. All *Garcinia* species fruit rind extracts evaluated showed potential antioxidant activity with IC_{50} values ranging from 18.78 µg/ml to 137.51 µg/ml (Table 4.26). Among them the species endemic to southern Western Ghats *G. travancorica* showed highest antioxidant potential among all species and standard trolox. Almost all species which are endemic to southern Western Ghats namely *G. imbertii*, *G. rubro-echinata*, *G. gamblei* and *G. pushpangadaniana* showed high antioxidant activity which is almost close to standard trolox. However, lowest antioxidant potential was found in *G. talbotii* species extract.

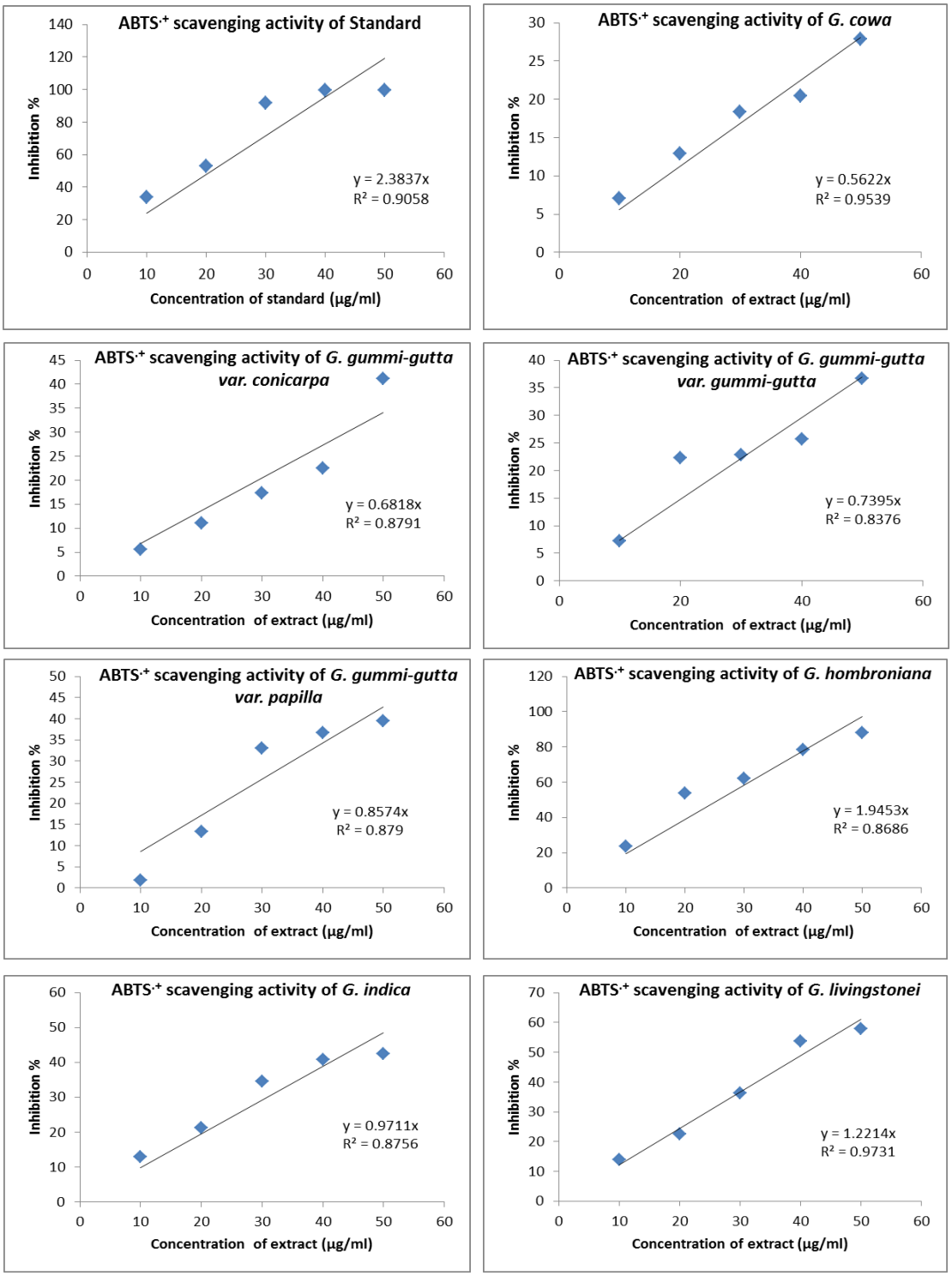


Figure 4.34: ABTS$^{\cdot+}$ radical scavenging activity of standard (trolox) and different *Garcinia* species fruit rind extracts.

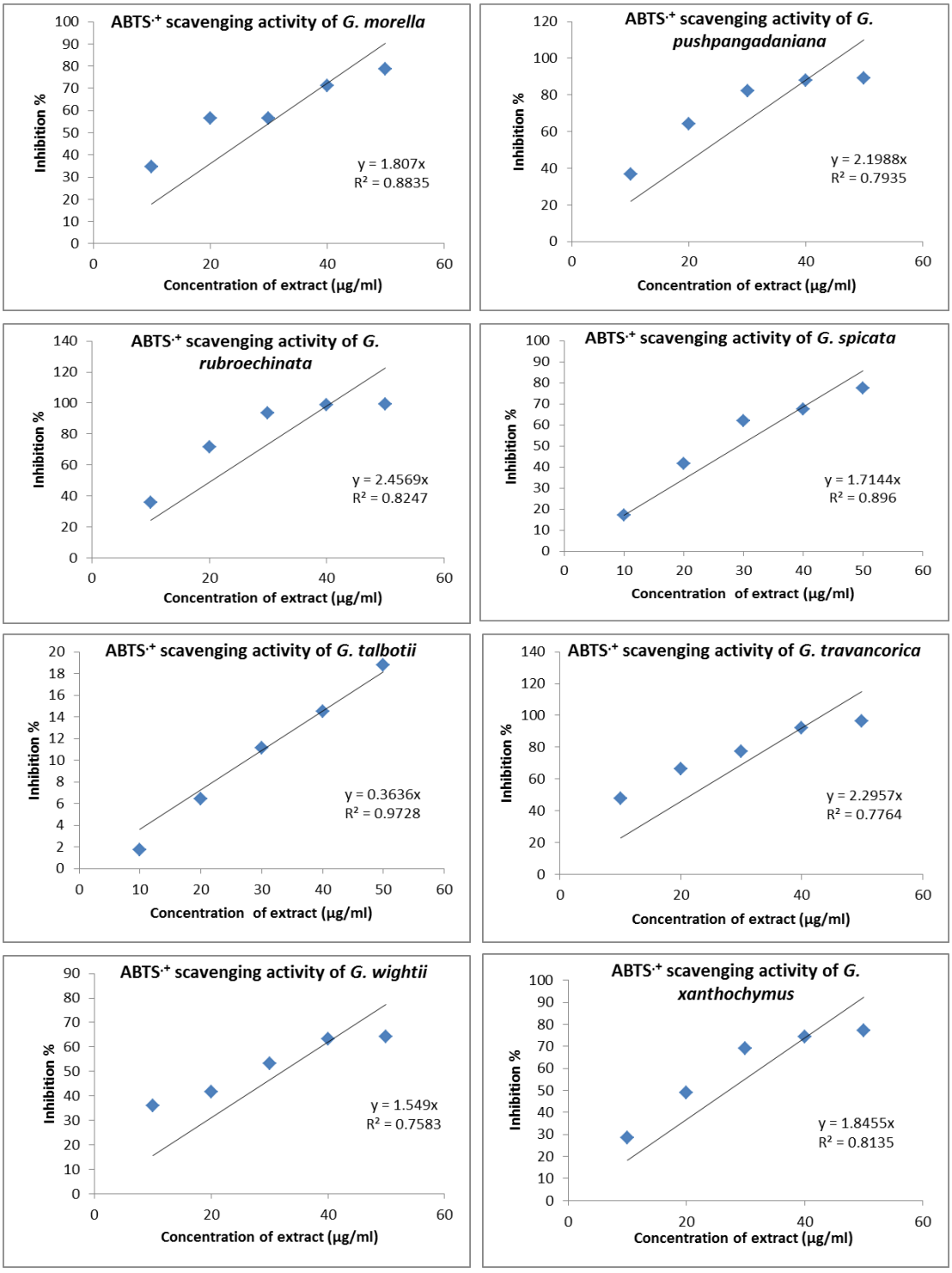


Figure 4.35: ABTS$^{\cdot+}$ radical scavenging activity of different *Garcinia* species fruit rind extracts.

Table 4.26: IC_{50} values showing antioxidant potential of standard (trolox) and different *Garcinia* species fruit rind extracts.

Sample	IC_{50} value (µg/ml)
Trolox	20.98
G. cowa Roxb. ex DC.	88.94
G. gamblei Shameer *et al*.	20.99
G. gummi-gutta var. *conicarpa* (Wight) N. P. Singh	73.34
G. gummi-gutta var. *gummi-gutta* Roxb.	67.61
G. gummi-gutta var. *papilla* (Wight) N. P. Singh	58.32
G. hombroniana Pierre	25.70
G. imbertii Bourd.	19.09
G. indica Choisy	51.49
G. livingstonei T. Anderson	40.94
G. mangostana L.	18.85
G. morella Desr.	27.67
G. pushpangadaniana T. Sabu *et al*.	22.74
G. rubro-echinata Kosterm.	20.35
G. spicata Hook. f.	29.16
G. talbotii Raiz.ex Sant	137.51
G. travancorica Bedd.	18.78
G. wightii T. Anderson	32.28
G. xanthochymus Hook. f.	27.09

4.4.2 Anti-cancer (cell line screening) activity

The cytotoxic activity of ethanolic fruit extracts of different *Garcinia* species were evaluated against human cancer cell lines namely A-549 (lung), HeLa (cervix), HT-29 (colon) and MCF-7 (breast) using SRB (sulforhodamine-B) protein stain assay. It is colorimetric cell density determination assay based on the measurement of cellular protein content. Results were expressed in terms of GI_{50} value i.e. concentration of drug required to inhibit 50% cell growth. Drug/ extract showing GI_{50} value ≤10 µg/ml was considered to demonstrate activity which is as equal to pure compounds and ≤20 µg/ml was considered to demonstrate activity which is as similar to plant extracts.

Fruit extracts of five *Garcinia* species namely *G. wightii*, *G. imbertii*, *G. morella*, *G. travancorica* and *G. mangostana* showed strong inhibition against growth of A-549 cells with less than 10 µg/ml GI_{50} values which is as equivalent to the value shown by positive control and pure compounds (Fig. 4.36a & c; Table 4.27). Another two species *G. spicata* and *G. pushpangadaniana* showed moderate inhibitory activity with 16.9 and 17.3 µg/ml GI_{50} values (Fig. 4.36a & b; Table 4.27). Different *Garcinia* species fruit extracts exhibited an inhibitory activity which led to morphological modifications in cells. A-549 cells are squamous epithelial in nature and after treating with drug/ extracts they showed cellular shrinkage, membrane blebbing during shrinkage and led to formation of apoptic bodies around cells (Plate 4.18). Decrease in cell number was also found after treatment with some species extracts.

Similarly, five species as mentioned above viz. *G. wightii*, *G. imbertii*, *G. morella*, *G. travancorica* and *G. mangostana* showed high cytotoxic activity against HeLa (cervix cancer) cell line with less than 10 µg/ml GI_{50} value (Fig. 4.37a & c; Table 4.28). Further, another three species namely *G. spicata*, *G. hombroniana* and *G. pushpangadaniana* showed moderate cytotoxicity with 11.9, 16.3 and 14 µg/ml GI_{50} values respectively (Fig. 4.37a & b; Table 4.28). HeLa cells are soft-irregular shaped with thin rim of cytoplasmic projected-filamentous cells. After treating with different fruit extracts and positive control drug, an elongated HeLa cells were shrunken and led to the formation of round blebs. Also, cell number got reduced due to inhibitory effect of positive control drug and different *Garcinia* fruit extracts as compared to control and same is represented in Plate 4.19.

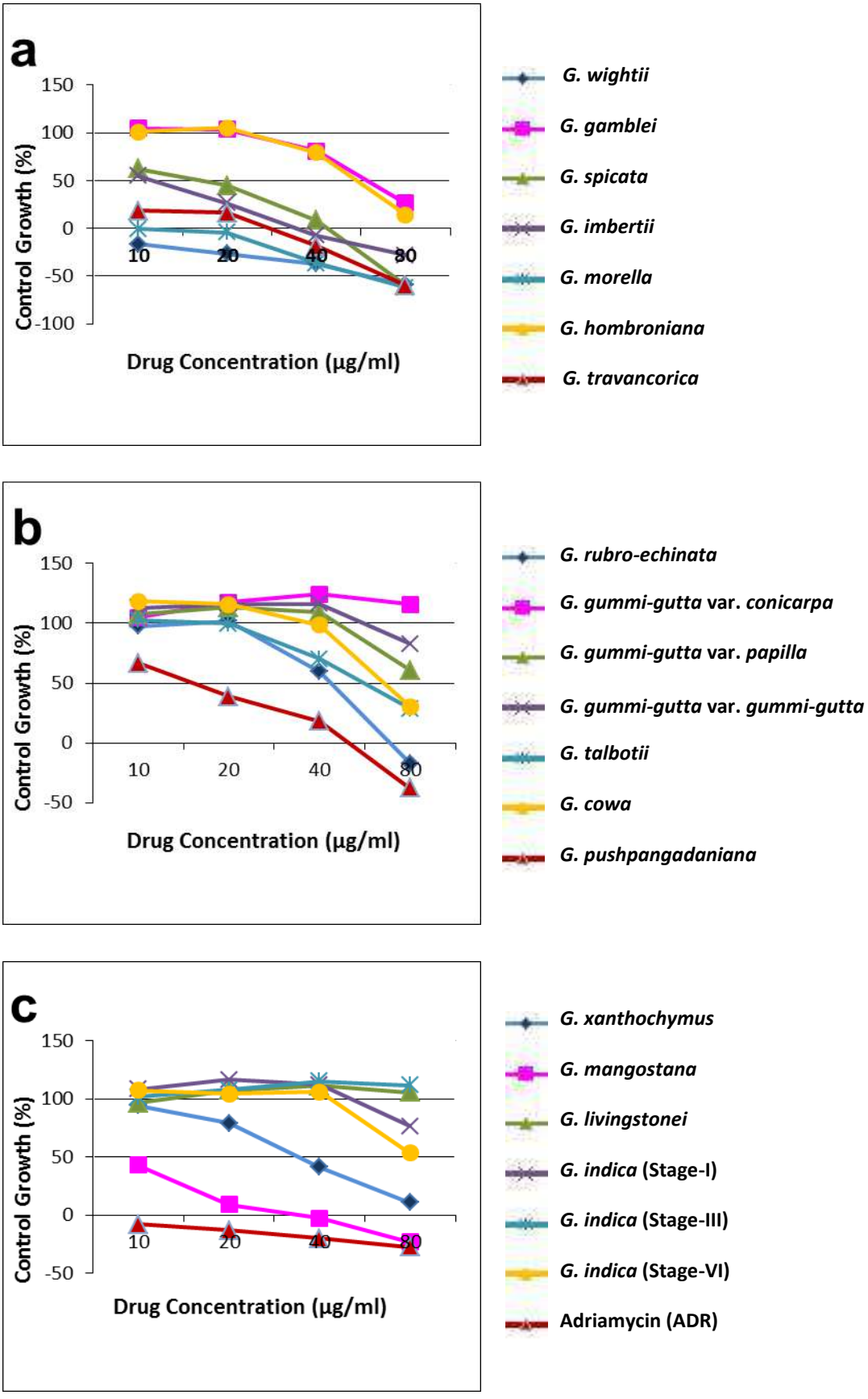


Figure 4.36: Cytotoxicity of positive control (adriamycin) and different *Garcinia* species fruit extracts against A-549 (lung) cancer cell line.

Table 4.27: LC_{50}, TGI and GI_{50} values showing cytotoxicity of positive control drug and different *Garcinia* species fruit extracts against A-549 (lung cancer) cell line.

A-549 cell line	Drug concentrations (µg/ml) calculated from graph		
Species name	**LC_{50} value**	**TGI value**	**GI_{50}* value**
G. wightii	63.0	<10	<10
G. gamblei	NE	NE	62.1
G. spicata	74.1	45.5	16.9
G. imbertii	>80	47.7	<10
G. morella	63.9	9.2	<10
G. hombroniana	NE	>80	56.2
G. travancorica	70.6	28.1	<10
G. rubro-echinata	NE	72.0	43.5
G. gummi-gutta var. *conicarpa*	NE	NE	NE
G. gummi-gutta var. *papilla*	NE	NE	NE
G. gummi-gutta var. *gummi-gutta*	NE	NE	NE
G. talbotii	NE	NE	60.3
G. cowa	NE	NE	68.9
G. pushpangadaniana	>80	52.6	17.30
G. xanthochymus	NE	>80	42.9
G.mangostana	NE	45.4	<10
G. livingstonei	NE	NE	NE
G. indica (I)	NE	NE	NE
G. indica (II)	NE	NE	NE
G. indica (VI)	NE	NE	>80
ADR (Adriamycin)	NE	<10	<10

Note: LC_{50}- concentration of drug causing 50% cell kill

TGI- concentration of drug causing total inhibition of cell growth

***GI_{50}- concentration of drug causing 50% of inhibition of cell growth (considered for activity)**

NE- non-evaluable data

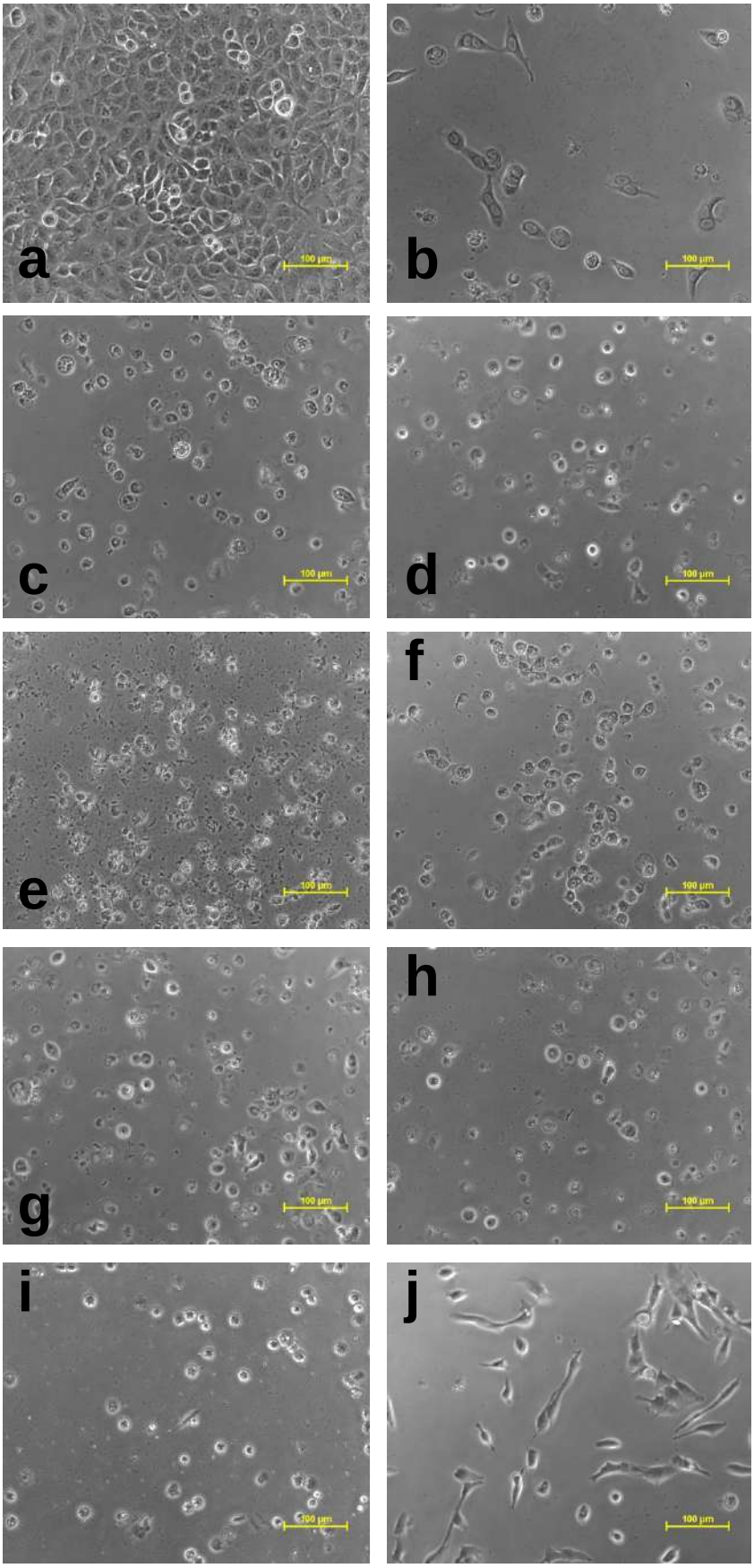


Plate 4.18: Effect of drugs on A-549 cells. a. Control; b. Positive control; c. *G. wightii*; d. *G. spicata*; e. *G. imbertii*; f. *G. morella*; g. *G. travancorica*; h. *G. pushpangadaniana*; i. *G. mangostana*; j. *G. xanthochymus*.

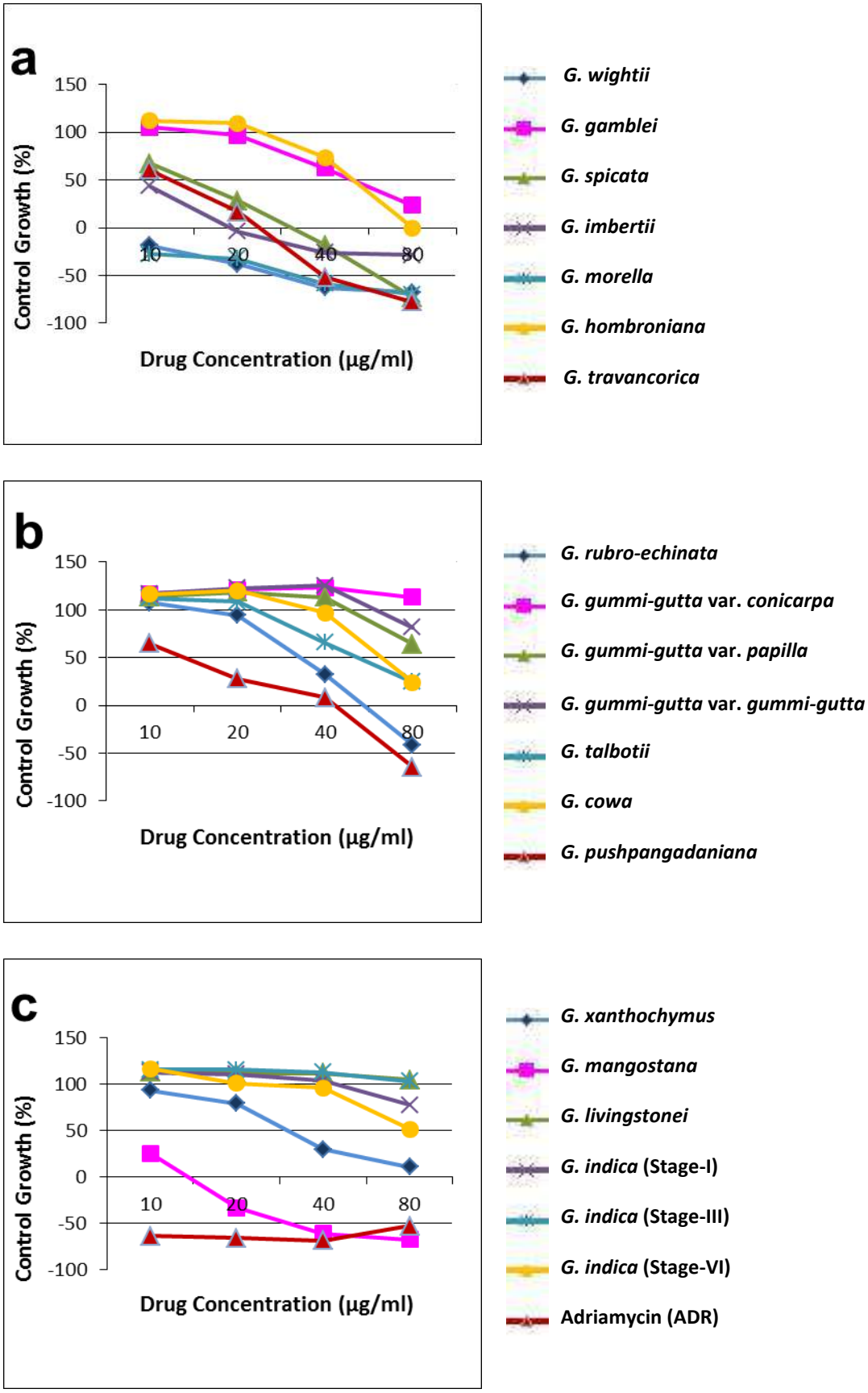


Figure 4.37: Cytotoxicity of positive control (adriamycin) and different *Garcinia* species fruit extracts against HeLa (cervix) cancer cell line.

Table 4.28: LC_{50}, TGI and GI_{50} values showing cytotoxicity of positive control drug and different *Garcinia* species fruit extracts against HeLa (cervix cancer) cell line.

HeLa cell line	Drug concentrations (µg/ml) calculated from graph		
Species name	**LC_{50} value**	**TGI value**	**GI_{50}* value**
G. wightii	NE	NE	<10
G. gamblei	NE	NE	55.9
G. spicata	NE	65.2	11.9
G. imbertii	>80	33.1	<10
G. morella	41.8	<10	<10
G. hombroniana	NE	NE	16.3
G. travancorica	57.1	30.5	<10
G. rubro-echinata	82.4	59.5	36.7
G. gummi-gutta var. *conicarpa*	NE	NE	NE
G. gummi-gutta var. *papilla*	NE	NE	>80
G. gummi-gutta var. *gummi-gutta*	NE	NE	>80
G. talbotii	NE	NE	58.6
G. cowa	NE	NE	65.7
G. pushpangadaniana	71.5	42.7	14.0
G. xanthochymus	NE	NE	40.5
G.mangostana	52.3	<10	<10
G. livingstonei	NE	NE	NE
G. indica (I)	NE	NE	NE
G. indica (II)	NE	NE	NE
G. indica (VI)	NE	NE	>80
ADR (Adriamycin)	<10	<10	<10

Note: LC_{50}- concentration of drug causing 50% cell kill

TGI- concentration of drug causing total inhibition of cell growth

***GI_{50}- concentration of drug causing 50% of inhibition of cell growth (considered for activity)**

NE- non-evaluable data

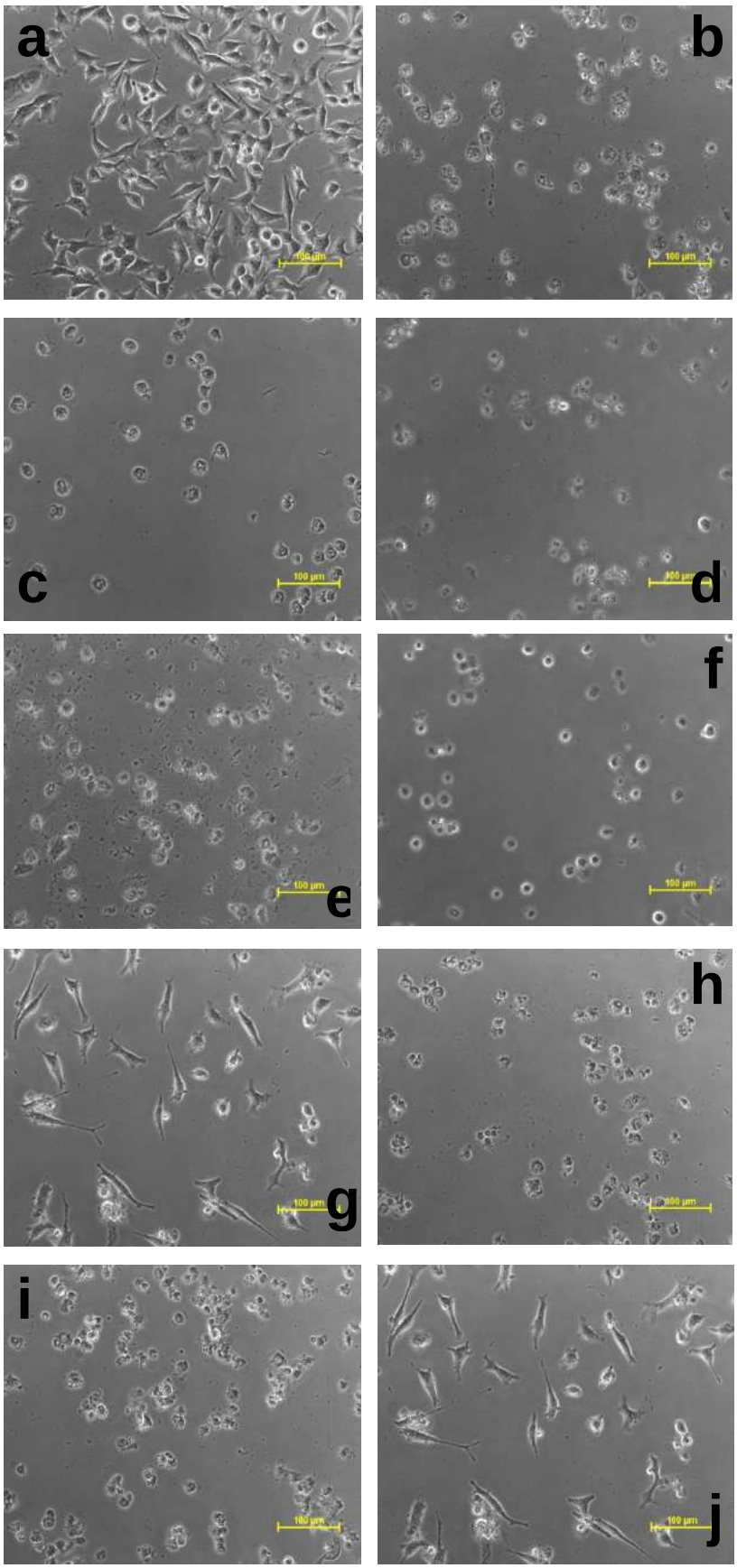


Plate 4.19: Effect of drugs on HeLa cells. a. Control; b. Positive control; c. *G. wightii*; d. *G. spicata*; e. *G. imbertii*; f. *G. morella*; g. *G. hombroniana*; h. *G. travancorica*; i. *G. pushpangadaniana*; j. *G. mangostana*.

In addition to the above five species fruit extracts namely *G. wightii*, *G. imbertii*, *G. morella*, *G. travancorica* and *G. mangostana*; another two species extracts *G. spicata* and *G. pushpangadaniana* exhibited strong inhibitory effect against colon cancer cell line HT-29 with less than 10 μg/ml GI_{50} value (Fig. 4.38, Table 4.29). The change in cell number and cell morphology after exposing HT-29 cells to positive control and fruit extracts was compared to control and presented in Plate 4.20. It shows significant morphological changes such as formation of apoptotic bodies and cell debris.

In case of breast cancer cell line MCF-7, the above mentioned seven species fruit extracts and additionally the species *G. hombroniana* proved to be effective with less than 10 μg/ml GI_{50} value. Further, the species *G. cowa* showed inhibition with less than 20 μg/ml GI_{50} value (Fig. 4.39, Table 4.30). Cobblestone shaped MCF-7 cells were shrunken, membrane blebbed and formed apoptotic bodies around cell after treating with positive control and *Garcinia* species extracts (Plate 4.21). Along with this reduction in cell number was also found.

Remaining species fruit extracts such as *G. gamblei*, *G. rubro-echinata* and *G. xanthochymus* revealed weak inhibition against all four cell lines. Widely used species *G. indica* and *G. gummi-gutta* showed very less cytotoxic effect towards three (A-549, HeLa, MCF-7) and two (HeLa, MCF-7) cell lines respectively. However, two varieties *G. gummi-gutta* var. *conicarpa* and *G. gummi-gutta* var. *papilla* indicated weak inhibitory effect against one and two cell lines respectively. Also, an endemic species *G. talbotii* and exotic species *G. livingstonei* showed less cytotoxicity towards three and one cell line respectively. Fruit extracts of species *G. hombroniana* and *G. cowa* depicted potent growth inhibition towards MCF-7 cell line and additionally former species towards HeLa cells; however, both species showed weak inhibition against remaining two and three cell lines respectively.

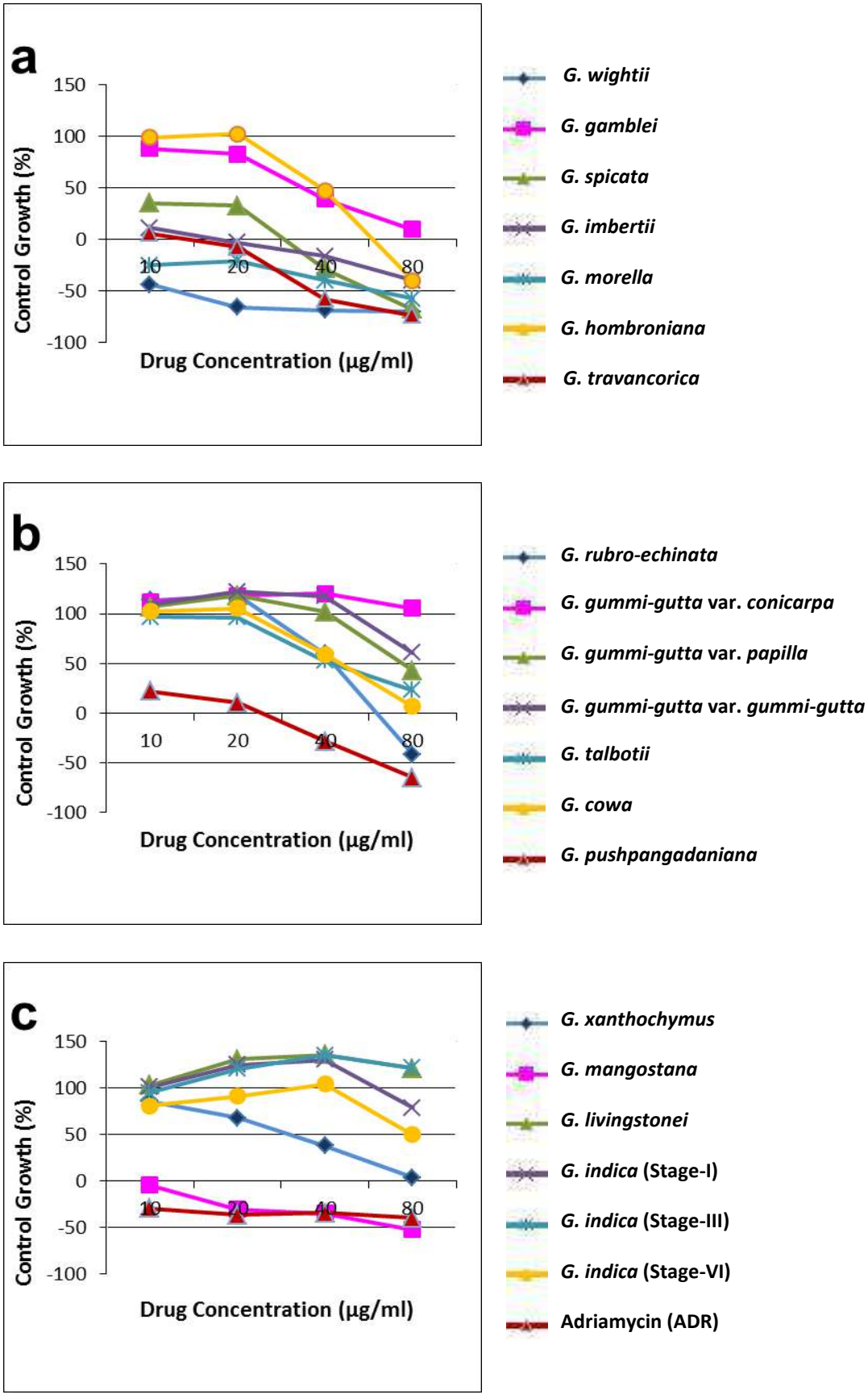


Figure 4.38: Cytotoxicity of positive control (adriamycin) and different *Garcinia* species fruit extracts against HT-29 (colon) cancer cell line.

Table 4.29: LC_{50}, TGI and GI_{50} values showing cytotoxicity of positive control drug and different *Garcinia* species fruit extracts against HT-29 (colon cancer) cell line.

HT-29 cell line	Drug concentrations (µg/ml) calculated from graph		
Species name	**LC_{50} value**	**TGI value**	**GI_{50}* value**
G. wightii	NE	NE	<10
G. gamblei	NE	NE	>80
G. spicata	65.0	33.0	<10
G. imbertii	>80	20.2	<10
G. morella	65.3	<10	<10
G. hombroniana	>80	62.2	38.6
G. travancorica	52.0	8.5	<10
G. rubro-echinata	>80	64.0	42.9
G. gummi-gutta var. *conicarpa*	NE	NE	NE
G. gummi-gutta var. *papilla*	NE	NE	NE
G. gummi-gutta var. *gummi-gutta*	NE	NE	NE
G. talbotii	NE	NE	NE
G. cowa	NE	NE	50.2
G. pushpangadaniana	65.4	25.5	<10
G. xanthochymus	NE	NE	36.4
G.mangostana	NE	NE	<10
G. livingstonei	NE	NE	NE
G. indica (I)	NE	NE	NE
G. indica (II)	NE	NE	NE
G. indica (VI)	NE	NE	NE
ADR (Adriamycin)	NE	<10	<10

Note: LC_{50}- concentration of drug causing 50% cell kill

TGI- concentration of drug causing total inhibition of cell growth

***GI_{50}- concentration of drug causing 50% of inhibition of cell growth (considered for activity)**

NE- non-evaluable data

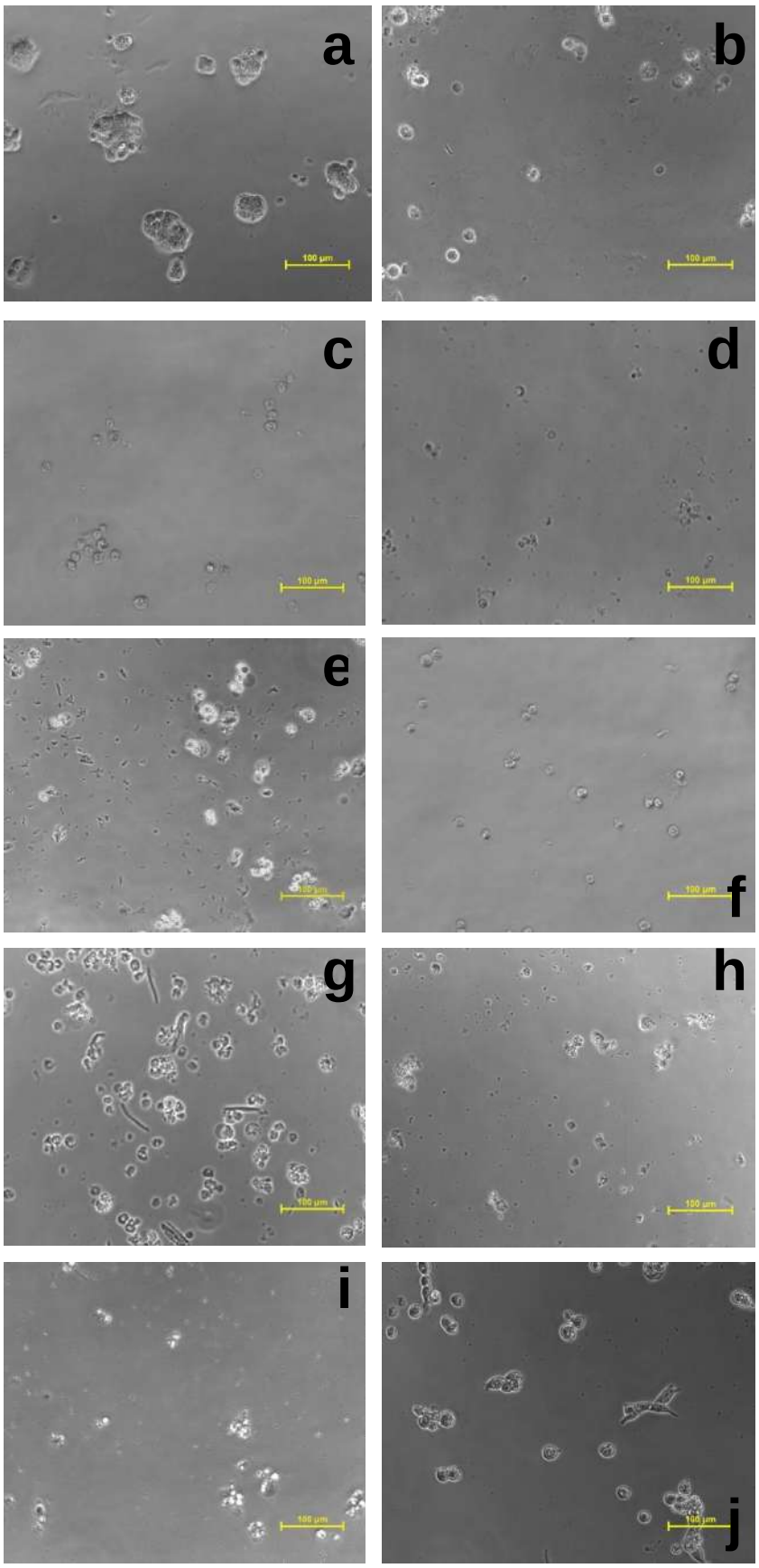


Plate 4.20: Effect of drugs on HT-29 cells. a. Control; b. Positive control; c. *G. wightii*; d. *G. spicata*; e. *G. imbertii*; f. *G. morella*; g. *G. travancorica*; h. *G. pushpangadaniana*; i. *G. mangostana*; j. *G. xanthochymus*.

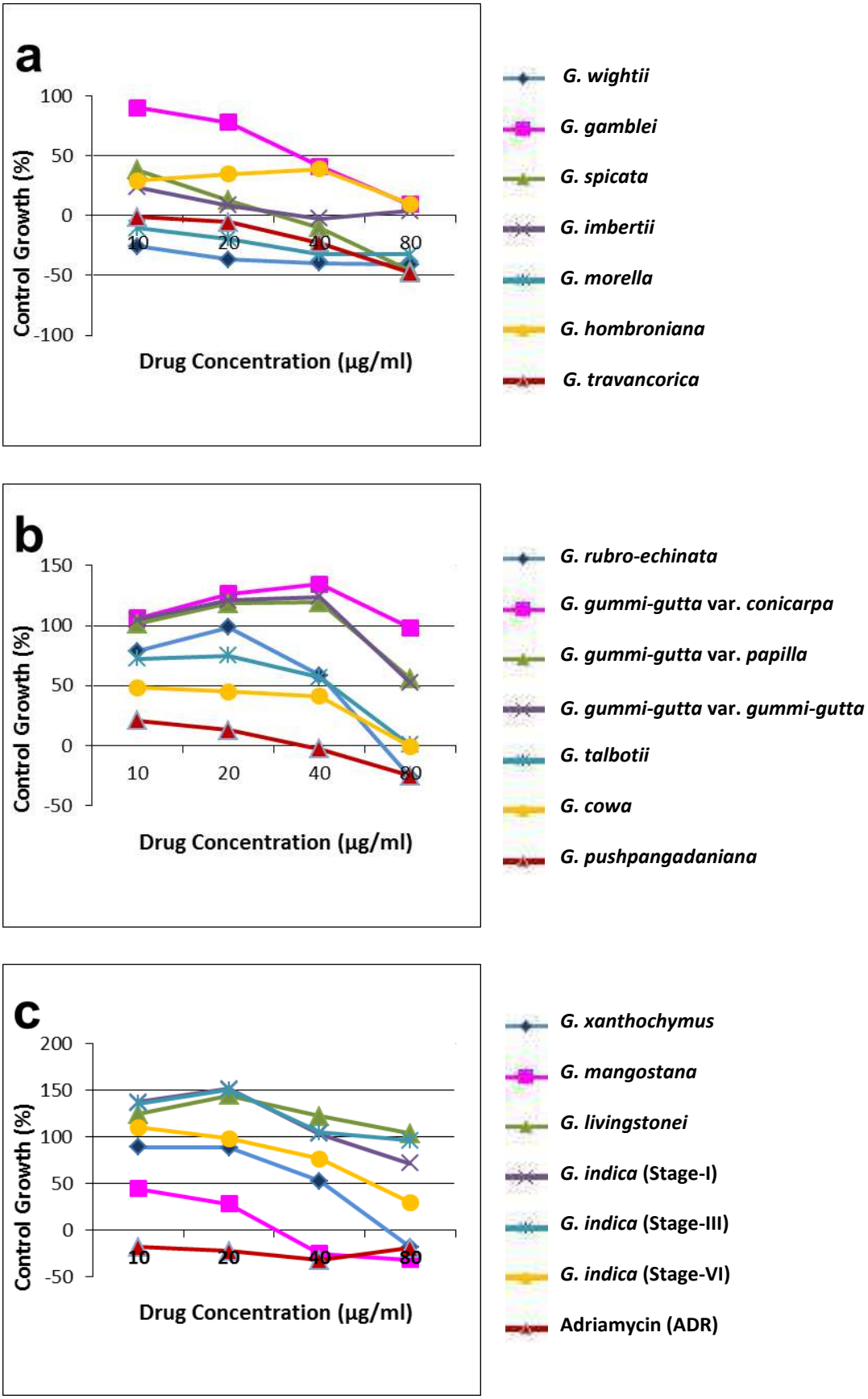


Figure 4.39: Cytotoxicity of positive control (adriamycin) and different *Garcinia* species fruit extracts against MCF-7 (breast) cancer cell line.

Table 4.30: LC_{50}, TGI and GI_{50} values showing cytotoxicity of positive control drug and different *Garcinia* species fruit extracts against MCF-7 (breast cancer) cell line.

MCF-7 cell line	Drug concentrations (µg/ml) calculated from graph		
Species name	**LC_{50} value**	**TGI value**	**GI_{50}* value**
G. wightii	NE	NE	<10
G. gamblei	NE	>80	41.4
G. spicata	NE	NE	<10
G. imbertii	NE	75.2	<10
G. morella	NE	>80	<10
G. hombroniana	NE	>80	<10
G. travancorica	>80	<10	<10
G. rubro-echinata	>80	68.6	39.0
G. gummi-gutta var. *conicarpa*	NE	NE	>80
G. gummi-gutta var. *papilla*	NE	NE	>80
G. gummi-gutta var. *gummi-gutta*	NE	NE	>80
G. talbotii	NE	>80	38.5
G. cowa	NE	>80	14.2
G. pushpangadaniana	NE	39.9	<10
G. xanthochymus	NE	70.5	39.4
G.mangostana	>80	41.2	<10
G. livingstonei	NE	NE	>80
G. indica (I)	NE	NE	>80
G. indica (II)	NE	53.7	>80
G. indica (VI)	NE	NE	62.6
ADR (Adriamycin)	NE	35.4	<10

Note: LC_{50}- concentration of drug causing 50% cell kill

TGI- concentration of drug causing total inhibition of cell growth

***GI_{50}- concentration of drug causing 50% of inhibition of cell growth (considered for activity)**

NE- non-evaluable data

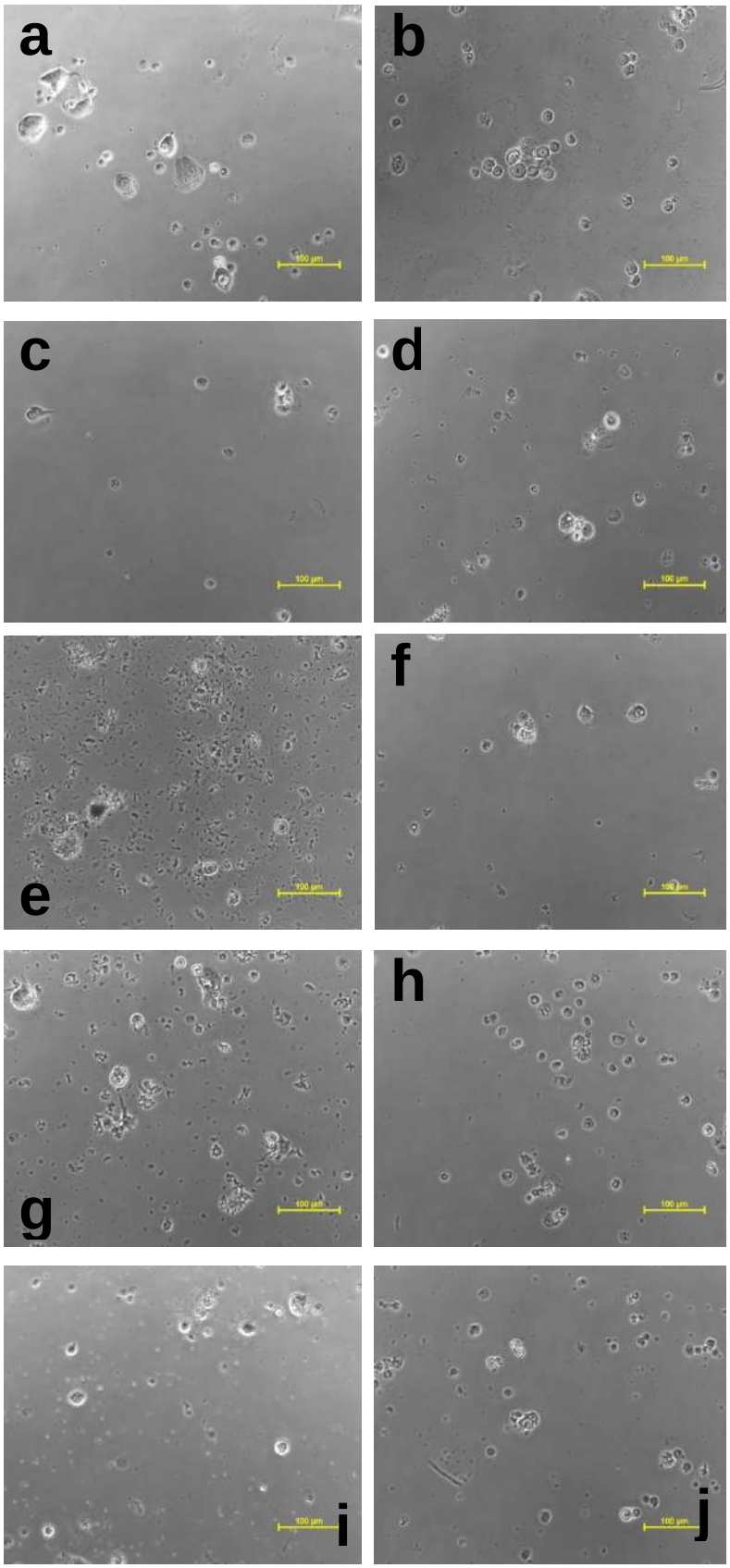


Plate 4.21: Effect of drugs on MCF-7 cells. a. Control; b. Positive control; c. *G. wightii*; d. *G. spicata*; e. *G. imbertii*; f. *G. morella*; g. *G. hombroniana*; h. *G. travancorica*; i. *G. cowa*; j. *G. pushpangadaniana*.

4.5 Molecular phylogeny of *Garcinia* species from the Western Ghats using nuclear and chloroplast genes.

In the present study silica gel dried as well as fresh leaf samples of all 16 *Garcinia* species with 3 varieties including recently published new species *G. gamblei* and *G. pushpangadaniana* from the entire Western Ghats were used for the gene sequencing.

4.5.1 PCR analysis, DNA sequencing and sequence submission

DNA isolated from leaf materials of different species showed good quality. Further it was used for PCR analysis and sequenced using universal primers of nuclear region (ITS) and chloroplast genes (*rbc*L, *mat*K) (Plate 4.22). Gene sequences for all the *Garcinia* species from the entire Western Ghats of India was deposited to NCBI (GenBank) database and the accession numbers obtained as well as the herbarium voucher specimens submitted to BSI (MH) are listed in Table 4.31. All 16 *Garcinia* species studied, ITS and chloroplast genes (*rbc*L, *mat*K) were amplified using universal primers however, the nuclear region (ITS) of *G. gummi-gutta* var. *papilla* could not amplify using universal primer. Additionally, in same species and *G. travancorica* samples, plastid gene (*mat*K) could not amplify with universal primers.

4.5.2 Molecular Phylogeny

For the phylogenetic analysis highly similar ITS region, *rbc*L and *mat*K gene sequences from different species of the genus *Garcinia* and an out-group *Pentadesma butyracea* were retrieved from NCBI (BLAST-n) database (Table 4.32) and further used. Most of the species used for the analysis were Old World species. To construct phylogenetic trees separate as well as combined DNA sequence datasets of ITS, *rbc*L and *mat*K genes were used in MEGA. The results for molecular phylogenetic analysis and their sequence characteristics were interpreted for three different gene sequence datasets and combined datasets.

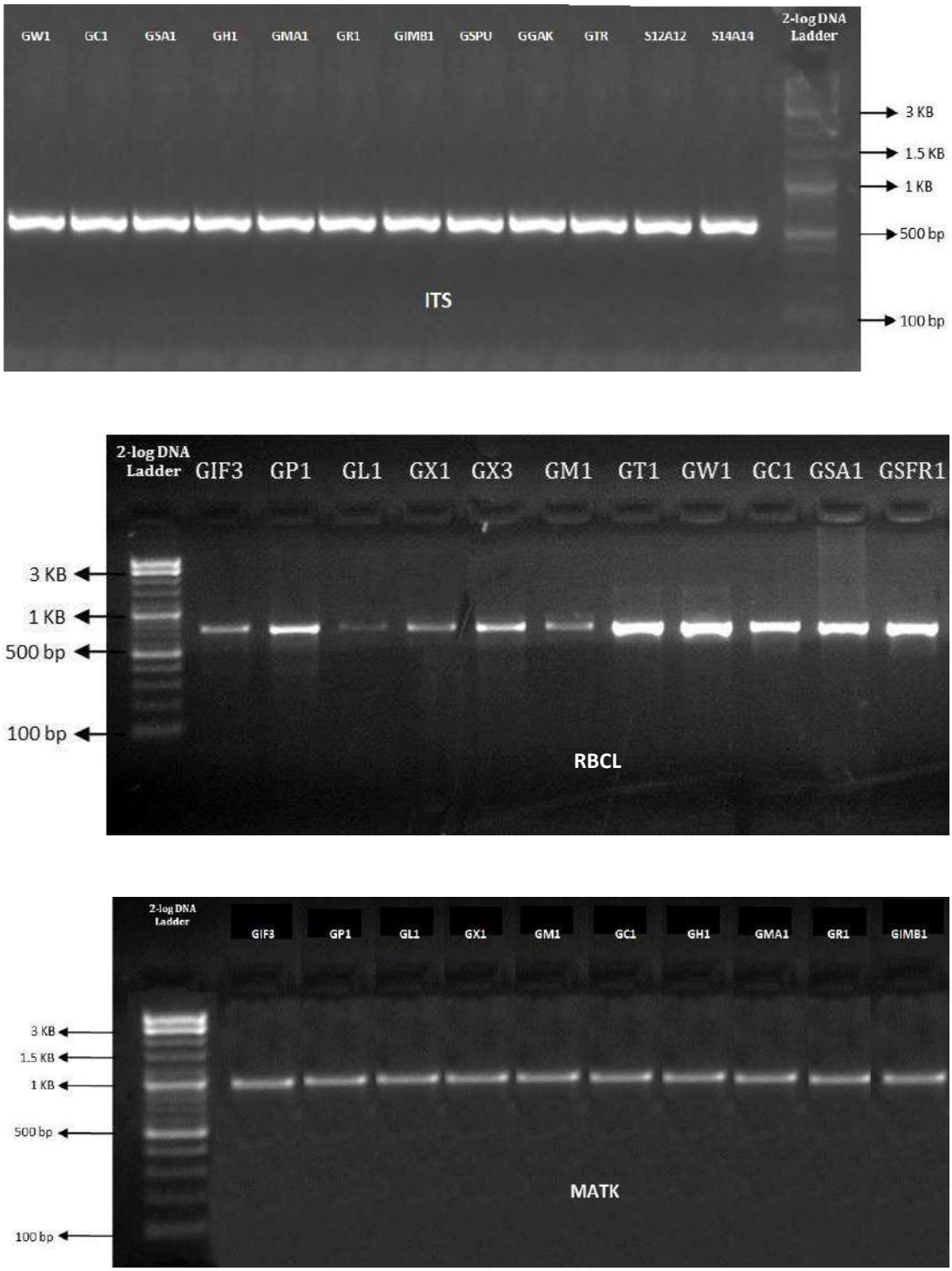


Plate 4.22: Gel photographs showing PCR amplified products for the different *Garcinia* species using universal primers of ITS, *rbc*L and *mat*K genes.

Table 4.31: Collection, voucher specimen information and GenBank accession numbers of ITS, *rbc*L and *mat*K gene sequences for the *Garcinia* species studied.

Sl. No.	Species	Voucher specimen submitted to BSI (MH)	GenBank accession numbers		
			ITS	*rbc*L	*mat*K
1	*G. cowa*	MH:177815	MT160372	MT441649	MT441667
2	*G. gamblei*	MH:177813	MT160373	MT441650	MT441668
3	*G. gummi-gutta* var. *conicarpa*	MH:177814	MT160374	MT441651	MT441669
4	*G. gummi-gutta* var. *gummi-gutta*	MH:177819	MT160375	MT441652	MT441670
5	*G. gummi-gutta* var. *papilla*	*	**	MT441653	**
6	*G. hombroniana*	MH:177810	MT160376	MT441654	MT441671
7	*G. imbertii*	MH:177812	MT160377	MT441655	MT441672
8	*G. indica*	MH:177818	MT160378	MT441656	MT441673
9	*G. livingstonei*	MH:177808	MT160379	MT441657	MT441674
10	*G. mangostana*	MH:177820	MT160380	MT441658	MT441675
11	*G. morella*	MH:177807	MT160381	MT441659	MT441676
12	*G. pushpangadaniana*	MH:177811	MT160382	MT441660	MT441677
13	*G. rubro-echinata*	MH:177816	MT160383	MT441661	MT441678
14	*G. spicata*	MH:177823	MT160384	MT441662	MT441679
15	*G. talbotii*	MH:177822	MT160385	MT441663	MT441680
16	*G. travancorica*	MH:177817	MT160386	MT441664	**
17	*G. wightii*	MH:177806	MT160387	MT441665	MT441681
18	*G. xanthochymus*	MH:177804	MT160388	MT441666	MT441682

Note: *No voucher specimen deposited to MH for *G. gummi-gutta* var. *papilla*.

****No amplification was found with universal primers.**

Table 4.32: Accession numbers of DNA sequences for the genes ITS, *rbc*L and *mat*K retrieved from NCBI database.

No.	Taxa	Location	ITS	*rbc*L	*mat*K
1	*G. nigrolineata*	Malaysia	AF367216.1	-	-
2	*G. globulosa*	Malaysia	AF367221.1	-	-
3	*G. porrecta*	Thailand	AB110812.1	-	-
4	*G. griffithii*	Malaysia	AF367223.1	-	-
5	*G. bancana*	Malaysia	AF367212.1	-	-
6	*G. lanceifolia*	India	KP318342.1	KF783273.1	MF990631.1
7	*G. atroviridis*	Malaysia	AF367211.1	MH069785.1	-
8	*G. pedunculata*	India	KP318346.1	KF783274.1	-
9	*G. malaccensis*	Malaysia	AB856031.1	-	-
10	*G. speciosa*	Thailand	AB110816.1	-	-
11	*G. acuminata*	Thailand, India	AB110796.1	MF806030.1	-
12	*G. rostrata*	Thailand	AB110813.1	HQ332061.1*	HQ331606.1*
13	*G. mannii*	Cameroon, Cameroon	EU128428.1*	KC628183.1	KC627647.1
14	*G. subelliptica*	China, Japan, Japan	KX421315.1	AB233850.1	AB233746.1
15	*G. kola*	China	KX421299.1	KC628604.1	-
16	*G. dulcis*	Thailand, Papua new guinea	AB110800.1	JF738433.1	-
17	*G. costata*	Thailand	AB110798.1	-	-
18	*G. vilersiana*	Thailand, Cambodia	AB110818.1	AB925655.1	AB925027.1
19	*G. nervosa*	Malaysia	AF367222.1	HQ332059.1*	HQ331604.1*
20	*G. intermedia*	China, Costa Rica	KX421332.1	JQ591097.1	HQ331600.1*
21	*G. bracteata*	China	KX421310.1	-	-
22	*G. oblongifolia*	China	KX421328.1	KJ440046.1	KJ510946.1
23	*G. multiflora*	China	KX421311.1	KP095023.1	KP094070.1
24	*G. opaca* var. *minor*	Malaysia	AF367227.1	-	-
25	*G. syzygiifolia*	Indonesia	AB110815.1	-	-
26	*G. penangiana*	Malaysia	AB856033.1	HQ332060.1*	HQ331605.1*

27	*G. yunnanensis*	China	KX421345.1	-	-
28	*G. xipshuanbannaensis*	China	KX421304.1	KR529360.1	-
29	*G. parvifolia*	Malaysia	AF367217.1	-	-
30	*G. lancilimba*	China	KX421307.1	KR529345.1	-
31	*G. tetralata*	China	KR532162.1	KR529348.1	KR530869.1
32	*G. punctata*	Africa	EU128391.1*	-	KC627616.1
33	*G. afzelii*	Africa, Africa	EU128424.1*	KC628029.1	KC627468.1
34	*G. conrauana*	Africa	EU128470.1*	KC628098.1	-
35	*G. lucida*	Africa	EU128449.1*	KC628282.1	-
36	*G. gnetoides*	Africa	EU128460.1*	KC628009.1	-
37	*G. hanburyi*	Cambodia	-	AB925386.1	AB924775.1
38	*G. scortechinii*	Singapore	-	KJ594721.1	HQ331607.1*
39	*G. urophylla*	-	-	HQ332066.1*	HQ331611.1*
40	*G. oliveri*	Cambodia	-	-	AB924719.1
41	*G. smeathmannii*	Africa	-	-	KC627414.1
42	*G. benthamii*	Cambodia	-	-	AB924708.1
43	*G. harmandii*	Cambodia	-	-	AB925070.1
44	*G. merguensis*	Cambodia	-	-	AB924684.1
45	*P. butyracea*	Africa	EU128369.1*	KC628674.1	HQ331669.1*

Note: *Location not available.

4.5.2.1 Molecular Phylogeny using Internal Transcribed Spacer (ITS) region sequences

The ITS DNA sequence dataset used for phylogeny contained 54 taxa and total aligned characters were 968. It was observed that 154 were conserved and 677 were variable sites. Also, 554 and 114 sites were found as parsim-informative and singleton sites respectively (Table 3.3).

Molecular phylogeny using ITS dataset revealed segregation of all *Garcinia* species in different clades. All Indian *Garcinia* species clustered in a large clade along with other Old World species. In the clade, all Western Ghats species grouped together in three different subclades and their segregation was supported mostly by endemism along with morphological characters. In subclade-I five species were clustered together, of which four species are endemic to the Western Ghats and consisted broad, long and leathery leaves except *G. indica*. In the subclade-I newly described high altitudinal, southern Western Ghats endemic species *G. gamblei* and *G. pushpangadaniana* were grouped together as their clustering is well supported by morphological data since they are allied species. The fifth species in clade-I is introduced species. An another subclade-II showed clustering of seven species, among them an endemic four species including a variety *G. gummi-gutta* var. *gummi-gutta* were clustered together with species *G. cowa*, *G. morella* and *G. hombroniana* which are mostly distributed in north-east and Andaman Island regions of India and extended distribution to Old World. However, an endemic high altitude variety *G. gummi-gutta* var. *conicarpa* was nested in another subclade-III with an Indian species *G. lanceifolia* which has extended distribution in Old World. A low altitude species *G. spicata* which is distributed in India and extended upto Sri Lanka nested in separate clade with another Indian species *G. xanthochymus* and other Old world species. A cultivated species *G. mangostana* found to occur in different clade with other Old World species which includes its progenitor *G. malaccensis* too. However, a species from high altitudes of the southern Western Ghats *G. travancorica* clustered separately in distinct clade with an north-east Indian species *G. acuminata* which is also found in other countries of Old World (Fig. 4.40).

4.5.2.2 Molecular Phylogeny using *rbc*L gene sequences

A total of 43 taxa and 1425 aligned characters were used to construct phylogenetic tree using *rbc*L dataset. Out of these 859 were conserved and 547 were variable sites. The parsim-informative and singleton sites found were 482 and 64 respectively (Table 3.3).

All Indian *Garcinia* species clustered in a large clade along with other Old World species except two endemic species *G. wightii* and *G. gummi-gutta* var. *gummi-gutta* which were found to occur in distinct clade. In the clade, it was found that all Western Ghats endemic species were grouped together along with non-endemic species too and formed two different subclades. One of the subclade contained two Old World species namely *G. livingstonei* and *G. xanthochymus* which were found to occur in cultivated and natural form in the Western Ghats. An endemic species *G. talbotii* and *G. gummi-gutta* var. *conicarpa* were nested in two different subclades apart from subclades containing Western Ghats species. Both species were clustered with other Old World and Indian species. In ITS molecular phylogeny, the later variety *G. gummi-gutta* var. *conicarpa* was grouped together with north-east Indian species *G. lanceifolia*, similar pattern was found using *rbc*L molecular phylogeny (Fig. 4.41).

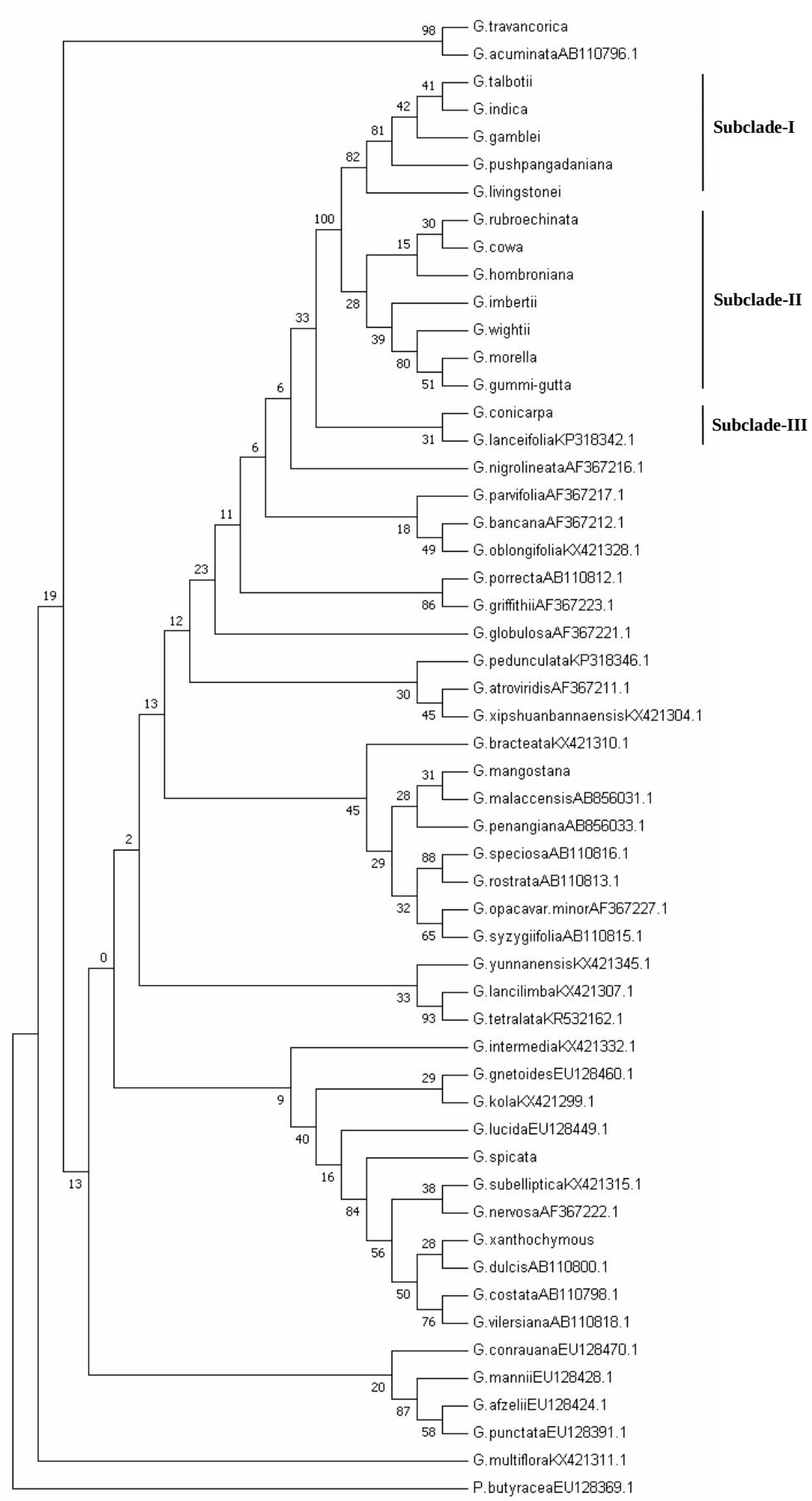


Figure 4.40: Phylogenetic tree constructed using ITS region sequences.

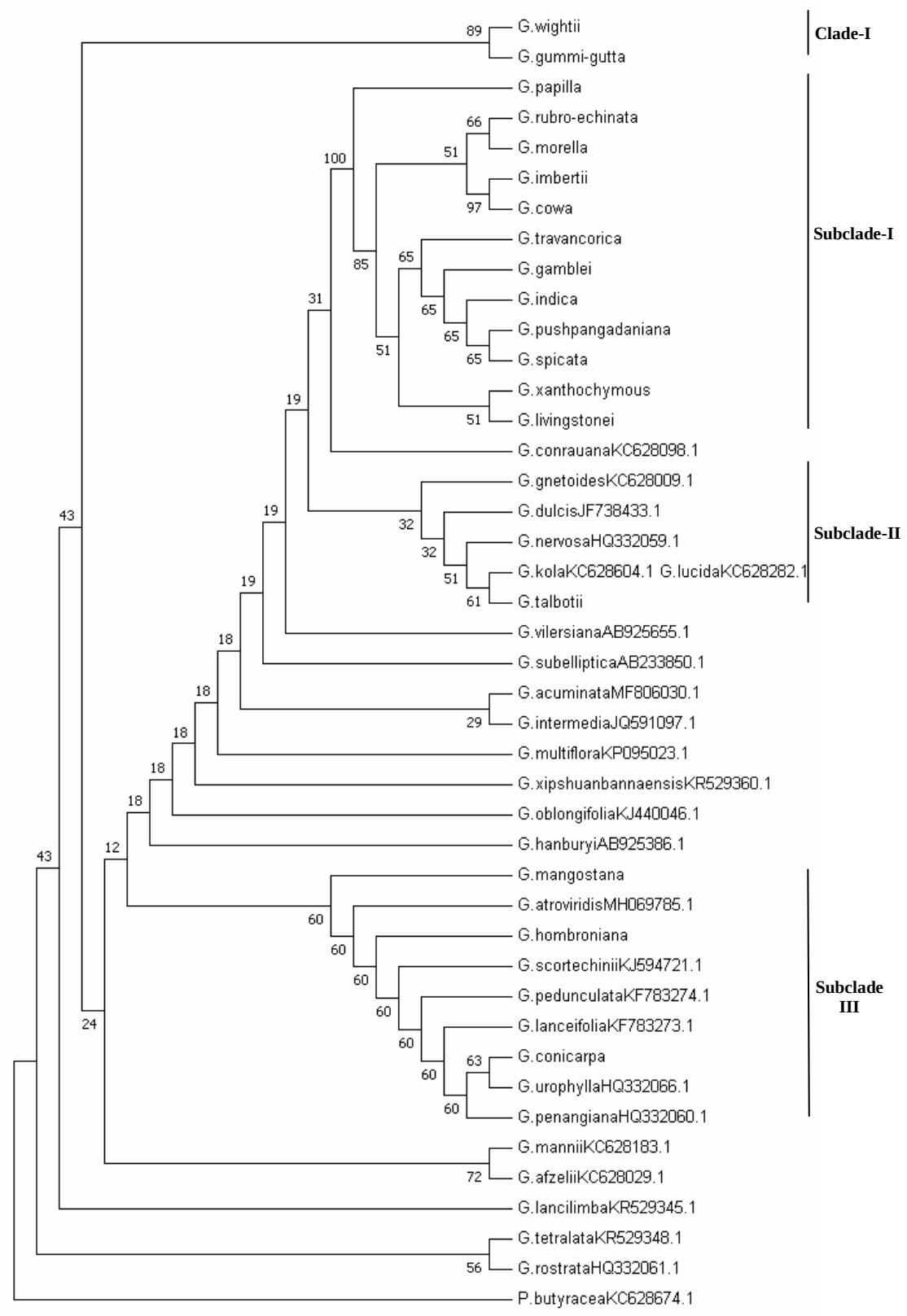


Figure 4.41: Phylogenetic tree constructed using *rbc*L gene sequences.

4.5.2.3 Molecular Phylogeny using *mat*K gene sequences

The *mat*K DNA sequence dataset containing 38 taxa was used for phylogeny. A total of 1531, 382, 805, 440 and 361 character sites were witnessed as aligned characters, conserved, variable, parsim-informative and singleton sites respectively in the dataset (Table 3.3).

Molecular phylogenetic tree constructed using *mat*K dataset revealed almost similar tree topology as ITS dataset with few differences in species segregation (Fig. 4.42). Four endemic Western Ghats species clustered together in one subclade with non-endemic species *G. spicata* since the species shown morphological similarities with them except the species *G. indica*. Two species namely *G. rubro-echinata* and *G. imbertii* restricted to high altitudes of the southern Western Ghats were clustered together in distinct subclade. However, other endemic species were clustered together in different subclades and segregated along with another Indian and Old World species. Species distribution in the phylogenetic tree was mostly influenced by morphological characters.

4.5.3.4 Molecular Phylogeny using combined dataset of ITS and *rbc*L gene sequences

The combined dataset of ITS and *rbc*L sequences consisted 43 taxa and 2300 total aligned characters. Among them 495 were observed as conserved and 1785 were variable sites. A total of 1594 and 187 sites were found as parsim-informative and singleton sites respectively (Table 3.3).

Molecular phylogenetic tree constructed using combined dataset revealed almost similar pattern of species segregation as using individual ITS and *rbc*L datasets (Fig. 4.43).

4.5.3.5 Molecular Phylogeny using combined dataset of ITS and *mat*K gene sequences

A total of 33 taxa and 2849 aligned characters were found in the ITS and *mat*K combined dataset. Out of which 851 and 1638 sites were observed as conserved and variable sites respectively. Also, parsim-informative and singleton sites found were 1175 and 425 respectively (Table 3.3).

Molecular phylogenetic tree constructed using combined dataset revealed similar tree topology for species segregation as shown in individual ITS and *mat*K datasets (Fig. 4.44). All the Western Ghats species were segregated in three subclades within single large clade except *G. rubro-echinata*. Two cultivated species namely *G. mangostana* and *G. hombroniana* were clustered together in a subclade which is sister to another subclade contained two endemic as well as north-east species.

Overall, data analysis reveals segregation of almost all species endemic to the Western Ghats in similar clades with different sub-clades however, some Old World species also nested in same clade. Hence, species segregation is based on endemism as well as other factors such as morphological characters, chromosome data etc.

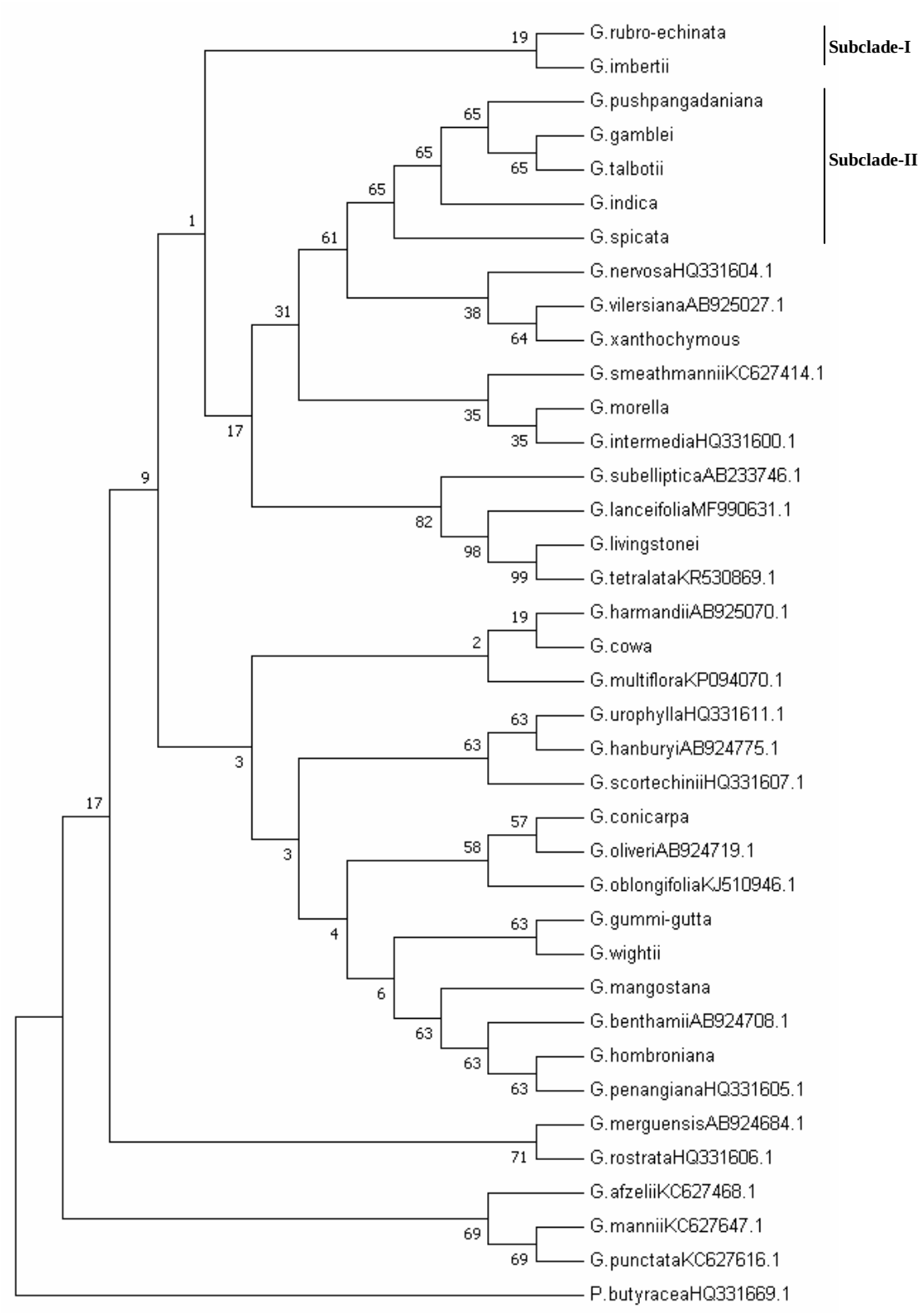


Figure 4.42: Phylogenetic tree constructed using *mat*K gene sequences.

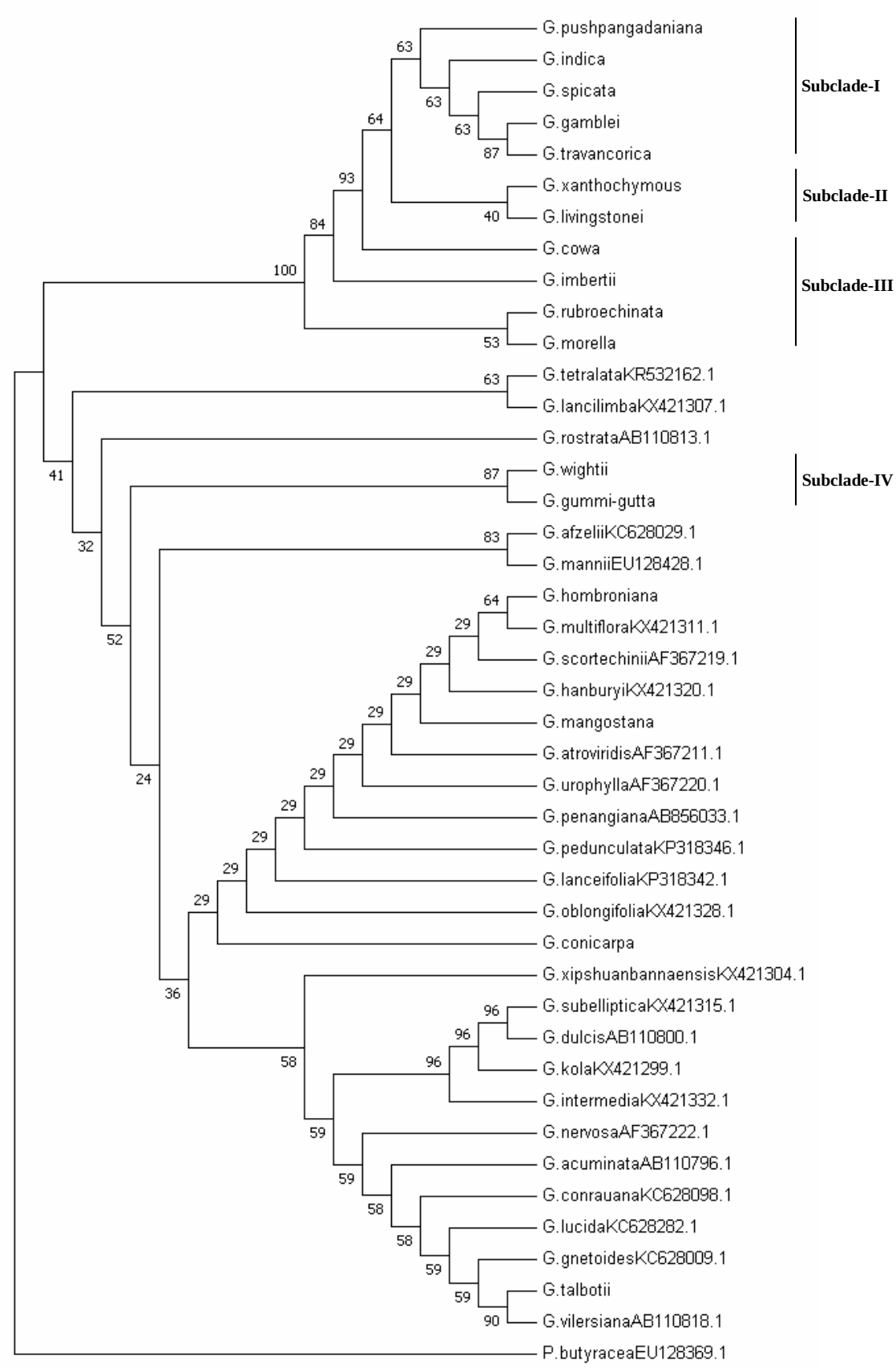


Figure 4.43: Phylogenetic tree constructed using combined dataset of ITS region and *rbc*L gene sequences.

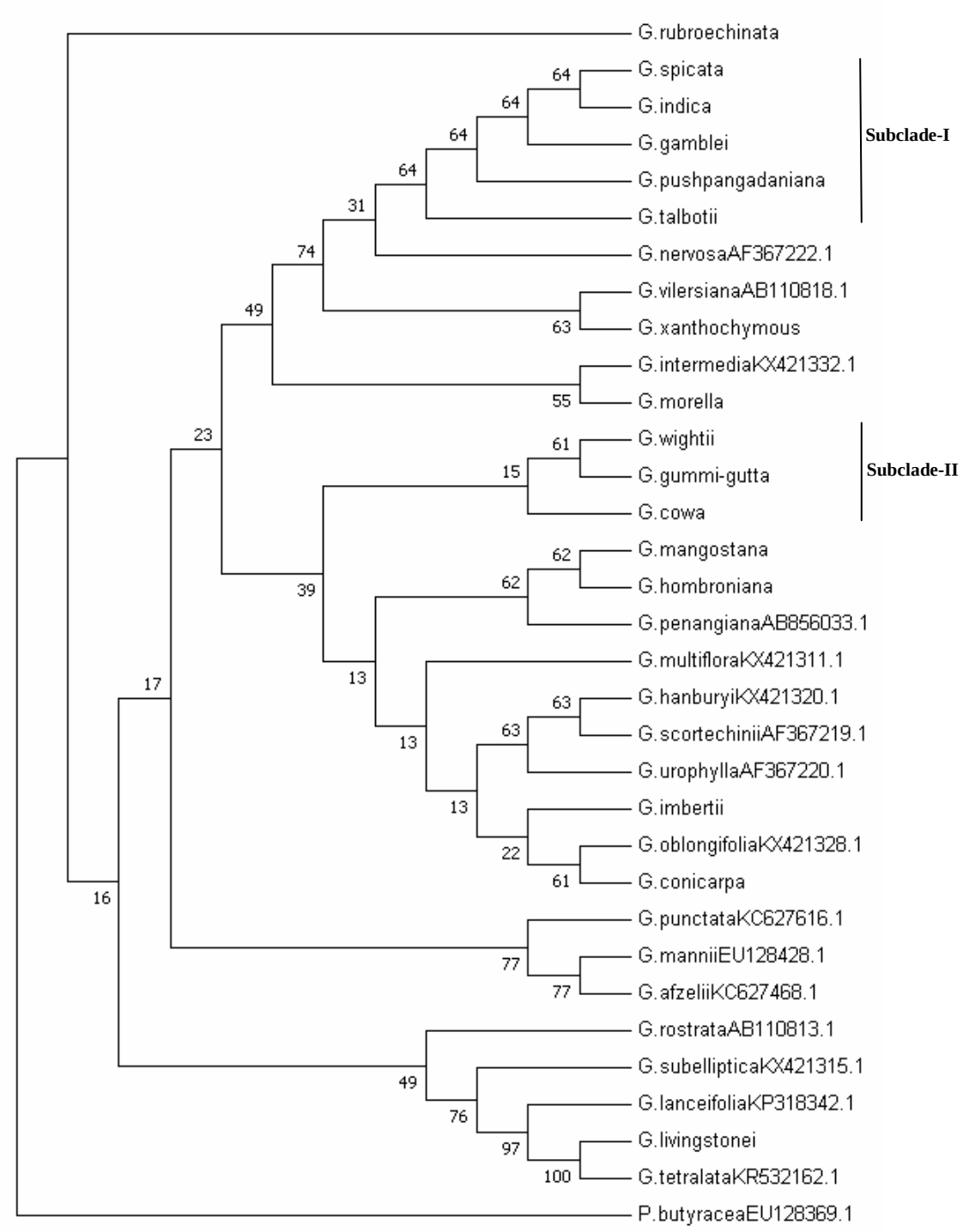


Figure 4.44: Phylogenetic tree constructed using combined dataset of ITS region and *mat*K gene sequences.

Chapter 5

Discussion

5.1 Field survey, documentation and taxonomic characterisation of *Garcinia* species from the Western Ghats of India

5.1.1 Field survey, collection and identification of plant species

Previous studies indicated the presence of *Garcinia* species in variable numbers. Hence, there were some ambiguities in number of species in the Western Ghats. Anderson (1874) reported the presence of 30 species in India and 8 species in the Western Ghats. Maheshwari (1964) reported 35 and 13 species in India and the Western Ghats respectively. In the Western Ghats region 11, 10 and 12 species were listed by Singh (1993), Gunaga *et al*. (2010) and Nayar *et al*. (2014) respectively. However, to date 41 *Garcinia* species with 7 varieties are reported to occur in India. Among them 17 species with 5 varieties are endemic to India. Out of which five species are endemic to Andaman and Nicobar Islands, three species are endemic to the north-east India and nine species are endemic to the Western Ghats region (Anderson, 1874; Maheshwari, 1964; Singh, 1993; Srivastava, 1994; Pandey & Diwakar, 2008; Sabu *et al*., 2013; Nayar *et al*., 2014; Sarma *et al*., 2016: Shameer *et al*., 2017). In the present study 16 species with 3 varieties were documented and collected from the entire Western Ghats (Table 4.1; Plates 4.1-4.16). Out of them 9 species with 3 varieties were found to occur as an endemic. *G. gummi-gutta* var. *gummi-gutta*, *G. indica* and *G. talbotii* were found as an endemic species to the region, distributed throughout and first two species occurs as natural population as well as cultivated. Additionally, the species *G. xanthochymus* was also located throughout the region including its cultivation in parks and gardens. The species *G. morella* was documented from the central and southern Western Ghats with occasional distribution in northern Western Ghats. Further, some introduced and cultivated species were also documented from botanical gardens and agricultural farms namely G. *cowa*, *G. hombroniana*, *G. livingstonei* and *G. mangostana*. Some

endemic species namely *G. gamblei*, *G. gummi-gutta* var. *conicarpa*, *G. gummi-gtta* var. *papilla*, *G. imbertii*, *G. pushpangadaniana*, *G. rubro-echinata*, *G. travancorica* and *G. wightii* were located from southern Western Ghats. All the species were found to be strictly endemic to the region and available at higher altitudes only, except *G. wightii* which is located at lower altitudes. Distribution of species *G. gamblei*, *G. imbertii*, *G. rubro-echinata* and *G. travancorica* were found strictly restricted to Agasthyamalai biosphere reserve, Kerala (Mohanan & Sivadasan, 2002; Shameer *et al*., 2016; 2017). *G. imbertii* and *G. travancorica* both species are endangered and the report on population status of both species was indicated extreme fluctuation in flowering-fruiting every year. This is due to disturbance in forest ecosystem and hence both species are included in threatened category of IUCN and need to be conserved (Manikandan & Ramasubbu, 2017).

Earlier data showed taxonomic ambiguities in occurrence and identification of some species. Hence, these ambiguities were solved with the help of morphological and anatomical characters. One of the issues solved on two most important species *G. talbotii* and *G. spicata*. The species *G. talbotii* Raizada ex Santapau is one of the species endemic to Western Ghats that produces edible fruits which can be used in culinary preparations. Quantitative HPLC-PDA analysis of benzophenones and biflavonoids in eight species of *Garcinia* showed highest amount of xanthochymol in *G. spicata* (Wight & Arn.) Hook.f. (Acuna *et al*., 2012). It also yields timber, used in construction and yellow coloured pigment from bark and young fruits as a dye. Taxonomic identity and occurrence of *G. spicata* and *G. talbotii* in peninsular India was worked out (Palkar *et al*., 2017). *G. talbotii* is often misidentified as *G. spicata* in the regional Floras of northern Western Ghats. This paper clearly delimits these two species with unambiguous morphological and anatomical characters to facilitate authentic identification. A critical examination of herbarium specimens of *G. spicata* housed at BSI, FRLH, CALI, MH, SUK and TBGT revealed that most of them were misidentification of *G. talbotii.* Similarly, scrutiny of descriptions of *G. spicata* in the earlier published Floras (Santapau, 1967; Almeida, 1990; Naithani *et al*., 1997; Godbole & Das Das, 2000; Yadav & Sardesai, 2002; Ganeshaiah *et al*., 2012) that recorded the occurrence of this species in the northern Western Ghats revealed that the diagnostic features provided for *G. spicata* fall well within the circumscription of *G. talbotii*. Inclusion of northern Western Ghats, especially Goa and Maharashtra

under the distribution range of *G. spicata* by other workers (Singh, 1993; Nayar *et al*., 2014) is also based on the above mentioned earlier works. An examination of fresh field collections corroborated that *G. spicata* does not occur in northern Western Ghats. An accurate identification of both these species was worked out based on detailed description, differentiating characters provided in the result section (4.1.3.13. & 4.1.3.14), photographs depicting morphological (Plates 4.12 & 4.13) and anatomical features (Fig. 4.1) and images of type specimens (Figs. 4.2 & 4.3). The study supports revisionary work earlier done by Maheshwari (1964) which showed absence of the species *G. spicata* in the northern Western Ghats. The mentioned species showed scattered distribution in peninsular India covering the states of Karnataka, Kerala & Tamil Nadu from the Western Ghats and coastal areas of Andhra Pradesh, Tamil Nadu & Union Territory of Pondicherry extends to Sri Lanka (Balachandran *et al*., 2014). The report on species diversity, population structure, abundance and dispersion patterns of woody plants were evaluated in tropical dry evergreen (sacred grove or temple) forests at the Coromandel coast of south India showed the existence of species richness, density, dominance, rarity and contribution to total density of *G. spicata* over other plants (Parthasarathy & Karthikeyan, 1997; Parthasarathy & Sethi, 1997).

An endemic varieties of the species *G. gummi-gutta* namely *G. gummi-gutta* var. *conicarpa* and *G. gummi-gutta* var. *papilla* collected from shola forests of Eravikulam National Park, Munnar, Idukki, Kerala and its surrounding area, possess similar morphological characters. However, these were identified using key distinguishing characters as mentioned in the result section (4.1.3.4 & 4.1.3.5) and also based on the earlier literature (Maheshwari, 1964; Singh, 1993; Shameer *et al*., 2016).

5.1.2 Ecological niche modeling study

Garcinia indica is one of the most important species in the genus because of its medicinal and economic value. It is distributed in forests of the Western Ghats of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. It is also cultivated predominantly in the states of Goa, Karnataka and Maharashtra covering the entire Konkan region for commercial purpose. The fruits and leaves of the species contain

hydroxycitric acid (HCA), garcinol, polyphenols, several acids and other phytochemicals (Baliga *et al*., 2011; Ramachandran, 2014). Due to the presence of these compounds, it has pharmaceutical values. These compounds possess many biological activities such as antiobesity (fat-reducing agent), anti-cancerous, antioxidant, antibacterial, antifungal, anti-HIV, anti-inflammatory, antipyretic and anti-Alzheimer's activity (Padhye *et al*., 2009). Considering the importance and due to overexploitation of the species the threat status of *G. indica* is categorized as vulnerable (Ravikumar & Ved, 2000). Hence, the prediction of potential distribution and understanding the climatic factors responsible for the niche of the species in wild including areas for its cultivation was worked out based on primary occurrence data (Palkar *et al*., 2020).

Ecological niche modeling was carried out using MaxEnt and BIOCLIM algorithms. Maximum entropy method (MaxEnt) for species distribution modeling is a simple and precise general purpose machine learning method and have a natural probabilistic interpretation (Phillips *et al*., 2004; 2006). BIOCLIM was the first widely used package to implement the basic species distribution modeling process and with WorldClim database which is source for climate data possessing all the most commonly used set of 19 bioclimatic variables. It has wide applications in conservation biogeography with measuring niche and assessing impacts of climate change (Booth *et al*., 2014; Booth, 2018).

To attain best fit model, quality was evaluated based on area under the receiver operating characteristic (ROC) curve or AUC values which indicates better discrimination in suitable and unsuitable areas for the niche of species (Phillips *et al*., 2006). Among both algorithms, MaxEnt performed better as compared to BIOCLIM model with high AUC value. However, predictions by both models were found to be almost similar. In addition to threshold-independent AUC value (ROC curve), the MaxEnt algorithm was validated with omission/commission rate which is a threshold-dependent binomial test based on omission and predicted area (Phillips *et al*., 2006; Elith *et al*., 2011). The analysis of omission/commission revealed the lines of average omission and predicted area are close to each other which indicates that the model performed better than random and showed that the model is more accurate (Fig. 4.4a & b).

Results of the analysis were interpreted based on jackknife evaluation, percent contribution and permutation importance of each variable and prediction maps (Barik & Adhikari, 2012; Patil & Janarthanam, 2013). Sample bias was attained by sample stratification to avoid overrepresentation of the training data set (West *et al*., 2016). Sample size, sample accuracy and environmental variables effects on predictive performance of MaxEnt model (Li & Ding, 2016).

The prediction maps obtained using both algorithms MaxEnt and BIOCLIM depicted the potential geographical distribution, range of richness and density of *G. indica* in the Western Ghats of India (Figs. 4.5 & 4.6). The MaxEnt prediction revealed the potential range for richness of the species and habitat suitability is excellent at the junction of northern-central Western Ghats mainly comprising Konkan region of Maharashtra, Goa and Karnataka. Also, these regions are popular for various kokum fruit processing industries and utilization of kokum products for culinary applications (Baliga *et al*., 2012). According to MaxEnt prediction, niche of the species prefers western part of Western Ghats, but entire stretch of Western Ghats is not suitable for the growth of *G. indica*. However, earlier study carried out using grid sampling method showed the presence of some *Garcinia* species such as *G. gummi-gutta*, *G. morella* and *G. talbotii* in the entire Western Ghats including *G. indica* (Gunaga *et al*., 2010).

The precipitation and temperature were the major factors defining the distribution of the species (Table 4.2). Other parameters were also responsible in a lesser extent. The data support that the species prefers specific environmental conditions which was revealed in earlier study. The study was carried out using Kira's warmth index, coldness index, Xu's humidity index and other climate indexes revealed that *G. pedunculata* Roxb. species richness was due to humidity and thermal factors (Ting *et al*., 2012). The species distribution modeling was used to estimate the probability of occurrence of species with climate data based on presence-only (or absence) data for many plant species (Ortega-Andrare *et al*., 2015; Thriveni *et al*., 2015).

5.2 Quantitative estimation of bio-active compounds garcinol and hydroxycitric acid (HCA) during various developmental stages of fruits of *Garcinia indica* and matured fruits of other *Garcinia* species using HPLC system

5.2.1 Plant material, extract preparation and extraction yield

The present study was carried out using mature fruit rinds of 10 different *Garcinia* species and 6 different developmental stages of fruit rinds of *G. indica*. Dried fruit rinds of all *Garcinia* samples were extracted using Soxhlet extraction method with methanol solvent; as earlier literature proved effects of plant parts, extraction methods and solvents used on extraction yield, quantity of bioactive compounds and bioactivities. Dried fruit rinds of different developmental stages of *G. indica* yielded semi-solid extracts ranging from 4.77 to 6.48 g per 10 g of fruit and among different *Garcinia* species the highest extraction yield was found in *G. livingstonei* fruit extract (8.1 g per 10 g fruits) (Tables 4.3 & 4.4).

The effect of extracting solvents on phenolic, flavonoid contents and antioxidant activity of three different Nigerian medicinal plants showed highest antioxidant activity was found in methanol extract of *Acalypha wilkesiana* leaves (Anokwuru *et al*., 2011). Solvent effects on phytochemical constituent profiles and antioxidant activities of *Bucida buceras* L. and *Phoradendron californicum* using four different extraction formulations was carried out and showed the highest total phenolic and flavonoid content in *P. californicum* (oak) methanol extract (Iloki-Assanga *et al*., 2015). Phytochemical and bioactive potential of *Ocimum gratissimum* L. extracts was evaluated and checked for the effect of different extraction temperature and solvent type on it. Increased concentrations of compounds in methanolic and ethanolic extracts were found during chemical profiling and also highest antibacterial activity was revealed in methanolic extract (Onyebuchi & Kavaz, 2020). In phytochemical studies both fresh and dried samples proved beneficial however, dried samples are preferable, considering the time period between plant sample collection and further evaluation (Azwanida, 2015). No significant effect was found in total phenolics but higher flavonoid content was found in dried samples when fresh and dried leaves of *Moringa oliefera* were compared (Vongsak *et al*., 2013). Further, the effect of different extraction techniques on extraction yield, total

phenolic and anti-radical capacity of extracts from *Pinus radiata* Bark were evaluated. Among the four extraction techniques, Soxhlet extraction showed highest extraction yield, total phenol and tannin content (Aspe & Fernandez, 2011). Further this method requires a smaller quantity of solvent compared to other methods of extraction (Handa *et al*., 2008). Numerous phytochemicals were reclaimed by using Soxhlet extraction from leaf methanolic extract of *Azadirachta indica* (Neem) (Hossain *et al*., 2013).

5.2.2 Thin-layer chromatography (TLC) for confirmation of hydroxycitric acid

Thin layer chromatography (TLC) is the chromatographic system exhibits widest applications in phytochemistry. It can be used for preliminary profiling of compounds from crude plant extracts (Harborne, 1998).

For thin layer chromatography solvent system was standardised and n-butanol:formic acid:water was used and presence of hydroxycitric acid (HCA) in different *Garcinia* methanolic extracts was confirmed by 1% sodium metavanadate spray. Formation of yellowish orange coloured spots after applying the spray was observed in all samples which confirmed the presence of HCA. In earlier study also orange coloured spot was observed and confirmed the presence of HCA on paper chromatogram and TLC plates after spraying 1% sodium metavanadate reagent (Revathi *et al*., 2010). It also revealed Rf values for HCA as 0.26 and 0.35 from *G. cambogia* fruit extract. During present study Rf values were found in the range of 0.26 to 0.35. Lewis and Neelakantan (1965) isolated the principal acid and identified it as (-)-HCA using chemical and spectroscopic studies from fruit rinds of *G. cambogia*. Paper chromatography was carried out to identify and separate hydroxycitric acid using n-butanol:acetic acid:water (4:1:5) and n-propanol:formic acid:water (4:1:5). After treatment of 5% metavanadate spray fruit extracts showed two spots on chromatograms and Rf values were 0.34 & 0.46 for HCA & HCA lactone respectively. In the earlier report, HCA an anti-obesity agent was identified from *G. cambogia* extract showed orange coloured spot and Rf value 0.34 for presence of HCA after applying a solution of 3% sodium metavanadate (Ferrara, 2014). To check nutraceutical properties of *Garcinia*, nutrient and medicinal composition was studied for some Indian species (Parthsarathy & Nandakishore, 2014). Paper chromatography was performed to detect organic acids from the samples

using butanol:formic acid:water (4:1.5:5) as solvent system and 1% bromocresol green as indicator. Rf values of sample spots were identified using standard Rf values of acids. Among six organic acids the Rf value for HCA was found to occur as 0.24. The report on phyto-physicochemical, acute and subacute toxicity of a rare ethno medicinal plant of Assam i.e. *G. lanceifolia* showed TLC profile of bark extract (Bora *et al*., 2017). Five different solvent systems and iodine and alcoholic $FeCl_3$ were used to visualize spots. It showed range of different Rf values for different solvent systems.

5.2.3 High performance liquid chromatography (HPLC) analysis

High performance liquid chromatography (HPLC) is an analytical technique also called as high pressure liquid chromatography. It is developed in the mid of 1970s and can be used for quantitative and qualitative data simultaneously. It is used for isolation and quantification of compounds which are non-volatile in nature for several studies in biological, food, pharmaceutical, industrial applications etc. (Raaman, 2006).

5.2.3.1 Quantification of hydroxycitric acid (HCA)

During the present study, suitable and easy HPLC method was developed and validated. 8 mM sulfuric acid as mobile phase, isocratic elution pattern with 1 ml/min flow rate and UV detector at 210 nm wavelength was used and it showed presence of HCA at retention time of 3.2 min (Table 4.5). The developed method was also validated by accomplishing carry over test, system suitability test and linearity within acceptable limit. Quantity of HCA was estimated using above method showed HCA amount varied from 776.94 mg to 2713 mg per 10 g of fruit rinds from different developmental stages of *G. indica* fruits and 15.39 mg to 1880.54 mg per 10 g of fruit rinds from different *Garcinia* species fruits (Tables 4.3 & 4.4).

Lewis *et al*. (1964) isolated HCA and identified using chemical and spectroscopic studies. A wide occurrence of HCA was noted in nature. It was found that in highly acidic fruits of *G. cambogia*, HCA is present as principal acid and same acid was found to occur in two other *Garcinia* species fruits and also in *Hibiscus cannabinus* leaves (Lewis & Neelakantan, 1965).

Organic acids in leaves and rinds of *Garcinia indica* (Kokum) were determined by high-performance liquid chromatography (HPLC). The major organic acid in leaves and rinds was found to be hydroxycitric acid (HCA) present to the extent of 4.1-4.6 and 10.3-12.7%, respectively (Jayaprakasha and Sakariah, 2002). For the quantification of HCA authors developed isocratic elution method using 8 mM sulfuric acid and it proved to be satisfactory in terms of reproducibility, accuracy and sensitivity hence, can be used for large number of samples of *Garcinia* routinely. Similarly, organic acids in fresh leaves, fruits and dried rinds of *G. cowa* were determined by high-performance liquid chromatography. The major organic acid was found to be hydroxycitric acid present in leaves, fruits and rinds to the extent of 1.7, 2.3 and 12.7% respectively. Along with HCA, hydroxycitric acid lactone, oxalic and citric acids are present in leaves, fruits and rinds in minor quantities (Jena *et al*., 2002). Determination of organic acids from different plant parts of *G. pedunculata* was carried out using reversed-phase HPLC. HCA was found as a major organic acid and quantity was 2.5%, 0.8%, 3% and 20.1% in leaf, pulp, fresh fruit and dried rind respectively (Jayaprakasha *et al*., 2003).

Chromatographic fingerprinting and estimation of organic acids in eight selected *Garcinia* species collectively from Western Ghats and north-east Himalayas of India was done with the help of paper chromatography and HPLC. The study showed presence of HCA along with other acids such as malic acid, citric acid, oxalic acid, acetic acid, ascorbic acid and tartaric acid with Rf value of 0.24 in paper chromatography and variation in number of peaks for HPLC. Among the species *G. cowa* indicated highest amount of total acids 28% while *G. gummi-gutta* indicated highest amount of HCA 10.48% (Parthasarathy *et al*., 2012). Another report indicated nutraceutical importance of eight different *Garcinia* species from the Western Ghats and north-east India. It showed importance of all *Garcinia* species with varied composition of primary metabolites, mineral, vitamins, organic acid, total phenols, xanthones and antioxidant activity. Highest amount of HCA 15.48% was observed in *G. gummi-gutta* fruit rind (Parthasarathy & Nandakishore, 2014). The comparative estimation of HCA from four different *Garcinia* species leaves and fruits was carried out with HPLC and titration method. It showed more quantity in leaves (3.1%) and fruits (5.98%) of *G. gummi-gutta* species by HPLC method. Titration method revealed

high quantity in leaves of *G. cowa* (3.26%) and fruits of *G. gummi-gutta* (6.13%) (Asish *et al*., 2008).

Simultaneous determination of 26 multi-class bioactive constituents from leaves of 11 *Garcinia* species was carried out using ultra high performance liquid chromatography-hybrid linear ion trap triple quadrupole mass spectrometry (UHPLC-QqQLIT-MS/MSR). For the analysis rapid and highly sensitive method was developed and validated and can be used for quality control studies. Results showed that leaf extracts of *G. cowa*, *G. echinocarpa*, *G. gummi-gutta*, *G. hombroniana* and *G. indica* possess organic acids as a major class of constituents. Also, it was found that biflavonoids and flavonoids were the major constituents in other species. However, highest total content of all the 26 bioactive constituents were found in *G. morella*. Also highest amount of HCA and garcinol was found in *G. cowa* and *G. gummi-gutta* leaf extract respectively (Pandey *et al*., 2015). For rapid detection and quantification of HCA in fruit extract of *Garcinia cambogia* HPLC- isocratic method was developed and standardized. In methanolic extract of the species 50-55% of HCA was found (Upadhyay *et al*., 2013).

Novel HPLC method was developed using water as mobile phase with UV detector for estimation of HCA from dried fruit rinds of *G. lanceaefolia*. Primarily HCA was detected using paper chromatography observing orange coloured spot and 0.34 Rf value. During HPLC analysis 4.56 ± 0.13 g of HCA per 10 g of sample was estimated (Gogoi *et al*., 2014).

Garcinia fruits and food supplements were authenticated using DNA barcoding and NMR spectroscopy. NMR was used to quantify HCA and HCA lactone from raw herbal drugs and *Garcinia* food supplements especially from *G. indica* and *G. gummi-gutta*. It revealed large variation in content of HCA and HCA lactone (Seethapathy *et al*., 2018).

5.2.3.2 Quantification of garcinol

In current study precise and feasible high performance liquid chromatographic method was developed and validated using isocratic elution pattern and UV detector

at 215 nm wavelength. Retention time for garcinol was found at 5.6 min (Table 4.6). The carry over test, system suitability test and linearity was found to occur within acceptable limits during method validation. In different developmental stages of *G. indica* fruits and different *Garcinia* species fruits garcinol quantity was varied from 42.49 mg to 676.02 mg and 0.71 mg to 1279.49 mg per 10 g of fruit rinds respectively (Tables 4.3 & 4.4).

Krishnamurthy *et al*. (1981) isolated new polyisoprenylated phenolic pigment known as garcinol and its colourless isomer isogarcinol from *G. indica* and their structural elucidation was carried out using chemical and spectral data.

The novel method of reversed-phase HPLC (RP-HPLC) for analysis of garcinol and isogarcinol from fruit rind of *G. indica* was developed and validated, using mixture of acetonitrile & 0.1% orthophosphoric acid in water as mobile phase and detector wavelength at 215 nm (Praveen Kumar *et al*., 2013). Developed and validated method was proved to be simple, efficient, rapid, precise, linear over the analysis range and was able to determine both the compounds simultaneously and can be used for routine analysis.

G. travancorica leaf extract was investigated for phytochemical analysis and antioxidant activity. The study revealed presence of polyisoprenylated benzophenone garcinol & bioflavonoids by using chromatographic and spectroscopic techniques (Anu Aravind *et al*., 2016).

Optimized extraction and quantification of two anticancer polyisoprenylated benzophenones garcinol and isogarcinol from fruits of *G. indica* was carried out using LC/ESI-MS/MS (Bharate *et al*., 2014). In the method of extraction optimization the highest amount of garcinol and isogarcinol was found in combination of dichloromethane-methanol solvent. The developed and validated method was found to be precise, easy and robust in terms of precision, recovery, limit of detection and limit of quantification.

Quantification of garcinol from *G. indica* fruit rinds was carried out by high performance thin layer chromatography (HPTLC) method. Method was validated and

used to estimate garcinol from fruit rinds of *G. indica* and its market formulations. Highest amount of garcinol was found to occur in fruit rind extract (2.5%) as compared to market formulations (Patel *et al*., 2015).

A dietary weight reducing compound hydroxycitric acid lactone (HCAL) and chemo preventive compounds isoxanthochymol (isoxan) and xanthochymol (xan) were identified and quantified using reversed-phase HPLC-PDA method. For identification and quantification simultaneous, rapid and sensitive method was developed and validated found to be robust in terms of linearity, accuracy and precision, limit of detection and limit of quantification. Also, this method was used to quantify the HCAL, isoxan and xan from extracts of different plant parts of *G. indica* (Kumar *et al*., 2013). Identification and quantification of two biologically active polyisoprenylated benzophenones xanthochymol and isoxanthochymol in different plant parts of *G. indica* was carried out with HPLC and LC-ESI-MS. The plant parts used were fruit rinds, leaves and seed pericarps; among them, fruit rinds showed high quantities of both compounds (Kumar & Chattopadhyay, 2007). Comparative metabolic profiling and bioactivities of the leaf, branch and fruit samples of *G. oblongifolia* were analysed using UPLC-QTOF-MS. A total of 40 compounds including 15 new compounds were identified first time from the species. Benzophenones were found in high concentration in all plant parts. In branch extract bioflavonoids, xanthones and benzophenones were found predominantly. Also the bark extracts showed strong cytotoxic and antioxidant activities (Li *et al*., 2016).

5.3 Phytochemical profiling of fruits of *Garcinia* species using GC-MS

GC-MS phytochemical profiling revealed the presence of several compounds in all *Garcinia* species. Some major and common as well as distinctive compounds were identified during the present study. Further, data was analysed to check chemical nature, chemical properties and applications of the identified compounds using several databases and previous literature. All detected and identified compounds were found to occur in different phytochemical groups based on their chemical nature. Across all the species, most common and major phytochemicals were found. Their chemical nature, industrial applications and previously reported bio-activities are discussed below: saturated and unsaturated fatty acids and their esters were used as

food flavouring agents, surfactants, in personal care products, in agricultural products; to prevent neurodevelopmental syndromes and psychiatric disorders (Mahmoudi *et al*., 2019) and possessed anti-inflammatory (Prasath *et al*., 2021) and antidiabetic (Choi *et al*., 2021) activities. Carboxylic acids and their esters were used as herbicide, food flavouring agents. Phenols and their derivatives were exhibited antifungal (Ren *et al*., 2019); antioxidant, anti-inflammatory, cytotoxicity, insecticidal, antiviral, antibacterial (Peng *et al*., 2017; Zhao *et al*., 2020) and antidiabetic (Bu *et al*., 2010) activities. Terpenes could be used as food preservative and possessed antifungal activity (Omoruyi *et al*., 2014). Organic acids and their esters were used as food preservative and showed cytotoxic (Kuete *et al*., 2015) and antibacterial (Seukep *et al*., 2016) activities. Hydrocarbons were used as adhesive, sealant, lubricant, moth repellent, fumigant and disclosed anti-allergic, anti-inflammatory (Choi *et al*., 2020); cytotoxic (Ashmawy *et al*., 2020) effect. Alkanes were found useful in food, textile and cosmetics industries and showed anti-protozoal (Naemi *et al*., 2014), anti-malarial (Nahrevanian *et al*., 2010), leishmanicidal (Delgado-Altamirano *et al*., 2019) activities. Sesquiterpenes were found useful in food flavouring agents, personal care products and disclosed larvicidal effect (Pandiyan *et al*., 2019) and cytotoxic, antimicrobial activities (Ramak & Talei, 2018). Phthalates were found to be useful in various industries such as personal care product, paint, colourant, resin, plastic and rubber. Chloroform was used as extraction solvent in food additive. Oxaspiro compounds unveiled anti-inflammatory (Lakshmegowda *et al*., 2020), antibacterial (Das *et al*., 2021) and anti-Alzheimer activities (Kareti & Pharm, 2020). Organosilane compounds were found useful in personal care products and possessed antifungal activity (Omoruyi *et al*., 2014). Phytoconstituents from terpenoids, phytosterol, furan, sulfone, tetraprinol, aromatic essential oils, glycosides, flavonoids, alkaloids, sugar alcohol (polyols), fatty alcohol, pyrimidines, diazine derivatives, chromen (pyran), steroid, triterpenoid, alkanol etc. groups were also detected.

Across all species the unique compounds were also recorded from each species. All these compounds have commercial applications as well as medicinal importance. The chemical nature of common compounds which were found to occur in high amounts as well as some unique phytoconstituents present in each species and their potential bio-activities and applications are discussed below.

Garcinia cowa, north-east Indian species showed presence of major compounds belongs to fatty acids and derivatives, carboxylic acids and derivatives, hydrocarbons and furans. Some distinct compounds found in the species extract were Dimethylisopropylsilyloxycyclobutane {trialkylheterosilane}; 1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disiloxanyl tris which is trialkylheterosilane possesses acute cytotoxicity (Lassen *et al*., 2005); 9-Octadecenoic acid (Z)-, methyl ester {syn: oleic acid, methyl ester}is unsaturated fatty acid ester useful in oleates and lotion preparations and possess anti-cholesterol activity (Lopez Huertas, 2010), anti-inflammatory activity (Harvey *et al*., 2010), an analgestic and reducing neurotrauma (Galán-Arriero *et al*., 2017), showed modulatory effects on infections, inflammation, cardiovascular diseases and skin repair (Sales-Campos *et al*., 2013). ***G. gamblei*** is newly described species endemic to the southern Western Ghats revealed presence of major group of compounds such as saturated fatty acids, carboxylic acids, sulfone, terpenes specially diterpenes, phthalate and oxaspiro compounds.

Garcinia gummi-gutta highly used species in Malabar regions of the Western Ghats for culinary purposes repeatedly showed presence of saturated fatty acid and derivatives, terpenes especially diterpenes, sulfone, organic acids & esters, glycoside and oxaspiro compound as a major group of compounds. Also some unique compounds were observed such as Hexadecanoic acid, 14-methyl-, methyl ester which is saturated fatty acid exhibits antifungal (Mohy El-Din *et al*., 2018), antiviral, antimicrobial and cytotoxic activities (Matloub *et al*., 2020); 7-Hexadecenoic acid, methyl ester, (Z)- a fatty acid has antioxidant activity (Qadir *et al*., 2020); Bromacil which is pyrimidine compound a broad spectrum herbicide; 4a,7,7,10a-Tetramethyldodecahydrobenzo[f]chromen-3-ol is chromen which is naphthopyran has an anxiolytic effect (Liu *et al*., 2015). Another variety ***G. gummi-gutta* var. *conicarpa***, southern Western Ghats species present at high altitudes showed major group of compounds such as saturated fatty acids, organic acid esters, terpenes especially diterpenes, sulfone, volatile oils and oxaspiro compounds as well as some unique compounds such as Citric acid, triethyl ester is an organic acid ester is useful in food industry as flavouring agent; personal care products such as cosmetics, perfume industry; beverage industry; pharma and medicine manufacturing; plastic product manufacturing (Park *et al*., 2004). 6-Ethyl-2,3-dimethyl-tetrahydro-thiopyran-4-ol is a flavonoid useful as volatile oil and Silane, dimethyldi(but-3-enyloxy)- is

terpene. In another rare, high altitude variety ***G. gummi-gutta* var. papill*a*** major group of compounds found were saturated fatty acids, organic acid esters, terpenes especially diterpenes, sulfone, sugar alcohol and oxaspiro compounds. Also, some unique compounds were found as- Cyclohexanecarboxylic acid, methyl ester is carboxylic acid ester useful as flavouring agent and food additive; Succinic acid, 2-decyl propyl ester is tricarboxylic acid ester possess tuberculocidal activity (Barry & Twomey, 1945); D-galactitol, 3,6-anhydro-1,2,4,5-tetra-o-methyl- {sugar alcohol}; Butanedioic acid, 3-hydroxy-2,2-dimethyl-, diethyl ester is carboxylic acid derivative found useful in cosmetics and therapeutic applications (Kornhauser *et al*., 2010); D-Galactitol, 2,3,4-tri-O-methyl-, triacetate a sugar alcohol possess anti-alzheimer activity; s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino- an alkaloid which is antitumor (Brzozowski *et al*., 2000), antipsychotic, anticholinergic agent and commercially useful compound; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- {syn: methyl linolenate} an unsaturated fatty acid was used as flavouring agent in food industry, perfume industry and it exhibited anti-melanogenesis (Ko *et al*., 2018), antibacterial, cytotoxic and antioxidant activities (Amado *et al*., 2018; Kotb *et al*., 2020); 4-Hydroxyphenyllactic acid, ethyl ester, di-TMS which is phenol derivative useful in food industry and possess antioxidant, antimicrobial activities (Zieniuk *et al*., 2020).

A cultivated species ***G. hombroniana*** possess major group of compounds such as saturated fatty acids, phenol, terpenes, diterpenes, carboxylic acid esters, sulfone, volatile oils, fatty acid lactone, alkane, oxaspiro and phthalate compounds. Moreover unique compounds found to occur in extract such as Nonane, 1-iodo- {volatile oil}; 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- {syn: Germacrene-D} a terpene exhibit antitumor, antiviral and anti-proliferative, cytotoxic and antimicrobial activities (Costa *et al*., 2015; Tan *et al*., 2020; Torky *et al*., 2021); Valeric acid, dodecyl ester a saturated fatty acid useful in perfumes, cosmetics and food additives; Hexadecane, 1-chloro- {halogenated hydrocarbon}; Nonadecane a volatile oil used in food industry as flavouring agent, perfume industry and showed antileishmanial and antibacterial (Oliveira *et al*., 2020), antimicrobial and anticancer (Marrez *et al*., 2019), antioxidant and α-glucosidase inhibition (Jelassi *et al*., 2017) activities; 2-Bromotetradecane an alkylhalide used in therapeutics; Pentadecanoic acid a saturated fatty acid useful in food and beverage, paper, organic chemical and plastic

manufacturing industries and exhibits antioxidant, anti-diabetic, anti-obesity and anti-hypertensive activities (Mayasankaravalli *et al*., 2020); 2H-Pyran-2-one, 6-heptyltetrahydro- {syn: .delta.-Dodecalactone} which is fatty acid lactone can be used in food industry as flavouring agent; Hexatriacontane an alkane useful in food industry and has anticancer activity (Mishra *et al*., 2019); Dodecahydrosqualene {syn: Cosbiol/ Squalane} a triterpene useful in personal care, pet care and lubricant product industries and disclosed anti-metastatic effect (Yoo *et al*., 2002), use in Parkinson's disease (Kabuto *et al*., 2013), anticancer, antioxidant, drug carrier, detoxifier, skin hydrating and emollient effects (Kim & Karadeniz, 2012) and Tridecanol, 2-ethyl-2-methyl- {fatty alcohol}.

A rare and endemic, high altitudinal species ***G. imbertii*** restricted to the southern Western Ghats showed presence of wide range of compounds as saturated fatty acids, phenols, carboxylic acid esters, terpenes, diterpenes, sulfone, chloroform, sesquiterpene, cyclic ketone, steroids, triterpenoid, oxaspiro and phthalate compounds. Some unique compounds were also found such as .alpha.-Caryophyllene {syn: humulene} a sesquiterpene useful in food and fragrance industry possess anti-influenza, antioxidant (Wu *et al*., 2012), antifungal (Shahina *et al*., 2018) activities; 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl- {syn: aromadendrane} a sesquiterpenoid showed cytotoxicity (Deng *et al*., 2020), insect repellent, anti-microbial, antiviral, cytotoxic, antioxidant and anti-inflammatory (Duran-Pena *et al*., 2015) activities; 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde {cyclic ketone}; 10,13-Octadecadienoic acid, methyl ester {syn: linoleic acid ester} a fatty acid ester revealed anti-inflammatory, hepatoprotective, nematicide, anticancer and antiarthritic activities (Krishnamoorthy & Subramaniam, 2014) and Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- {epoxide}.

A well-known species from Konkan region of Goa, Maharashtra, Karnataka i.e. ***G. indica*** (Kokum) showed presence of fatty acid esters, diterpene, organic acid esters, carboxylic anhydride, volatile oils, sulfone and unsaturated hydrocarbons. An unique compounds were also found to occur in the species extract such as 3,5-Octadiyne {unsaturated hydrocarbon}; Benzene, 1,3-dimethyl- {hydrocarbon}; an volatile oil Azulene possess anti-inflammatory, antineoplastic, anti-diabetes, antiretroviral, antimicrobial and antifungal activities (Bakun *et al*., 2021); Phthalic

anhydride a dicarboxylic anhydride useful in artificial resin, plastic, rubber, personal care products, dyes, paint, and lubricant industries; 2-Propenoic acid, tridecyl ester a carboxylic acid ester has applications in food, cosmetic and pharmaceutical industries; Pentadecane an alkane volatile oil can be used as flavouring agent in food industry possess antimicrobial and antitumor activities (Essien *et al*., 2012; Lin *et al*., 2012).

Saturated and unsaturated fatty acids, carboxylic acids, phenols, steroid, fatty alcohol, triterpenoid, chloroform, phthalate and oxaspiro compounds were found as major group of compounds in ***Garcinia livingstonei***, a cultivated species. Along with common and major group of compounds it showed range of unique compounds such as Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.beta.,8.alpha.(1E,5E)] is polycyclic hydrocarbon; 1-Triacontanol {syn: Myricyl alcohol} a fatty alcohol useful in cosmetics preparation and showed anti-neoplastic (Zhou *et al*., 2018), anti-ulcer and anti-inflammatory (Srivastava *et al*., 2009) activities and 9-Octadecene, 1-[2-(octadecyloxy)ethoxy]-, (Z)- {dialkyl ether}.

Another cultivated species ***G. mangostana*** fruit extract revealed presence of range of major phytochemicals such as saturated and unsaturated fatty acids, terpene, diterpene, sesquiterpene, carboxylic acids, chloroform, terpenoid, alkanol, sulfone, silane, phthalate and oxaspiro compounds. Distinctive compounds found were Copaene a sesquiterpene useful as pesticide; the sesquiterpene namely Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- {syn: Eudesma-3,7(11)-diene} useful as insecticidal essential oil; Triacontane a long chain alkane useful in cosmetic, perfume industries and exhibited cytotoxic and antioxidant (Paudel *et al*., 2019), mosquito larvicidal (Satyan *et al*., 2012) and antitumor (Figueiredo *et al*., 2014) activities; 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12,16,20,24,28,32-octamethyl-3,7,11,15,19,23,27,31-tritriacontaoctaenyl)-, [r-(all-E)]- {syn: gamma-tocotrienol} a terpenoid and vitamin-E derivative showed antioxidant, antineoplastic and antitumor (Ghanem *et al*., 2019), anti-apoptosis (Suman *et al*., 2013) activities and Disiloxane, hexabutoxy- {silane compound}.

Fruit extract of ***G. morella*** species showed presence of organic acids, fatty alcohol, sugar, sesquiterpenes, volatile compounds, ethane derivative, alkane, carboxylic acid and polyol, glycoside, saturated fatty acid, triterpene, phytosterol as a major group of compounds. Among them some unique compounds were also found in major quantities such as 2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)- {fatty acid}; Ethane, 1-chloro-1-fluoro- {halogenated hydrocarbons}; 4-Penten-2-ol {fatty alcohol}; Isopropyl Alcohol a secondary alcohol used in personal care product, pharmaceutical, paint, adhesive, organic chemical, dye preparations and possess antibacterial activity; Propane, 2-fluoro-2-methyl- {alkane}; Acetic acid a carboxylic acid useful in food industry as food acidity regulator, food additive also showed antimicrobial and anti-fungal activities; Sucrose a glycosyl glycoside useful as sweetening agent in food, beverage industries and cosmetics, personal care products and pharmaceuticals manufacturing industries; 3-Deoxy-d-mannoic lactone {cyclic ester}; (1R,3R,4R,5R)-(-)-Quinic acid a carboxylic acid and polyol exhibited neuroprotective (Hur *et al*., 2001), antifungal (Ma *et al*., 2010) and hepatocyte protective (Xiang *et al*., 2001) activities; a sesquiterpenoid Caryophyllene oxide useful as a flavouring agent in food, perfume industries and showed larvicidal effect (Moussavi *et al*., 2015), antimicrobial, anti-inflammatory, antioxidant, antibacterial and cytotoxic activities (Essien *et al*., 2012; Cote *et al*., 2017).

Newly described southern Western Ghats endemic species, ***G. pushpangadaniana*** fruit extract exhibited major and common group of compounds namely saturated and unsaturated fatty acids, organic acid esters, terpenes and triterpenoid. Also some unique compounds found to occur in the species such as Decanoic acid, 2-oxo-, methyl ester {fatty acid ester}; a nutraceutical unsaturated fatty acid ester 8,11,14-eicosatrienoic acid, methyl has anticancer, vasodilatory activities; Heptadecanoic acid, 14-methyl-, methyl ester {fatty acid ester}; 1-Phenanthrenecarboxylic acid, 7-ethyltetradecahydro {carboxylic acid}; 2,5,5,8a-Tetramethyl-3-oxo-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, methyl ester {carboxylic acid ester}; a sesquiterpene Isozonarol possess an antioxidant (Kumagai *et al*., 2018) and algicidal (Ishibashi *et al*., 2013) activities; fatty acid halide 1,16-Dibromohexadecane an antimalarial compound; Lupan-3-ol, acetate a triterpenoid showed an antiprotozoal, antimicrobial, anti-inflammatory, antitumour activities (Yamuna *et al*., 2017); Handianol a triterpene showed an antidiabetic (Nair

et al., 2020), cytotoxic (Ragasa *et al*., 2013) and anti-inflammatory (Ahumada *et al*., 1997) activities and Lanosterol a novel triterpenoid exhibits therapeutic approach against neurodegenerative and protein conformational diseases (Upadhyay *et al*., 2018; Zhou *et al*., 2019).

G. rubro-echinata, the species restricted to the southern Western Ghats showed presence of saturated fatty acids, sulfone, carboxylic acid, terpenes, diterpenes, sesquiterpenes, sesquiterpenoids and heterocyclic compound with benzene ring and diazepine ring as major group of compounds. Among them, along with common compounds some unique compounds also found to occur in major quantities. Some unique compounds found were 1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undec-3-ene {syn: Humulen-(v1)} a sesquiterpenoid possess anti-inflammatory, anticancer, antioxidant, antibacterial, antifungal activities (Cote *et al*., 2017); another sesquiterpenoid Caryophyllenyl alcohol used as flavouring agent showed antiasthamatic, antibacterial, antifungal, anti-inflammatory activities (Yang *et al*., 1999; Sain *et al*., 2014; Ghavam *et al*., 2020); 3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl- {syn: Humulol} a sesquiterpenoid useful as surfactant, emulsifier in industries; .alpha.-Cadinol a sesquirerpenoid exhibits hepatoprotective, trypanocidal activities (Tung *et al*., 2011; de Souza *et al*., 2017); 1,5-Heptadien-4-ol, 3,3,6-trimethyl- {syn: Artemisia alcohol} a terpene useful in fragrance industry and possess antioxidant, anticancer activities (Koul *et al*., 2017); D-Glycero-D-gulo-heptonic acid, 2,3,5,6,7-pentakis-O-(trimethylsilyl)-, .gamma.-lactone a carbohydrate useful in chemical, diagnostic and pharmaceutical industries; 3-Acetyl-1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-3H-2,3-benzodiazepine {benzodiazepines}; Chrom-6-ol-4-one, 2,3-dihydroxy-2,2,7,8-tetramethyl- {benzopyran}; a sesquiterpene Naphthalene, 1,6-dimethyl-4-(1-methylethyl)- {syn: Cadalin} possess antioxidant (Kim *et al*., 2004), anticancer (Jin *et al*., 2007), anti-inflammatory (Martins *et al*., 2008), anti-feedant (Espinoza *et al*., 2018), antiplasmodial (Assis *et al*., 2020) activities and Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.beta.) {steroid}.

Palkar *et al*. (2017) solved the problem of distinguishing ***G. spicata*** and ***G. talbotii*** by using various morphological and anatomical characteristics. Also occurrence and distribution of both species was worked out. Both the species indicated presence of same range of common and major group of compounds such as

phenols, carboxylic acid esters and oxaspiro compound. However, former species showed more amounts of saturated fatty acids as compared to later species. But later species showed presence of diterpenes in large quantities as compared to former one. Along with the above sulfone, organophosphorus, triterpene and volatile oil was another major group of compounds found in *G. spicata* fruit extract. However, dialkylthioether, saturated fatty acids, phthalate, terpenes were found in *G. talbotii* fruits. Both the species revealed the presence of some unique compounds across all species such as Phospholane {organophosporus}; Nonane, 1,9-dibromo {halogenated hydrocarbon}; 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol {syn: Oleanan-3-ol} a triterpene with insecticidal, anti-inflammatory and anticancer activity (Kutney *et al*., 1993; Parikh *et al*., 2014) were found in *G. spicata* fruit extract and 1,2-Cyclopentanedione a ketone possess antioxidant activity (Alghamdi *et al*., 2018); Propane, 2-methyl-1-(methylthio)- {dialkylthioether} were found in *G. talbotii* fruit extract.

Another southern Western Ghats endemic and high altitudinal species ***G. travancorica*** fruit extract indicated presence of major group of compounds such as sesquiterpenes, glycosides, saturated fatty acids, carboxylic acid esters, terpenes, diterpene, triterpene, phytosterol, polyacetylene, hydrocarbon derivative, carbohydrate derivative, alkyne, amino acid ester. Among them some compounds were unique in nature too. Unique compounds found were dl-Glyceraldehyde dimer {carbohydrate derivative}; 2,4-Hexadiyne-1,6-diol {syn: Diacetylene glycol} an alkyne; Ylangene a sesquiterpene showed anti-inflammatory (Zhang *et al*., 2014), antibacterial and antioxidant (Chen *et al*., 2012; Teng & Lee, 2014) activities; 5-Aminomethylene-6-hydroxy-4-methyl-2-oxo-2,5-dihydro-3-pyridinecarbonitrile useful as colourant in food, dye and cosmetic industries; Benzene, 1-ethoxymethyl-4-tert-butyl- {phenylpropane}; 1,4-Benzenedicarboxylic acid, diethyl ester {carboxylic acid}; Quinic acid a carboxylic acid useful in cosmetics possess antifungal (Ma *et al*., 2010), anticancer (Murugesan *et al*., 2020), neuroprotective (Liu *et al*., 2020), anti-inflammatory and anti-neuropathic (Raafat, 2019) activities; Galactopyranoside, 1-octylthio-1-deoxy- {syn: n-octyl-beta-D-thioglucopyranoside} a glucoside useful as non-ionic detergent for membrane biochemistry; 2,4-Hexadien-1-one, 1,3,5-triphenyl- {lignans}; Undecane, 5-ethyl- {alkane}; 1,2,3-Propanetriol, 1-acetate {fatty acid}; Bis(2-ethylhexyl) phthalate a phthalate useful in personal care product, paints and

resin industries; Octadecanoic acid, ethenyl ester {syn: Vinyl stearate} a fatty acid ester possess antioxidant activity (Jiang *et al*., 2021); a fatty alcohol 1-Heptacosanol showed hypocholesterolemic effect (Singh *et al*., 2006), anti-ulcerative (Awaad *et al*., 2013), antibacterial and antifungal (Awaad *et al*., 2017), antioxidant and antidiabetic (Unnikrishnan *et al*., 2014), anti-proliferative and anti-obesity (Heendeniya *et al*., 2020) activities; 17-Oxo-4-propyl-3,4-seco-5.alpha.-androstan-3-oic acid methyl ester {terpene}; 1-Tetradecen-3-yne {unsaturated hydrocarbon}; an volatile oil Cubedol exhibits antibacterial and cytotoxic activities (Omoruyi & Muchenje, 2017) and Benahorin a coumarin possess cytotoxic effect (Gonzalez *et al*., 1977).

G. wightii the species restricted to the southern Western Ghats displayed presence of several major compounds belongs to sulfone, sesquiterpene, sesquiterpenoid, carboxylic acid ester, volatile oil, saturated fatty acids, glycoside, essential aromatic amino acid, fatty acyl, phytosterol, oxaspiro and phthalate group. The distinct compounds present were Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- {syn: alpha-Bulnesene/ D-guaiene} a sesquiterpenoid used as surfactant, emulsifier in food industry and it showed antagonist, anti-platelet aggregation (Tsai *et al*., 2007), insecticidal (Albuquerque *et al*., 2013), larvicidal (Lima *et al*., 2019) effects; 1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]- {sesquiterpenoid}; Docosane an alkane showed antiviral (Chathuranga *et al*., 2021), antimicrobial, antihyperglycemic and antioxidant (Rizwan *et al*., 2012; El-Kashoury *et al*., 2013) activities; a fatty alcohol Z-2-Octadecen-1-ol useful as plant growth regulator, fragrant, lubricant, additive, emollient in several industries and possess cytotoxicity, antifungal, antioxidant activities; l-Phenylalanine, n-heptafluorobutyryl-, hexadecyl ester {essential aromatic amino acid} and a fatty acyl Z,Z-5,16-Octadecadien-1-ol acetate used as pest control agent possess anticolotis and antiautistic activities.

The common species ***G. xanthochymus*** fruit extract exhibited range of major group of compounds such as saturated and unsaturated fatty acids, organic acid ester, terpenes, diterpene, steroids. Some unique compounds were also found viz. 13,27-Cycloursan-3-one {triterpenoid}; Pentanoic acid, 4-oxo- {syn: Levulinic acid} which is saturated fatty acid showed activities against cancer cell lines and influenza A

(Aydin *et al*., 2013); an unsaturated fatty acid cis-Vaccenic acid {syn: cis-11-octadecenoic acid/ omega-7-fatty acid} exhibits anticancer, anti-inflammatory activities (Field *et al*., 2009; Jacome-Sosa *et al*., 2016); Pregnane-3,11,20-trione, (5.alpha.)- {steroid} and an unique steroid 4,22-Stigmastadiene-3-one possess antioxidant and cytotoxic activities (Pornprasertpol *et al*., 2015).

5.4 Evaluation of anti-oxidant and anti-cancer (cell line screening) activities of fruit extracts of *Garcinia* species

Garcinol is the main medicinal component from the genus *Garcinia* responsible for antioxidant, anti-inflammatory and anti-cancer properties (Liu *et al*., 2015). Several previous studies showed medicinal importance of few commonly used species such as *G. indica*, *G. mangostana* and *G. hombroniana* w.r.t. different activities (Yamaguchi *et al*., 2000; Chen *et al*., 2008; Darji *et al*., 2010; Magadula *et al*., 2010; Jamila *et al*., 2013). However, in present study antioxidant and anti-cancer activities were investigated for all *Garcinia* species including rare and endemic species to the Western Ghats. Most of the species showed good antioxidant and anti-cancer potential however, earlier reported spp. showed intermediate activity.

5.4.1 Anti-oxidant activity

Recently, the research on plant based natural antioxidants has become more popular as plant contains several phytochemicals such as flavonoids, polyphenols, vitamins, volatile components etc. Considering the importance of antioxidants as nutraceuticals for many health benefits (Droge, 2002; Lee *et al*., 2004; Valko *et al*., 2007), different assay methods are developed, analysed and reported for several plant extracts in previous literature. The efficiency of these assay methods are varying from each other in terms of solvents used to prepare plant extract and for assay, reagents and their concentrations used for assay, experimental conditions and analytical evaluation methods. Concerning about solvent, higher absorbance was found to occur in methanolic solution which implies better sensitivity (Sharma & Bhat, 2009). Low values for the extent of reduction were found using other solvents as compared to methanol (Guo *et al.*, 2001).

In the present study antioxidant potential of methanolic fruit rind extracts of all *Garcinia* species was determined using DPPH and ABTS assays.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay has become quite popular among all assays due to easy and highly sensitive performance. Due to delocalization of spare electron DPPH is stable free radical and it governs the occurrence of purple colour around 520 nm wavelength. As DPPH free radical reacts with hydrogen atom from an antioxidant, reduced form of DPPH is getting generated and antioxidant effect on DPPH is proportional to decolourisation of purple colour to colourless solution (Molyneux, 2004; Moon & Shibamoto, 2009; Pisoschi & Negulescu, 2011).

In the present study L-ascorbic acid was used as reference standard and it showed free radical scavenging activity against DPPH with IC_{50} value 7.18 µg/ml. However, IC_{50} values for DPPH radical scavenging using L-ascorbic acid reported previously were 11.8 µM (Sharma & Bhat, 2009), 56 µM (Kano *et al*., 2005), 629 µM (Ricci *et al*., 2005) and 3.2 µg/ml (Anu Aravind *et al*., 2016).

Methanolic extracts of mature fruit rinds of all the *Garcinia* species from the study area showed remarkable scavenging potential against DPPH free radical (Table 4.25). Among them an endemic and rare species *G. pushpangadaniana* showed highest antioxidant potential with 15.01 µg/ml IC_{50} value however, it was found lowest in an endemic species *G. wightii* with 49.52 µg/ml IC_{50} value. Previous report on leaf extract of nine *Garcinia* species from the Western Ghats showed 9.04 and 16 µg/ml IC_{50} values for the species *G. pushpangadaniana* and *G. wightii* respectively. Among the nine species, *G. talbotii* showed highest antioxidant potential with 2.8±0.6 µg/ml IC_{50} value (Anu Aravind *et al*., 2016). Another report on antioxidant potential of five wild fruits of Manipur, India showed DPPH radical scavenging activities for *G. xanthochymus* and *G. pedunculata* fruits with 92.08 and 493.30 µg/ml IC_{50} values respectively (Sharma *et al*., 2015). Some previous studies showed antioxidant potential of different species and their plant parts and expressed in terms of IC_{50} values such as *G. mangostana* fruit peel 9.19 mg/ml (Ling *et al*., 2010), pericarp 52.62 µg/ml (Zarena & Sankar, 2009), hull 20.50 µg/ml (Kosem *et al*., 2007), fruit peel, rind and aril 28.9, 20.7 and 1347 µg respectively (Naczk *et al*., 2011); *G. pedunculata* fruit pulp 5 mg/ml (Mudoi *et al*., 2012) and 47.03 µg/ml (Gogoi *et al*.,

2012); *G. xanthochymus* fruit pulp 428.60 µg/ml (Gogoi *et al*., 2012), seed oil 120 µg/ml (Manohar *et al*., 2014); *G. morella* fruit pulp 303.63 µg/ml (Gogoi *et al*., 2012); *G. gummi-gutta* fruit rind 36.20 µg/ml (Subhashini *et al*., 2011); *G. indica* fruit rind 50.34 µg/ml (Minakshi *et al*., 2015) and *G. imbertii* leaf 218.11 µg/mL, stem bark 274.24 µg/mL (Rajkumar *et al*., 2015). These studies indicate that different plant parts of different *Garcinia* species have antioxidant potential.

ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] assay is decolourisation assay to check potential of lipophilic and hydrophilic antioxidants both. It is spectrophotometric method applied to evaluate antioxidant potential of natural products such as cereals (Hu *et al*., 2007; Abdel-Aal *et al*., 2008), beverages (Pellegrini *et al*., 2003; Piccinelli *et al*., 2004; Gómez-Ruiz *et al*., 2007; Rivero-Perez *et al*., 2008), essential oils (Zhao *et al*., 2008; Erkan *et al*., 2008), fruits and vegetables (Thaipong *et al*., 2006; Sun *et al*., 2007) and solutions of pure substances (Rice-Evans *et al*., 1995; 1996).

Trolox was used as a reference standard in the present study and it unveiled antioxidant potential using ABTS assay with 20.98 µg/ml IC_{50} value. All *Garcinia* species methanolic fruit extracts showed significant antioxidant activity using ABTS assay however, the species which are endemic to southern Western Ghats namely *G. imbertii*, *G. rubro-echinata*, *G. gamblei* and *G. pushpangadaniana* showed high antioxidant activity which is almost close to reference standard trolox (Table 4.26). All these species showed presence of polyphenols such as flavonoids, phenolic acids, tannins and coumarins; vitamins and carotenoids during phytochemical profiling due to which they demonstrated high antioxidant activity (Sies *et al*., 1992; Stahl & Sies, 2003; Manach *et al*., 2004; Pandey & Rizvi, 2009; Tsao, 2010; Fiedor & Burda, 2014).

Among all the *Garcinia* species fruit rind extract of *G. travancorica* showed highest antioxidant potential while it was lowest in *G. talbotii* species extract using ABTS assay. Previous studies on some species and their different plant parts from the genus *Garcinia* showed antioxidant potential with $ABTS^{\cdot+}$ radical scavenging such as *G. mangostana* peel 41.85 mg/ml (Ling *et al*., 2010), pericarp 31.25 µM (Zarena & Sankar, 2009); *G. oblongifolia* branch 0.64 mg/ml (Li *et al*., 2016); *G. xanthochymus*

pericarp 54.29 µg/ml (Gogoi *et al*., 2015); *G. lanceaefolia* pericarp 58 µg/ml (Gogoi *et al*., 2015); *G. parvifolia* fruit peel 22.6 mg, fruit flesh 32.7 mg (Hassan *et al*., 2013) and *G. imbertii* leaf 198.74 µg/mL, stem bark 232.14 µg/mL (Rajkumar *et al*., 2015) with IC_{50} values.

Recently, the synthetic antioxidants are substituted by natural ones which lead to the applications of plant derived antioxidants. Some of the examples include hydroxyl radical scavenging of curcumin (*Curcuma longa*) with 2.3 µM (Ruby *et al*., 1995); peroxynitrite-induced lipid peroxidation of eugenol (*Eugenia caryophyllus*) with 13.1 µM (Chericoni *et al*., 2005); superoxide scavenger for piperine (*Piper nigrum*) with 1.82 mM, lipid peroxidation with 1.23 mM (Reddy & Lokesh, 1992); DPPH radical scavenging for gingerol and paradol (*Zingiber officinale*) with 0.64 µg/ml (Surh, 2002; Stoilova *et al*., 2007); LDL oxidation for theaflavins (black tea) with 5-40 µM/L (Leung *et al*., 2001); ferric reducing power for black fermented tea with 132-654 µM/g, oolong semifermented tea with 233-532 µM/g, green non-fermented tea with 272-1144 µM/g (Benzie & Szeto, 1999) IC_{50} values.

5.4.2 Anti-cancer (cell line screening) activity

For over a decade, several plant-derived anticancer agents are available such as the vinca bisindole alkaloids, the epipodophyllotoxin analogs, the taxanes and the camptothecins (Chabner *et al*., 2005; Cragg *et al*., 2005; DeVita *et al*., 2008). Also, a dietary polyphenol from *Curcuma longa* L. namely curcumin is considered a potential anticancer agent (Kelloff *et al*., 1997). Chemopreventive agents garcinol and curcumin were investigated for their effectiveness against HL-60 cells which showed strong inhibition. Also, it was found that garcinol was more effective to induce apoptosis as compared to curcumin (Pan *et al*., 2001). Differential effects on histone and p53 modifications in MCF-7 breast tumour cells were found after garcinol and curcumin treatment (Collins *et al*., 2013).

Garcinol, an isoprenylated benzophenone revealed *in vitro* and *in vivo* anticancer effects in various types of cancers. The anticancer effect of garcinol is triggered by mechanisms such as apoptosis induction, cell growth inhibition, proliferation of cell cycle arrest and prevention of cancer cell metastasis (Liu *et al*.,

2015). The apoptosis induction was found using garcinol and its two reaction products (GDPPH-1 & GDPPH-2) on HL-60 cells which indicated chemo preventive ability of garcinol and its reaction products (Sang *et al*., 2001).

Breast cancer is the most common cancer and according to WHO data, worldwide 6,27,000 women died from this disease in 2018 alone. Nutrition can promote anticancer effects. Hence two nutritional supplements such as gamma-tocotrienol an unsaturated vitamin E analogue and Spirulina were evaluated for their anticancer effects in breast cancer (Subramaiam *et al*., 2021). MCF-7 human breast cancer treated with tocotrienol caused differential expression of genes which may be potential targets for the treatment of breast cancer (Ramdas *et al*., 2011).

The cytotoxic activity of ethanolic fruit rind extracts of different *Garcinia* species were evaluated against four human cancer cell lines namely A-549 (lung), HeLa (cervix), HT-29 (colon) and MCF-7 (breast) using SRB protein stain assay. SRB (sulforhodamine-B) protein assay is one of the most widely used method for screening of *in vitro* cytotoxicity and it is based on the measurement of cellular protein content as SRB dye binds to amino acid residues. SRB assay is always compared with another widely used MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay which is dependent on cell metabolic activity. However, the SRB assay is independent on cellular metabolic activity, delivered higher sensitivity and better linearity with cell number (Keepers *et al*., 1991; Vichai & Kirtikara, 2006).

During the present study, fruit extracts of five *Garcinia* species namely *G. travancorica, G. wightii, G. imbertii, G. morella* and *G. mangostana* showed strong inhibition against four human cancer cell lines with less than 10 µg/ml GI_{50} value which is considered as effective as pure compounds. Also, another two species namely *G. spicata* and *G. pushpangadaniana* were found effective against HT-29 & MCF-7 and A-549 & HeLa cancer cell lines with less than 10 and 20 µg/ml GI_{50} values respectively. *G. hombroniana* species extract revealed potent activity against two cell lines such as HeLa and MCF-7. Another species *G. cowa* indicated effect against breast cancer cell line MCF-7. Remaining species extracts including widely used *G. indica* and *G. gummi-gutta* showed very less activity against above cell lines

(Tables 4.27-4.30). However, in earlier studies both species extract shown potent anticancer activities against different cancer cell lines such as *G. indica* fruit rind extract against HL-60 with 9.42 µM (Pan *et al*., 2001); fruit rind extract against COLO-320-DM, MCF-7 & WRL-68 with 0.08 µM, 0.12 µM & 6.43 µM respectively (Kumar *et al*., 2007) and *G. gummi-gutta* fruit extract against N-2A with 0.23 mg/ml (Mazzio & Soliman, 2009) IC_{50} values.

Earlier reports indicated cytotoxicity against different cell lines by using crude extract and isolated compounds of different *Garcinia* species and their plant parts and expressed as IC_{50}/ LD_{50} values such as *G. morella* leaf extract against KB, MCF-7, COLO, CaCO2, PA-1 & WRL-68 with 1, 40, 10, 3, 0.9 & 8 µg/ml respectively (Garg *et al*., 2007); *G. mangostana* fruit pericarp extract against A-431 & SK-MEL-28 with 5.07 & 6.89 µg/ml respectively (Wang *et al*., 2011) and fruit pericarp extract against A549, Hep-G2 & MCF-7 with 12.5, 20.0 & 13.8 µM respectively (Zhou *et al*., 2011); *G. rubro-echinata* leaf extract against Hep G2 & H9C2 with 100 µM & 50 µM respectively (Menon *et al*., 2018); *G. cowa* stem bark extract against T47D with 5.10 µg/ml (Husni *et al*., 2015) and twig extract against HT-29, HCT116 with 5.1, 5.4 µM (Xu *et al*., 2010); *G. nervosa* leaf extract against HeLa, MCF-7 & HT-29 with 149, 111 & 103 µg/ml and bark extract against HeLa, MCF-7 & HT-29 with 66, 22.5 & 57.5 µg/ml respectively (Seruji *et al*., 2013); *G. subelliptica* pericarp extract against MCF-7, Hep 3B & HT-29 with 6.8, 6.3 & 11.2 µg/ml respectively (Wu *et al*., 2005); *G. xanthochymus* fruit extract against SW-480 with 7.85-185 µM (Baggett *et al*., 2005); *G. livingstonei* fruit extract against HCT-116, HT-29 & SW-480 with 5-10, 5-10 & 18-25 µM respectively (Yang *et al*., 2010); *G. hanbury* resin extract against K562/S with 0.11-2.41 µg/ml (Han *et al*., 2006) and *G. travancorica* leaf extract against A-431 & HeLa with 150 & 82.80 µg/mL respectively (Anu Arvind & Rameshkumar, 2016).

5.5 Molecular Phylogeny

Phylogenetics is the study of the evolutionary history and relationships of biological taxa using DNA sequence data (Savolainen & Chase, 2003). Along with phylogeny, DNA sequence and DNA fingerprinting data was used to study population dynamics (Fay & Krauss, 2003), hybridization (Clarkson *et al*., 2004), species

delimitation (Richardson *et al*., 2003) and identification (Bradford & Barnes, 2001), also recently to authenticate the herbal drug products (Joshi *et al*., 2004; Yip *et al*., 2007; Sucher & Carles, 2008; Balachandran *et al*., 2015). Kress *et al*. (2005) first proposed identification of flowering plants using nuclear internal transcribed spacer region and plastid regions as DNA barcodes. Consortium for the Barcode of Life (CBOL) plant working group (2009) compared the performance of seven plastid DNA regions and recommended 2-locus combination of *rbc*L and *mat*K to identify land plants for routine use.

Internal transcribed region of nuclear ribosomal DNA (ITS) is most widely used in plant molecular systematics since it showed high levels of interspecific sequence divergence and biparentally-inherited (Hillis & Dixon 1991; Baldwin *et al*., 1995). Yao *et al*. (2010) proposed ITS2 locus of nuclear ribosomal DNA should be used as universal DNA barcode in eukaryotes since it possess number of valuable characteristics to design universal primers. In addition ITS2 region was validated as a novel DNA barcode to identify medicinal plant species, broader range of plant taxa and their related species (Chen *et al*., 2010).

In the present study, molecular phylogeny using ITS dataset revealed segregation of all *Garcinia* species based on morphological characters and endemism (Fig. 4.40). Four endemic species such as *G. talbotii*, *G. indica*, *G. gamblei* and *G. pushpangadaniana* clustered together in subclade-I consists broad, long and leathery leaves except *G. indica*. The later two species were newly described from higher elevations in southern Western Ghats were grouped together. Since *G. gamblei* is allied species to *G. pushpangadaniana*, both species shown similarities w.r.t. habit, leaves, branchlets, latex, stigma lobes (Shameer *et al*., 2017). Another subclade-II showed clustering of seven species, which includes four endemic species. Among them species *G. wightii* and *G. imbertii* were clustered together as both species distribution is restricted to the southern Western Ghats and indicates morphological similarities w.r.t. leaf; also, these species formed cluster with the species *G. morella* which has similarities with *G. wightii* w.r.t. fruit (Singh, 1993). Two varieties namely *G. gummi-gutta* var. *gummi-gutta* and *G. gummi-gutta* var. *conicarpa* were nested in two different sister subclades. Later species clustered together with north-east Indian species *G. lanceafolia* since both possess lanceolate leaves. The species *G. spicata*

and *G. xanthochymus* both are non-endemic species but present in India in natural form and possess smooth, yellow coloured mature fruits were found to occur in same cluster along with other Old world species (Gogoi *et al*., 2016; Dutta *et al*., 2017; Palkar *et al*., 2017). Another clade showed presence of a cultivated species *G. mangostana* with its progenitor *G. malaccensis* as discussed in earlier studies based on morphological and ITS sequence data (Yapwattanaphun & Subhadrabandhu, 2004; Nazre, 2014) and ITS, trnL and accD-psaL phylogeny (Abdullah *et al*., 2012).

The barcode region *rbc*L, proved to be useful in many plants since it is easy to amplify, sequence, align and indicated good recovery and sequence quality (Hollingsworth, 2011). *rbc*L molecular phylogeny revealed similar segregation pattern like ITS phylogeny except some differences (Fig. 4.41). Two endemic species *G. wightii* and *G. gummi-gutta* var. *gummi-gutta* were nested in distinct clade apart from Indian species clade since both possess similarities w.r.t. leaf shape, flower colour etc. (Singh, 1993). Also it was observed that the species *G. spicata* present in India and extended distribution upto Sri Lanka was clustered together with *G. pushangadaniana* in a subclade along with other endemic species due to similarities in habit, leaf, petiole, pedicel and other characters (Sabu *et al*., 2013).

The plastid genome *mat*K is the most rapidly evolving coding region which possesses highest species discrimination as compared to *rbc*L region but, not easily retrievable using universal primers (Hilu & Liang, 1997; Hollingsworth *et al*., 2011). Molecular phylogenetic tree constructed using *mat*K dataset revealed almost similar tree topology as using ITS dataset with few differences in species segregation (Fig. 4.42). Four endemic Western Ghats species clustered together in one subclade with non-endemic species *G. spicata* as similar clustering pattern was found in *rbc*L phylogeny. However, *G. rubro-echinata* and *G. imbertii* were clustered together in distinct subclade since distribution of both species restricted to high altitudes of the southern Western Ghats (Maheshwari, 1964; Singh, 1993; Nayar *et al*., 2014; Shameer *et al*., 2016).

Recent years, multigene combined phylogenetic analyses were used commonly to address the evolution in several plants. In most of the cases single-gene analyses did not provide sufficient phylogenetic resolution which lead to conflicting

results due to limited number of alignable nucleotides (Capesius & Bopp, 1997; Nei *et al*., 1998; Poe & Swofford, 1999; Nickrent *et al*., 2000). Thus, combined approaches were proved to be helpful w.r.t. phylogenetic accuracy and resolution due to large number of characters (Hillis, 1996). During the present study combined analyses with ITS+*rbc*L (Fig. 4.43) and ITS+*mat*K (Fig. 4.44) were carried out which showed almost similar tree topologies as individual analyses of each locus.

Chapter 6

Conclusion

- In the present study all 16 *Garcinia* species with 3 varieties from the Western Ghats of India were collected, documented, characterised taxonomically and deposited in the national herbarium (MH). Out of 16 species, 9 species with 3 varieties were found to be endemic to the region.

- Ecological niche modeling study was carried out using MaxEnt and BIOCLIM algorithms predicted that niche of the species *G. indica* is predominantly found to be Konkan regions of Maharashtra, Goa & Karnataka and the precipitation & temperature are the major factors which influence the habitat suitability for the species. As this species is commercially exploited, the outcome of present study will help in large-scale cultivation and sustainable utilization of the species.

- HPLC quantification of anti-obesity compound HCA and anti-cancer compound garcinol among 10 species mature fruit rind extracts studied and it revealed the highest quantity of HCA and garcinol in *G. indica* and *G. pushpangadaniana* fruit extracts respectively which indicates the potential for medicinal value.

- HPLC analysis using different developmental stages of *G. indica* fruit rind extracts were carried out and recorded the highest amount of HCA and garcinol in developmental Stage-I (15 DAF) and III (45 DAF) respectively. Hence, harvesting of fruits in these stages may be useful for pharmaceutical preparations.

- Among the 10 species studied using matured fruits and among the different developmental stages of *G. indica* fruits, the HCA content was found to be the

highest in Stage-I of *G. indica* fruits which indicates that the young fruits are highly acidic in nature.

- GC-MS study revealed presence of several common and unique phytoconstituents across mature fruit rind extracts of all *Garcinia* species which shown the potential chemical compounds for various medicinal and commercial importance. Among the species studied, *G. travancorica* revealed with highest number of unique phytochemicals indicating its distinctiveness.

- Both DPPH and ABTS assays revealed the high anti-oxidant potential of most of the *Garcinia* species fruit rind extracts from the Western Ghats as compared to earlier reported studies using different species and their plant parts from the genus. Among the species studied *G. pushpangadaniana* and *G. travancorica* showed the highest anti-oxidant activity as per DPPH and ABTS assays respectively. Some of the southern Western Ghats endemic species fruit extracts showed high potential as equivalent to standard antioxidants. *G. indica* and *G. gummi-gutta* extracts showed intermediate anti-oxidant activity. Hence, along with well-known *Garcinia* species and their plant parts, other species and fruit rind extracts from the genus could also be used in medicines and pharmaceutical industries.

- *G. pushpangadaniana* fruit extract shown positive correlation between garcinol content and its antioxidant potential. However, the other *Garcinia* species studied, no significant positive correlation could be established between garcinol content and its antioxidant potential may be due to the presence of other phytochemical compounds which requires further study.

- Mature fruit rind extracts of five species namely *G. travancorica, G. wightii, G. imbertii, G. morella* and *G. mangostana* showed highest inhibition against four cancer cell lines indicating the potential for the preparation of anti-cancer drug.

- Phylogenetic studies of the genus *Garcinia* revealed the clustering of species based on endemism, morphological characteristics and distributional patterns.

Chapter 7

Summary

The genus *Garcinia* L. belongs to the family Clusiaceae includes ca. 260 species worldwide. It is one of the most important genera due to its medicinal and economic uses. The species namely *G. indica*, *G. gummi-gutta* and *G. mangostana* are the most important species in the genus with proven medicinal and economic values. Among them former two species are an endemic and found to occur abundant in the Western Ghats as natural populations as well as in cultivated form. Along with these species, the study on other *Garcinia* species including an endemic species to the Western Ghats needs to be understood thoroughly to acquire their potential benefits.

During this study, the entire Western Ghats area was explored based on previous literature and details from various herbaria available w.r.t. the genus *Garcinia*. A total of 16 *Garcinia* species with 3 varieties were documented and collected from the Western Ghats of India. Among them, 9 species with 3 varieties namely *G. gamblei*, *G. gummi-gutta* var. *conicarpa*, *G. gummi-gutta* var. *gummi-gutta*, *G. gummi-gtta* var. *papilla*, *G. imbertii*, *G. indica*, *G. pushpangadaniana*, *G. rubro-echinata*, *G. talbotii*, *G. travancorica* and *G. wightii* are endemic to the region. Overall 8, 10 and 16 species with 3 varieties were documented in natural as well as cultivated form from the northern, central and southern Western Ghats respectively. Taxonomic identification of all the species was carried out and herbarium specimens of all species were deposited at Madras Herbarium (MH), Coimbatore, Southern Circle, Botanical Survey of India. Also, taxonomic ambiguities and occurrence of the species *G. spicata* and *G. talbotii* in peninsular India was worked out using morphological and anatomical characters.

An endemic species *G. indica* (Kokum) possess high medicinal values due to which it is overexploited in the Western Ghats especially in Konkan region. Considering the importance and due to overexploitation, the prediction of potential distribution and understanding the climatic factors responsible for the niche of the species in wild including areas for its cultivation was worked out based on the species

occurrence data and environmental variables using predictive modeling approaches in MaxEnt and BIOCLIM. The prediction using both MaxEnt and BIOCLIM algorithms revealed that the species habitat suitability is in the Konkan regions of Maharashtra, Goa and Karnataka. The precipitation and temperature were the major factors defining the distribution of the species. As the species is medicinally important and major industries are involved in processing, developing and marketing various Kokum products; the large-scale cultivation is essential. Further, the output of the study may help in conservation and sustainable utilization of the species by using appropriate management programs.

Methanolic extracts of dried mature fruit rinds of different *Garcinia* species and fruit rinds from six developmental stages of *G. indica* fruits were prepared using Soxhlet extraction method and concentrated extracts were used for the further quantification studies. During the study, extraction yield was also calculated and highest extraction yield was found using *G. livingstonei* fruit rinds however, lowest using *G. morella* fruit rinds. Dried fruit rinds of developmental Stage-VI (90 DAF) and IV (60 DAF) of *G. indica* generated highest and lowest extraction yield respectively. Different methanolic extracts were subjected to TLC for confirmation of HCA in the samples and it was confirmed based on colour & Rf values of the spots on chromatogram.

HPLC methods were developed and validated to quantify the bioactive compounds HCA and garcinol separately from different methanolic fruit rind extracts. The parameters used for method validation were found within acceptable limits. Among selected species extracts, HCA quantity was observed highest in *G. indica* and lowest in *G. mangostana* using HPLC method. Also, garcinol quantification was carried out which showed highest amount of garcinol in *G. pushpangadaniana* and lowest in *G. hombroniana*. HCA and garcinol quantity was also estimated in different developmental stages of *G. indica* fruit rinds. Highest amount of HCA and garcinol was found in developmental Stage-I (15 DAF) and III (45 DAF) respectively. However, lowest quantities of HCA and garcinol were observed in developmental Stage-IV (60 DAF).

GC-MS analysis was carried out to check phytochemical profile of all collected *Garcinia* species. Concentrated methanolic extracts from dried mature fruit rinds of all *Garcinia* species revealed presence of range of common as well as unique phytochemicals in major and minor quantities. Among all these compounds most common and major phytochemicals were saturated and unsaturated fatty acids and their esters, carboxylic acids and their esters, phenols and their derivatives, terpenes, organic acids and their esters, hydrocarbons, alkanes, sesquiterpenes, phthalates, chloroform, oxaspiro compounds, organosilane compounds, terpenoids, phytosterol, furan, sulfone, tetraprinol, aromatic essential oils, glycosides, flavonoids, alkaloids, polyols, fatty alcohol, pyrimidines, diazine derivatives, pyran, steroid, triterpenoid, alkanol etc. across all the species. All common as well as unique phytochemicals exhibits potential for various medicinal and commercial applications.

Anti-oxidant activity of different fruit rind methanolic extracts was evaluated using DPPH and ABTS assay methods. Among the species studied with former assay, the highest anti-oxidant potential was shown by *G. pushpangadaniana* followed by *G. morella*, *G. hombroniana*, *G. xanthochymus*, *G. imbertii* fruit extracts and the least was recorded in *G. wightii*. However, with ABTS assay the highest anti-oxidant activity was observed in *G. travancorica* extract which was higher than the standard anti-oxidant trolox. Also, few species namely *G. mangostana*, *G. imbertii*, *G. rubro-echinata* and *G. gamblei* showed anti-oxidant potential equivalent to reference standard. The least inhibition was recorded in *G. talbotii* extract. The highly used species *G. indica* and *G. gummi-gutta* fruit extracts displayed intermediate anti-oxidant activity with both assays.

For anti-cancer cell-line screening activity, dried fruit rind extracts of all *Garcinia* species were used to check inhibition against four human cancer cell-lines namely A-549 (lung), HeLa (cervix), HT-29 (colon) and MCF-7 (breast). Among all the species extracts, five species extracts namely *G. travancorica, G. wightii*, *G. imbertii*, *G. morella* and *G. mangostana* showed strong inhibition against all four cancer cell lines which was found as effective as pure compounds. Along with these species extracts other species extracts such as *G. spicata*, *G. pushpangadaniana*, *G. hombroniana*, *G. cowa* revealed potent activity against these cancer cell lines with values comparable to pure compounds and crude extracts. Remaining species extracts

including widely used *G. indica* and *G. gummi-gutta* showed very less activity against above cell-lines.

Molecular phylogenetic study of all *Garcinia* species including newly discovered species *G. gamblei* and *G. pushpangadaniana* from the entire Western Ghats was carried out to check species relationship. For the study, nuclear region (ITS) and chloroplast gene (*rbc*L, *mat*K) sequences amplified using universal primers were used to construct maximum likelihood phylogenetic tree. Molecular phylogeny carried out using nuclear and chloroplast genes individually as well as combined analysis showed segregation of all the Western Ghats species along with Old World species. It revealed clustering of all the Western Ghats *Garcinia* species based on an endemism, morphological characters and distribution pattern.

Chapter 8

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APPENDIX

Appendix 1: RPMI 1640 medium

Ingredients	Concentration (mM)
Inorganic salts	
Calcium nitrate	0.42
Magnesium sulphate anhydrous	0.41
Potassium chloride	5.33
Sodium bicarbonate	23.81
Sodium chloride	103.45
Sodium phosphate dibasic anhydrous	5.63
Amino acids	
Glycine	0.13
L-Arginine	1.15
L-Asparagine	0.38
L-Aspartic acid	0.15
L-Cystine dihydrochloride	0.21
L-Glutamic acid	0.14
L-Glutamine	2.05
L-Histidine	0.10
L-Hydroxyproline	0.15
L-Isoleucine	0.38
L-Leucine	0.38
L-Lysine hydrochloride	0.29
L-Methionine	0.10
L-Phenylalanine	0.09
L-Proline	0.17
L-Serine	0.29
L-Threonine	0.17
L-Tryptophan	0.02
L-Tyrosine disodium salt	0.11

L-Valine	0.17
Vitamins	
Choline chloride	0.02
D-Biotin	8.20
D-Calcium pantothenate	5.24
Folic acid	0.002
Niacinamide	0.008
Pyridoxine hydrochloride	0.004
Riboflavin	5.32
Thiamine hydrochloride	0.003
Vitamin B12	3.69
i-Inositol	0.19
para-Amino benzoic acid (PABA)	0.007
Other components	
D-Glucose	11.11
Glutathione (reduced)	0.003
Phenol red sodium salt	0.01

Appendix 2: CTAB lysis buffer (PL1)

Chemicals	Concentration (%)
Sodium chloride	5 - <10

Appendix 3: Binding buffer (PC)

Chemicals	Concentration (%)
Guanidine hydrochloride	24 - <36
Ethanol	35 - <55

Appendix 4: Washing buffer (PW1)

Chemicals	Concentration (%)
Guanidine hydrochloride	36 - <50
2-propanol	20 - <35

Appendix 5: TBE buffer

Chemicals
Tris-HCl
Boric acid
0.5 M EDTA

Appendix 6: Gel loading dye

Chemicals
Bromophenol blue
Sucrose
EDTA
SDS

RESEARCH ARTICLES PUBLISHED

1. **Rutuja S. Palkar**, M. K. Janarthanam and S. Krishnan. 2017. Taxonomic identity and occurrence of *Garcinia spicata* and *G. talbotii* (Clusiaceae) in Peninsular India. *Rheedea* 27(2): 143-151. (https://dx.doi.org/10.22244/ rheedea.2017.27.2.28) (**Scopus indexed journal**) (IF: 0.13).

2. **Palkar R. S.** and Sellappan K. 2019. Genetic diversity between and within the natural populations of *Garcinia indica* (Thouars) Choisy: A high value medicinal plant from Northern Western Ghats of India using ISSR markers. *Journal of Applied Research on Medicinal and Aromatic Plants* 15. (https://doi.org/10.1016/j.jarmap.2019.100219) (**Scopus indexed journal**) (IF: 1.96).

3. **Palkar R. S.**, Janarthanam M. K. and Sellappan K. 2020. Prediction of potential distribution and climatic factors influencing *Garcinia indica* in the Western Ghats of India using ecological niche modeling. *National Academy Science Letters* 43(6): 585-591. (https://doi.org/10.1007/s40009-020-00918-y) (**Scopus indexed journal**) (IF: 0.548).

PAPERS PRESENTED AT INTERNATIONAL AND NATIONAL CONFERENCES/ SEMINARS/ SYMPOSIA

1. **Oral presentation**: **Palkar Rutuja S**., M. K. Janarthanam and S. Krishnan. Phylogeny of *Garcinia pushpangadaniana* and other *Garcinia* species from Western Ghats of India using molecular data. 24th Annual Conference of Indian Association of Angiosperm Taxonomy (IAAT) and International Conference on "Trends in Plant Systematics (TIPS)" organized by Department of Plant Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India during 31st October to 2nd November, 2014.

2. **Poster presentation**: **Palkar Rutuja S**., Janarthanam M. K. and S. Krishnan. Preliminary Phylogenetic studies of genus *Garcinia* from the Western Ghats of India using molecular data. National Seminar on "New Frontiers in Plant Science and Biotechnology" organized by Department of Botany, Goa University, Goa, India during 29 to 30th January, 2015.

3. **Poster presentation**: **Palkar Rutuja S**., M. K. Janarthanam and S. Krishnan. Distributional status and economic importance of Genus *Garcinia* from the Western Ghats of India. "4th Bharatiya Vigyan Sammelan & Expo" organized by Vijnana Bharati and Goa University, Goa, India during 5 to 8th February, 2015.

4. **Poster presentation**: **Palkar Rutuja S.**, Janarthanam M. K. and S. Krishnan. Antioxidant potential of different *Garcinia* species from the Western Ghats of India. National Conference of Young Researchers on "New Frontiers in Life Sciences & Environment" organized by Faculty of Life Sciences & Environment, Goa University, Goa, India during 16 to 17th March, 2017.

5. **Poster presentation**: Diksha Damodar Naik, **Rutuja S. Palkar** and S. Krishnan. Anatomical bases for identification of *Garcinia* L. species from the Western Ghats of India. National Conference of Young Researchers on "New Frontiers in Life Sciences & Environment“ organized by Faculty of Life Sciences & Environment, Goa University, Goa, India during 16 to 17th March, 2017.

6. **Oral presentation: Rutuja S. Palkar**, M. K. Janarthanam and S. Krishnan. Quantification of bio-active compounds and antioxidant potential of some *Garcinia* species from the Western Ghats of India. National Symposium on “Current Trends in Plant Sciences” organized by Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India during 22nd to 23rd February, 2018.

7. **Oral presentation: Rutuja S. Palkar** and S. Krishnan. Antioxidant potential of different *Garcinia* species from the Western Ghats of India. National Seminar on “New Vistas in Botany” organized by Department of Botany, Goa University, Goa, India during 13 to 14th February, 2020.

HANDS-ON WORKSHOPS/ TRAINING COURSES ATTENDED

1. Workshop on "**Molecular Phylogenetics**" organized by Centre for Ecological Sciences, Indian Institute of Sciences, Bangalore, India during 1st to 5th August, 2014.

2. Workshop on "**Scientific Writing and Effective Communication**" organized by Department of Biotechnology, Goa University, Goa, India in association with University of Florida during 5 to 6th January, 2015.

3. Workshop on "**Open Source and Mobile GIS**" organized by Department of Computer Science and Technology, Goa University, Goa in association with Open Geospatial consortium, India on 23rd January, 2016.

4. Workshop on "**Plant Molecular Farming-III**" organized by Department of Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India in association with St. George's University of London, UK. UKIERI sponsored Indo-UK joint workshop during 27 to 28th July, 2016.

5. Workshop on "**Chemical Analysis of Biomolecules and Computation**" organized by Department of Biotechnology, Goa University, Goa, India during 23rd to 24th November, 2017.

6. Hands-on training on "**Mendeley Reference Manager**" organized by Department of Biotechnology, Goa University, Goa, India on 24th October, 2019.

CONFERENCES/ SEMINARS/ SYMPOSIA/ OTHER EVENTS ATTENDED/ PARTICIPATED

1. National Seminar on "**New Frontiers in Plant Science and Biotechnology**" organized by Department of Botany, Goa University, Goa, India during 29 to 30th January, 2015 participated as Registration committee member and assisted in overall organization of the seminar.

2. National Symposium on "**Asian Mycological Congress- Challenges to Plant Pathologists under Changing Disease Scenario**" organized by Goa University, Goa, India during 5 to 7th October, 2015 participated as Registration committee member.

3. National Conference of Young Researchers on "**New Frontiers in Life Sciences & Environment**" organized by Faculty of Life Sciences & Environment, Goa University, Goa, India during 16 to 17th March, 2017 participated as Registration committee member.

4. An event "**Nobel exhibition- Nobel media prize series, India 2018**" organized by Nobel Media AB, Department of Biotechnology, Govt. of India and Department of Sciences and Technology, Govt. of Goa, India during 1st to 28th February, 2018 participated as Volunteer.

5. Seminar on "**Biosafety and Intellectual property rights**" organized by Department of Biotechnology, Goa University, Goa, India attended on 20th March, 2018.

6. The seminar on "**Novel Vistas in Plant Sciences**" organized by Department of Botany, Goa University, Goa, India attended on 25th January, 2019.

7. The National Seminar on "**New Vistas in Botany**" organized by Department of Botany, Goa University, Goa, India during 13 to 14th February, 2020 as Technical committee member.

ADDITIONAL ACTIVITIES

1. Worked as a **Junior Research Fellow** (JRF) in the research project entitled "**Large scale screening for high Garcinol and hydroxycitric acid, *in vitro* culture and molecular characterization of *Garcinia* species from the Western Ghats of India**" sponsored by **CSIR [No.: 38(1348)/12/ EMR-II dated 16/10/2012], New Delhi** from 16th August 2013 to 31st March 2016.

2. Working as a **Senior Research Fellow** (SRF) in the research project entitled "**Histochemical and Biotechnological Characterization of High Value Medicinal Plants *Annona muricata* and *Rauvolfia verticillata* from the Western Ghats of India**" sponsored by **CSIR [No.: 38(1471)/18/EMR-II dated 04/04/2018], New Delhi** from 16th October 2018 to till date.

3. **Manuscript reviewed** for the journal "**Plant Science**" entitled "Advances and challenges in medicinal plant breeding" during 2020.

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2019/Tech./713 दिनांक/Date: 9 July 2019

सेवा में / To

Dr. S. Krishnan
Professor
Department of Botany, Goa University
Taleigao Plateau, Goa – 403 206

विषय/Sub.: Acknowledgement of herbarium specimens deposited at MH - reg.
निर्देश/Ref.: This office Lr. BSI/SRC/5/23/2019/Tech./485 dtd. 12.06.2019.

महोदय/Sir,

With reference to your E-mail dated 20.06.2019, I am sending herewith list of herbarium specimens for incorporation in the Madras Herbarium (MH), as desired, after making necessary corrections. Kindly acknowledge the receipt.

S.N.	Species Name	Family	Collector	Field No.	Acc. No. of MH
1	*Garcinia xanthochymus* Hook. f.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	11	177804
2	*G. indica* (Thouars) Choisy (male sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	147	177805
3	*G. wightii* T. Anderson	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	209	177806
4	*G. morella* (Gaertn.) Dest.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	227	177807
5	*G. livingstonei* T. Anderson	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	241	177808
6	*G. spicata* (Wight & Arn.) Hook. f.) (Male sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	251	177809
7	*G. hombroniana* Pierre	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	270	177810
8	*G. pushpangadaniana* Sabu *et al.*	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	271	177811
9	*G. imbertii* Bourd.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	278	177812
10	*G. gamblei* Shameer *et al.*	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	279	177813
11	*G. gummi-gutta* var. *conicarpa* (Wight) N.P. Singh	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	281	177814
12	*G. cowa* Roxb. exDC.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	291	177815
13	*G. rubro-echinata* Kosterm.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	293	177816
14	*G. travancorica* Bedd.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	295	177817
15	*G. indica* (Thours) Choisy (Female sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	299	177818
16	*G. gummi-gutta* var. *gummi gutta* (L.) N. Robson	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	304	177819
17	*G. mangostana* L.	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	306	177820
18	*G. talbotii* Raizada ex Santapau (Male sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	308	177821
19	*G. talbotii* Raizada ex Santapau (Bisexual sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	313	177822
20	*G. spicata* (Wight & Arn.) Hook. f.) (Female sp.)	CLUSIACEAE	Rutuja S. Palkar & S. Krishnan	321	177823

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

डॉ सी मुरुगन/Dr. C. Murugan
वैज्ञानिक 'ई' एवं कार्यालय अध्यक्ष /
Scientist 'E' & Head of Office

Rheedea
Vol. 27(2)
143–151
2017
ISSN: 0971-2313
https://dx.doi.org/10.22244/rheedea.2017.27.2.28

Taxonomic identity and occurrence of *Garcinia spicata* and *G. talbotii* (Clusiaceae) in peninsular India

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Abstract

Garcinia talbotii Raizada ex Santapau is often misidentified as *G. spicata* (Wight & Arn.) Hook.f. in the regional Floras of northern Western Ghats. This paper clearly delimits these two species with unambiguous morphological and anatomical characters to facilitate authentic identification.

Keywords: Distribution, *Garcinia spicata, Garcinia talbotii,* Misidentification, Northern Western Ghats

Introduction

Garcinia L. (Clusiaceae) is one of the medicinally and economically important genera due to the presence of two major bioactive compounds namely hydroxycitric acid and garcinol. The genus consists of *c*. 260 species, and distributed predominantly in tropical regions, especially Asia (Mabberley, 2017). In India, the genus is represented by 39 species and 7 varieties, of which 18 species and 5 varieties are endemic (Maheshwari, 1965; Singh, 1993; Sabu *et al*., 2013; Nayar *et al*., 2014; Sarma *et al*., 2016; Shameer *et al*., 2017). *Garcinia talbotii* Raizada ex Santapau is one of the species endemic to Western Ghats that produces edible fruits which can be used in culinary preparations. Quantitative HPLC-PDA analysis of benzophenones and biflavonoids in eight species of *Garcinia* showed highest amount of xanthochymol in *G. spicata* (Wight & Arn.) Hook.f. (Acuna *et al*., 2012). It also yields timber, used in construction and yellow-coloured pigment from bark and young fruits as a dye.

A critical examination of herbarium specimens of *G. spicata* housed at BSI, FRLH, CALI, MH, SUK and TBGT revealed that most of them were misidentification of *G. talbotii.* Similarly, scrutiny of descriptions of *G. spicata* in the earlier published Floras (Santapau, 1967; Almeida, 1990; Naithani *et al*., 1997; Godbole & Das Das, 2000; Yadav & Sardesai, 2002; Ganeshaiah *et al*., 2012) that recorded the occurrence of this species in the northern Western Ghats revealed that the diagnostic features provided for *G. spicata* fall well within the circumscription of *G. talbotii*. Inclusion of northern Western Ghats, especially Goa and Maharashtra under the distribution range of *G. spicata* by other workers (Singh, 1993; Nayar *et al*., 2014) is also based on the above mentioned earlier works. Further, an examination of fresh field collections corroborates that *G. spicata* does not occur in northern Western Ghats. The present communication provides detailed description, differentiating characters (**Table 1**), photographs depicting morphological and anatomical features and images of type specimens to facilitate the accurate identification of both these species.

Garcinia spicata (Wight & Arn.) Hook.f., J. Linn. Soc., Bot. 14: 486. 1875; Brandis, Indian Trees: 49. 1906; Gamble, Fl. Madras: 53. 1915; Maheshw., Bull. Bot. Surv. India 6: 112. 1965; N.P. Singh in B.D. Sharma & Sanjappa, Fl. India 3: 125. 1993. *Xanthochymus spicatus* Wight & Arn., Prodr. Fl. Ind. Orient. 1: 102. 1834. Type: *s.loc.*, *s.die*, *R. Wight* 346A (Syn, E, image!). **Figs. 1,3,5**

Tree, dioecious, 5–20 m high; trunk straight; bark smooth, olive green to brown; latex white; branchlets 4-angled, angles flat. Leaves broadly elliptic-oblong or ovate, 9–22 × 4–8 cm, base rounded or obtuse, margins entire, apex retuse, obtuse or acute-acuminate, thick-coriaceous, smooth, dark green; midrib prominent, raised; lateral veins 15–18 pairs, prominent on both surfaces, slightly curved, parallel, clearly visible in dry specimens; petioles to 2 cm long with stem-clasping ligule [anatomy: incurved open vascular strand with large gap]. Flowers in male plant on both spicate and fasciculate inflorescence; spicate inflorescence 2–11-flowered, 1.4–9.5 cm long; fasciculate inflorescence with 5–15 short pedicellate flowers arising directly from the stem. Female



Fig. 1. Syntype of ***Garcinia spicata*** (Wight & Arn.) Hook.f. (≡ *Xanthochymus spicatus* Wight & Arn. [*R. Wight* 346A, E00179065] @Royal Botanic Garden, Edinburgh.



Fig. 2. Holotype of ***Garcinia talbotii*** Raizada ex Santapau (*G. ovalifolia* var. *macrantha* Vesque) [*W.A. Talbot* 3713, BSI0000000003] @Botanical Survey of India, Western Regional Centre, Pune.

flowers solitary or in pairs. Flowers pale green or whitish yellow; sepals 4 or 5, suborbicular, 2.5–3 mm long, less than half the length of petal, margins ciliate, membranous, pubescent outside; petals 5, obovate, 6–7 mm long, concave, membranous, pale green or whitish yellow. Male flowers: Pedicels 1–5 mm long; stamens in 5 spathulate bundles, each bundle with 8–10 stamens, long-clawed, opposite petals; filaments very short, free. Rudimentary pistil absent. Female flowers: Pedicels 1.2–3.5 cm long; staminodes 5, small with weak anthers; ovary oblong, 2–4 locular; style short, *c*. 1 mm long; stigma 3–5-lobed, peltate, spreading, 2–3 mm in diameter, persistent, thick at peltate region, black. Fruits broadly globose, subglobose or depressed-globose, shallowly angular, to 4 cm in diameter; immature fruits with mild ridges and furrows, pale green; ripened fruits smooth, yellow, with mild unpleasant odour; pulp acidic, bitter taste; seeds 1–3, broadly oblongoid, 1.5–2.5 × 1–1.3 cm, dark brown, black when dried.

Flowering & fruiting: January–August.

Distribution: India (Andhra Pradesh, Karnataka, Kerala and Tamil Nadu) and Sri Lanka.

Specimens examined: INDIA, **Andhra Pradesh**: Nellore district, Sriharikota forest, May 1904, *s.coll.* 3034, 3035 (MH). **Karnataka**: Agumbe district, Barakana, Balehalli, 19.5.1960, *R. Sundara Raghavan* 62720 (BSI). **Kerala**: Kasargod district, Cherpady, Uduma, 12.3.1993, *A. Nazarudeen* 16408 (TBGT); Kollam district, Karingalodathodu area, Tenmalai range, 7.12.1961, *K.N. Subramanian* 77529 (BSI); Kottayam district, Koruthode, 200 m, 12.11.1995, *Jomy Augustine* 16906 (CALI); Puthupalli, *s.die*, *G.S. Puri* 36572 (BSI); Palakkad district, Mukkali forest, 7.3.1975, *E. Vajravelu* 46240 (MH); Pathanamthitta district, Pamba, 200 m, 12.2.1994, *Jomy Augustine* 13370 (CALI); Pamba, 18.3.1994, *A. Nazarudeen* 19587 (TBGT). **Tamil Nadu**: Cuddalore district, Puthupet, 12°03′ N, 79°52′ E, 5 m, 18.5.2017, *R.S. Palkar & S. Krishnan* 5177 (Goa University Herbarium); Pudukkottai district, Pudukkottai, November 1909, *C.A. Barber* 8498 (MH); Villupuram district, Marakkanam, 3.6.1907, *C.A. Barber* 8321 (MH); Marakkanam, Kurumbaram Reserve Forest, 12°12′ N, 79°56′ E, 10 m, 5.5.1995, *J.D.H., P.B. & W.F.G.* 02757 (FRLH). SRI LANKA, **Ceylon Southern Province**: Hambantota district, Yala, Talgasmankade, 26.4.1973, *L.H. Cramer* 4124 (MH).

Garcinia talbotii Raizada ex Santapau, Rec. Bot. Surv. India 16(1): 14. 1960; Maheshw., Bull. Bot. Surv. India 6: 113. 1965; N.P. Singh in B.D. Sharma & Sanjappa, Fl. India 3: 127. 1993; M.R. Almeida, Fl. Maharashtra 1: 88. 1996; Godbole & Das Das in N.P. Singh & Karthik., Fl. Maharashtra 1: 277. 2000. *G. ovalifolius* (Roxb.) Hook.f. ex T. Anderson var. *macrantha* T. Anderson in Hook.f., Fl. Brit. India 1: 269. 1874. *G. spicata* (Wight & Arn.) Hook.f. var. *macrantha* Vesque in A. DC., Monogr. Phan. 8: 311. 1893; T. Cooke, Fl. Bombay 1: 79. 1901. *G. malabarica* Talbot, J. Bombay Nat. Hist. Soc. 11: 234, 1897, non Desr. 1792. *Xanthochymus ovalifolius* J. Graham, Cat. Pl. Bombay: 26. 1839, non Roxb. 1832.

Garcinia spicata auct. non (Wight & Arn.) Hook.f. 1875: T. Cooke, Fl. Bombay 1: 78. 1901; Santapau, Rec. Bot. Surv. India 16(1): 14. 1967, incl. spec. *Blatter* 624; S.M. Almeida, Fl. Savantwadi 1: 54. 1990, incl. spec. *G.A. Gammie* 15025; H.B. Naithan *et al*., Forest Fl. Goa: 65. 1997; Godbole & Das Das in N.P. Singh & Karthik., Fl. Maharashtra 1: 276. 2000.

Type: INDIA, **Karnataka**: North Kanara district, Garrsoppah Ghat, 20.1.18??, *W.A. Talbot* 3713 (holo, BSI0000000003, image!). **Figs. 2,4,5**

Tree, dioecious, 6–18 m high; trunk straight; bark smooth, olive green; latex sticky, turning into brownish; branches angular, slender, twiggy; branchlets 6-angled, angles concave. Leaves elliptic-oblong or ovate, 5–18 × 4–9 cm, base rounded, margins entire, apex obtuse, retuse or acute-acuminate, coriaceous, shiny, dark green; midrib prominent; lateral veins 16–18 or more pairs, distinct, parallel; petioles to 1.8 cm long [anatomy: curved open vascular strand with no gap or minute gap]. Spikes in male plant on old wood, numerous, 5–8 mm long; spikes absent in female plant. Flowers 3–12, on fasciculate inflorescence in both male and female plant. Flowers creamy-white, white to greenish-yellow; sepals 5, orbicular, concave, green, 3–5 mm across; petals 5, orbicular or suborbicular, concave, *c*. 10 × 8 mm, pale green or whitish yellow. Male flowers: Pedicels 5–8 mm long; stamens in 5 spathulate bundles, thick, 8–12 anthers on each phalange, reddish, opposite petals; filaments very short, free. Rudimentary pistil absent. Female flowers: Pedicels 0.4–1 cm long; staminodes 5, thin, flattened bundles, 4 or 5 anthers on each phalange, similar to male flower; ovary globose, 2 or 3-locular; stigma 3-lobed, 1–3 mm in diameter, narrow at base, persistent, thick, black. Fruits globular, 4–6 cm; immature fruits smooth, without any ridges and furrows, dark green; ripened fruits smooth, yellow, with bad odour; pulp acidic, bitter; seeds 1 or 2, narrowly oblongoid, 2.5–3 × 1.2–1.5 cm, brown.

Flowering & fruiting: January–June.

Distribution: India (Goa, Karnataka, Kerala and Maharashtra). **Endemic**.

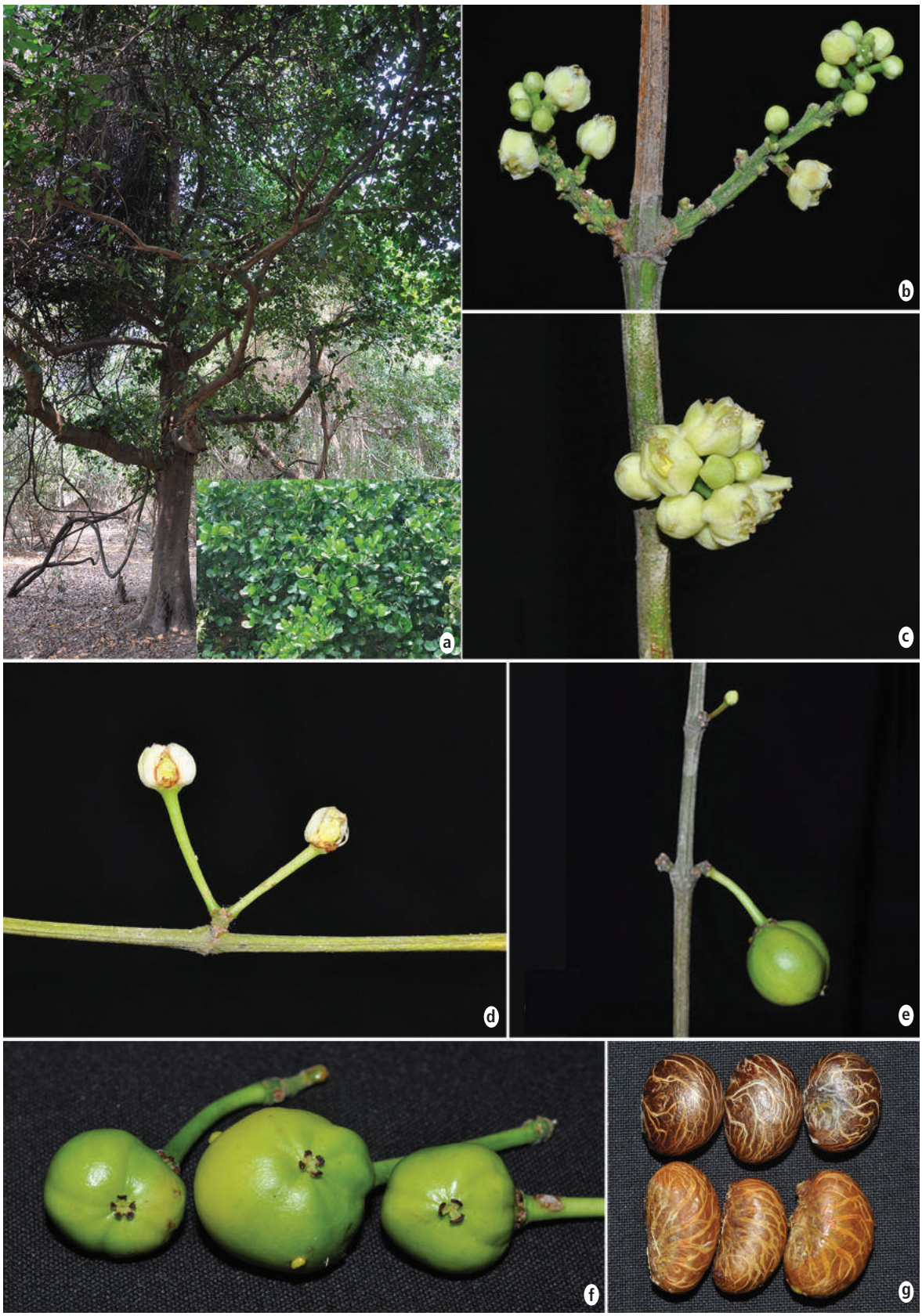


Fig. 3. *Garcinia spicata* Hook.f.: **a.** Habit (Insert: close-up of branch); **b.** Spicate inflorescence (male); **c.** Fasciculate inflorescence (male); **d.** Female flower; **e.** Immature fruit with ridges and furrows; **f.** Fruits with 3–5 stigmatic lobes; **g.** Matured and dried seeds: upper row – *G. spicata*, lower row – *G. talbotii*.

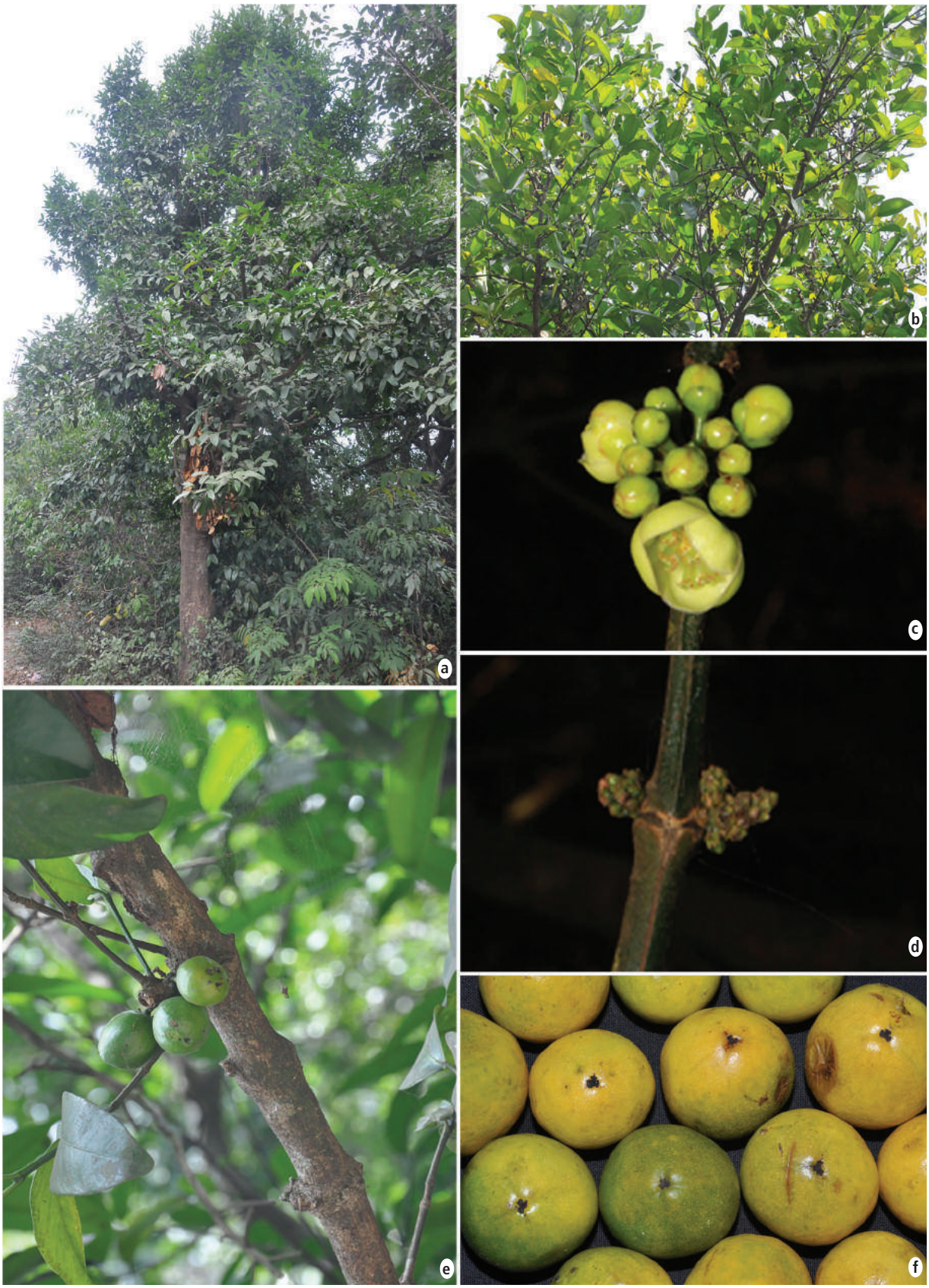


Fig. 4. *Garcinia talbotii* Raizada ex Santapau: **a.** Habit; **b.** Branch showing numerous spikes in male plant; **c.** Fasciculate inflorescence (male); **d.** Close-up of spike; **e.** Immature fruit with smooth surface; **f.** Mature fruits showing 3-lobed stigma.

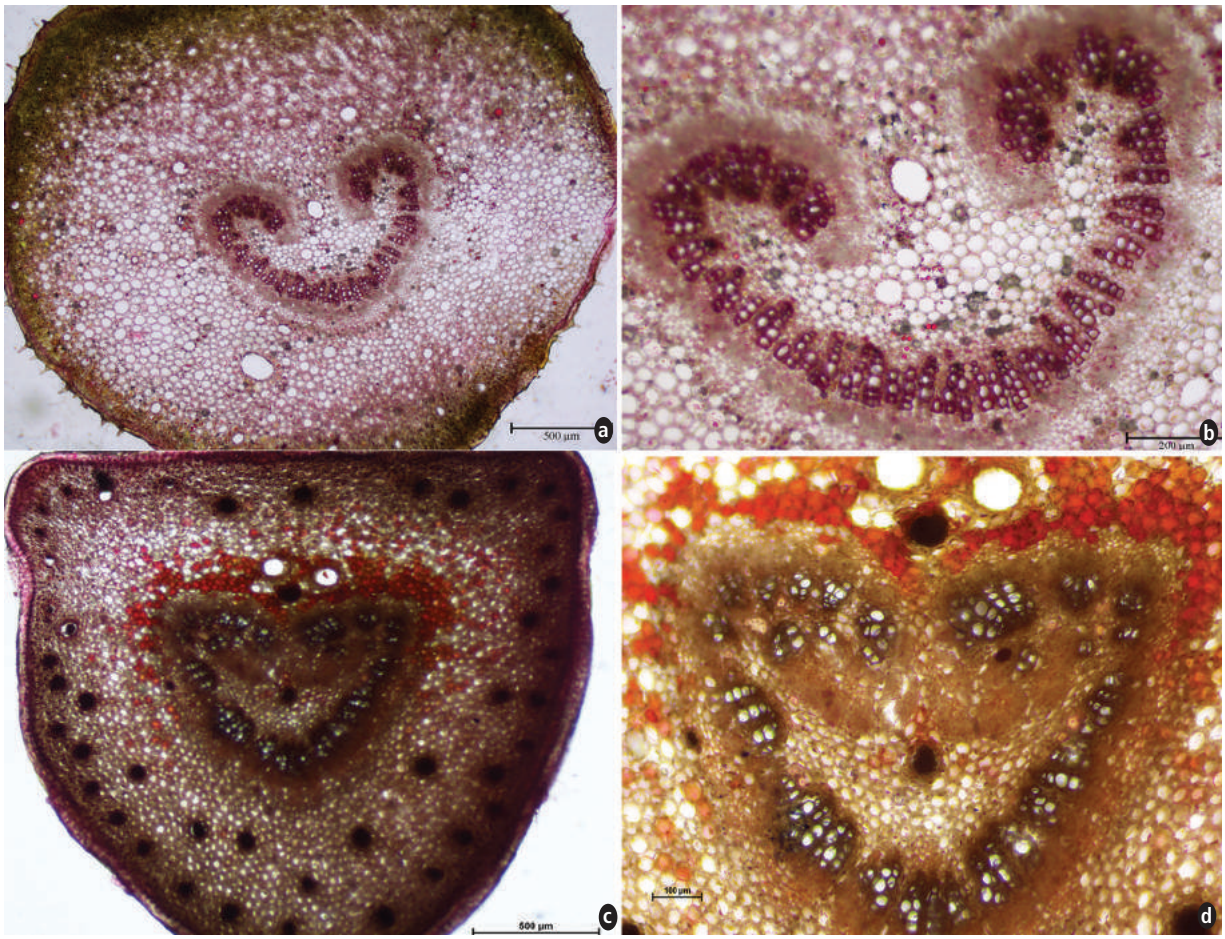


Fig. 5. T.S. of petioles stained with safranin: a,b. *Garcinia spicata* showing incurved open vascular strand with large distinct gap; c,d. *Garcinia talbotii* with curved vascular strand with very minute gap.

Table 1. Comparative key characteristics of *Garcinia spicata* and *G. talbotii*

Characters	***G. spicata***	***G. talbotii***
Branchlets	4-angled; angles flat	6-angled; angles concave
Flowers in male plant	Spicate and fasciculate. Spikes 1.4–9.5 cm long	Fasciculate. Also arranged in numerous pseudospikes of 0.5–0.8 cm long
Flowers in female plant	Solitary or in pairs	Fasciculate
Length of pedicels in female flowers	1.2–3.5 cm	0.4–1 cm
Ovary	2–4-locular	2 or 3-locular
Stigmatic lobes	3–5	3
Fruits	Shallowly angular	Smooth
Seeds	Broadly oblongoid, 1.5–2.5 × 1–1.3 cm, dark brown, black when dry	Narrowly oblongoid, 2.5–3 × 1.2–1.5 cm, brown
Vascular strand in petiole	Incurved open with large gap	Curved open with no gap or minute gap

Specimens examined: INDIA, **Goa**: North Goa district, Vagheri hills, 19.12.1997, *Vaishali C. Joshi* 1296 (Goa University Herbarium). **Karnataka**: Belgaum district, Hemadga, *s.die*, *Nilesh V. Malpure* NVM 3766 (SUK); Sada plateau, 15°40.18′ N, 74°7.10′ E, 806 m, 8.5.2016, *R.S. Palkar* 5172 (Goa University Herbarium). **Kerala**: Palakkad district, Nelliampathy ghat, 29.6.1914, *S.T. Dunn* s.n. (MH Acc. No. 3018). **Maharashtra**: Kolhapur district, Amba, *s.die*, *Priti Mahekar* SUK–392 (SUK); Gajapur, *s.die*, *Milind Sardesai* 831 (SUK); Manoli, Amba, 11.5.1904, *R.K. Bhide* s.n. (BSI); Palghar district, Shirgaon, 21.4.1957, *L.A. Vasavada* 17031 (BSI); Pune district, Auhpe forest, 23.3.1907, *G.M. Ryan* 1751* (BSI); Bhimashankar, 25.10.1956, *L.A. Vasavade* 8575* (BSI); Bhimashankar, 13.6.1961, *K.P. Janardhanan* 72256 (BSI); Bhimashankar, 7.6.1962, *K.P. Janardhanan* 76642 (BSI); Bhimashankar, *s.die*, *J.A.V.* 9314* (BSI); Choura hill, Bhimashankar, 24.2.1961, *K.P. Janardhanan* 69201 (BSI); Choura hill, Bhimashankar, 22.4.1961, *K.P. Janardhanan* 72108 (BSI); Choura hill, Bhimashankar, 4.4.1962, *K.P. Janardhanan* 76581 (BSI); Bhimashankar, 2.2.1999, *S.R. Yadav* 5845 (SUK); Raigad district, Matheran, 2.11.1907, *H.P. Paranjpe* s.n.* (BSI Acc. No. 6751); Matheran, 9.11.2006, *S.C. Majumdar & S.K. Das Das* 192443 (BSI); Sudagarh Fort, Pali, 9.3.1979, *M.J. Kothari* 156423 (BSI); Satara district, Khandala, 21.3.1903, *G.A. Gammie* 16163* (BSI); Koyna, 23.11.1978, *R.K. Kochhar* 157811 (BSI); Jaigad, Koyna, 11.2.1979, *R.K. Kochhar* 158332* (BSI); Kusavade, May 1995, *M.P. Bachulkar Cholekar* 20040* (SUK); Vasota, April 1993, *M.P. Bachulkar Cholekar* 5550 (SUK); Sindhudurg district, Amboli, 28.4.1902, *G.A. Gammie* 15025* (BSI); Amboli, 15°56.28′ N, 73°59.99′ E, 821 m, 27.5.2015, *R.S. Palkar* 5173 (Goa University Herbarium); Chaukul, Amboli, 9.11.1965, *B.G. Kulkarni* 107936 (BSI). (*Specimens of *G. talbotii* wrongly identified as *G. spicata*).

Acknowledgements

Authors gratefully acknowledge the financial support provided by the CSIR (No. 38(1348)/13/EMR-II), New Delhi, to carry out the research work. They are thankful to the Scientist/Professor-in-Charge of BSI, CALI, FRLH, MH, SUK and TBGT, for providing permission to consult the herbarium specimens. Authors are thankful to the authorities of BSI and E for permission to reproduce type images. Authors are grateful to: Dr. N. Parthasarathy, Professor, Department of Ecology and Environmental Sciences, for his help in the collection of *G. spicata*, Dr. Sharad S. Kambale, Assistant Professor, Department of Botany, MVP Samaj's Arts, Commerce and Science College, Nashik, Maharashtra, for his help and suggestions and Ms. Diksha D. Naik, Department of Botany, Goa University, for her help in anatomical studies.

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Received: 13.8.2017
Revised and Accepted: 29.12.2017

Journal of Applied Research on Medicinal and Aromatic Plants 15 (2019) 100219

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Genetic diversity between and within the natural populations of *Garcinia indica* (Thouars) Choisy: A high value medicinal plant from Northern Western Ghats of India using ISSR markers

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ARTICLE INFO

Keywords:
Germplasm conservation
Kokum
Medicinal properties
Polymorphism
PopGene
Sustainable utilization

ABSTRACT

The present study reveals genetic diversity between and within the natural populations of *Garcinia indica* which is an endemic and medicinally important tree species from the Western Ghats of India. During the study extensive field survey was carried out to different parts of the region and collected leaf samples from 11 different locations to assess genetic diversity between populations. To understand the genetic diversity within the population, leaf samples were collected from seven different locations (accessions) spread over the single large population at Amboli, Maharashtra. Out of 21 ISSR primers screened, 12 primers produced reproducible, clear banding pattern. A total of 127 bands amplified for the samples analyzed in between the populations, among them 119 were polymorphic and percent polymorphism varied from 80 to 100% per primer. Genetic diversity within the population shown a total of 85 amplified bands, out of which 74 were polymorphic and polymorphic percentages varied from 60 to 100% per primer. The other genetic diversity parameters revealed high diversity and differentiation in both between populations (percent polymorphism = 93.87%, h = 0.3196, na = 1.82, ne = 1.37, I = 0.41, Ht = 0.26 and G_{ST} = 0.81) and within population (percent polymorphism = 84.80%, h = 0.36, na = 1.88, ne = 1.56, I = 0.48, Ht = 0.32 and G_{ST} = 0.86). Dendrogram revealed two main clusters for both between and within the natural populations based on altitudinal variation and geographical distribution of the species which is significantly supported by Mantel correlation test for geographic and genetic distance among populations. *G. indica* is highly exploited for various traditional and pharmaceutical preparations and have demand in the national and international markets, the understanding of genetic diversity among the natural populations especially in Konkan regions where plants are abundant will help in conservation of potential germplasm and utilization for breeding programme for large scale cultivation by farmers.

1. Introduction

Garcinia indica (Thouars) Choisy is an endemic plant species to the Western Ghats of India belongs to the family Clusiaceae. It is one of the medicinally important tree species occurs particularly in the Konkan region of Maharashtra, Goa, Karnataka and some extent to Kerala. *G. indica* is popularly known as Kokum in Konkan regions and it is also cultivated in these States. It is commercially exploited due to the presence of two pharmaceutically important bioactive compounds such as hydroxycitric acid (HCA) and garcinol which have antiobesity and anticancerous activities respectively (Ramachandran, 2014). Besides this, Kokum has been used in several culinary preparations such as solkadhi, amsol, aagal, juice, squash, butter as edible oil; industrial applications of Kokum in confectionery, cosmetics and medicinal uses such as antioxidant, antibacterial, antifungal, anti-HIV, anti-inflammatory, antipyretic, anti-Alzheimer's activity (Padhye et al., 2009; Koppad and Shivanna, 2010; Baliga et al., 2011; Ramachandran, 2014). Kokum is propagated through seeds which lead to heterozygosity, along with heterozygous character, cross-pollination and polygamodioecious nature are responsible for variation in habit, branching pattern, leaf morphology, flowering-fruiting season, fruit shape, size and color, thickness of fruit rind and biochemical composition of fruit (Korikanthimath and Desai, 2005; Rawat and Bhatnagar, 2005; Hegde et al., 2010). Many genotypes phenotypically look similar although they are endowed by unique genetic features.

Molecular markers are widely used in plant genome analysis, plant ecological research, evolutionary biology, management and conservation of plant genetic resources, biodiversity, improvement of plants by breeding and genetic engineering techniques (Anolles, 2001). Molecular markers proved to be useful in genetic studies of wild plant

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https://doi.org/10.1016/j.jarmap.2019.100219
Received 9 April 2019; Received in revised form 15 August 2019; Accepted 19 August 2019
Available online 07 September 2019
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population by answering speciation; genotypic diversity within and between populations; gene flow characteristics and levels of outcrossing (Lee et al., 2001). DNA based markers are advantages over other marker systems because they are neutral and independent of any environmental factors. Regions of microsatellite abundance are source of ISSR markers and microsatellites are proved excellent markers due to high level of allelic variation, co-dominant way of inheritance and potential for automated analysis (Sarwat et al., 2012). ISSR (inter simple sequence repeats) are reliable, fast, simple to use, economic markers. These markers are proved advantages over other markers by much more informative than RAPD (Korbin et al., 2002; Galvan et al., 2003) and possess more polymorphic information content (PIC) than AFLP (Blair et al., 1999).

These are used in medicinal plants such as *Jatropha* species, *Changium smyrnioids* and *Chuanminshen violaceum* to ascertain population genetic structure and molecular characterization (Qiu et al., 2004; Senthil et al., 2009). Earlier studies are reported with other species of *Garcinia* with RAPD and ISSR markers (Mansyah et al., 2010; Parthasarathy et al., 2013; Tharachand et al., 2015). This study presents the comprehensive account of genetic diversity between and within natural populations of *G. indica* using ISSR markers covering the entire Northern Western Ghats of India predominantly Konkan region where the population is very abundant.

2. Material and methods

2.1. Exploration and collection of plant material

The extensive field survey carried out covering the entire Northern Western Ghats of India specifically the Konkan regions where *G. indica* is predominantly found and 11 different natural populations (different locations) were selected randomly. Representative plant leaf samples from each population were used to understand the genetic diversity between populations. To study genetic diversity within population, leaf samples from 7 different plants were collected at different locations (accessions) covering the single large population at Amboli, Sindhudurg district, Maharashtra, India. The characteristics of plants such as habit, floral and fruit morphology are provided in Fig. 1. List of geographical coordinates where leaf samples collected for the study are provided in Tables 1 and 2.

2.2. DNA isolation

Genomic DNA was isolated from leaf samples using CTAB (cetyl trimethyl ammonium bromide) method with partial modifications (Henry, 2001; Parab et al., 2008). Five gram of young, green, fresh leaves from different locations were thoroughly cleaned, cut into small pieces and ground to make fine powder using liquid nitrogen. Powder was suspended in 25 ml of pre-warmed (50–60 °C) extraction buffer (2% CTAB, 2% β-mercaptoethanol, 2 M NaCl, 20 mM EDTA at pH 8.0, 100 mM Tris-HCl at pH 8.0, 4% Polyvinylpyrrolidone). The content was mixed properly, incubated at 60 °C for 45 min, after incubation equal volume of chloroform : isoamyl alcohol (24:1) was added and followed by 20 min centrifugation at 12,000 rpm. Supernatant was collected carefully and precipitated by 0.6 volume of ice cold isopropanol at -20 °C for 2 h. The DNA was pelleted by centrifugation at 12,000 rpm for 20 min and suspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Further the genomic DNA was purified using initially phenol : chloroform mixture (1:1) followed by three to four times chloroform washes by centrifugation at 8000 rpm for 10 min. The supernatant was transferred to fresh tube, added 1/10 volume of 3 M sodium acetate and three volumes of ice cold ethanol and incubated at −20 °C for overnight. Next day pellet was collected after centrifugation at 8000 rpm for 20 min, washed using 1 ml of ethanol (70%) with gentle inversion. Final pellet was dried and suspended in appropriate quantity of TE buffer for further application.

2.3. DNA quality and purity

The quality of DNA was checked on 1% (w/v) agarose gel electrophoresis in 1X TBE buffer (Tris-HCl, Boric acid, 0.5 M EDTA at pH 8.0) using ethidium bromide stain. Also, purity was estimated using UV spectrophotometer by taking absorbance at the wavelength 260 and 280 nm and 260/280 ratio was calculated (A260/A280 = 1.8 for pure DNA).

2.4. ISSR-PCR amplification

Initially 21 inter simple sequence repeat (ISSR) primers (Genei, Bangalore, India) were screened. Out of 21 primers 12 primers produced reproducible and clear banding pattern which were used for further amplification. PCR amplification was carried out using 25 µl of reaction volume containing 50 ng of genomic DNA, 2 µl of primer, 10X Taq assay buffer (Tris at pH 9.0, KCl, Gelatin), 25 mM $MgCl_2$, 100 mM dNTP's (Genei), 5U Taq DNA polymerase (Genei). The amplification reactions were carried out in Mastercycler gradient (Eppendorf, Germany) using reaction conditions as initial denaturation step of 2 min at 94 °C, followed by 35 cycles of 15 s at 94 °C (denaturation), primer specific annealing temperature for 2 min (annealing), 1 min at 72 °C (extension), 10 min at 72 °C (final extension step) and kept in hold at 4 °C till further use. After amplification PCR products were separated using 1.4% (w/v) agarose gel electrophoresis containing ethidium bromide stain at 120 V. Bands obtained were visualized and documented under UV light using gel documentation system (Gel Doc™EZ System, BIO RAD, USA). A 100 bp DNA ladder (Genei) was used as molecular weight marker.

2.5. Data analysis

The reproducible and clear amplified bands were scored as 1 or 0 for presence or absence and binary data matrix was constructed. Various molecular profile characteristics such as total number of bands, number of polymorphic bands and percent polymorphism were calculated. PopGene 1.32 version software (Yeh et al., 1997) was used to generate dendrogram based on Nei's genetic distance by unweighted pair-group method with arithmetic averages (UPGMA) and to determine genetic diversity parameters such as Nei's gene diversity (h), observed number of alleles (na), effective number of alleles (ne), Shannon's information index (*I*), total heterozygosity (Ht) and Nei's differentiation coefficient (G_{ST}). The Mantel correlation between geographical distance and genetic distance was assessed using online version of Spatial Data Calculator (https://digiarhiiv.ut.ee/kalkulaator/Mantel).

3. Results

A total of 21 primers were screened, out of them 12 primers produced reproducible and clear bands. The amplified banding patterns of some of the primers are provided in Fig. 2. Also, the details of amplified primers and their molecular profile characteristics for both between and within populations of *G. indica* are presented in Tables 3 and 4.

3.1. Genetic diversity between populations

Eleven different natural populations of *G. indica* in the States of Goa and Maharashtra predominantly Konkan regions produced a total number of 127 amplified bands using 12 primers, varied from 06 to 15 (average 10.58) bands per primer. Out of total number of bands, 119 were polymorphic found from all samples, varied from 06 to 14 (average 9.91) bands per primer. The average percent polymorphism was 93.87% and it varied from 80% to 100% per primer. Six primers namely PKBT-7, ISSR-7, 857a, ISSR-2, PKBT-8 and PKBT-11 revealed with 100% polymorphism. Primer ISSR-3 showed minimum polymorphism. The Nei's genetic diversity value (h) varied from 0.1902 to

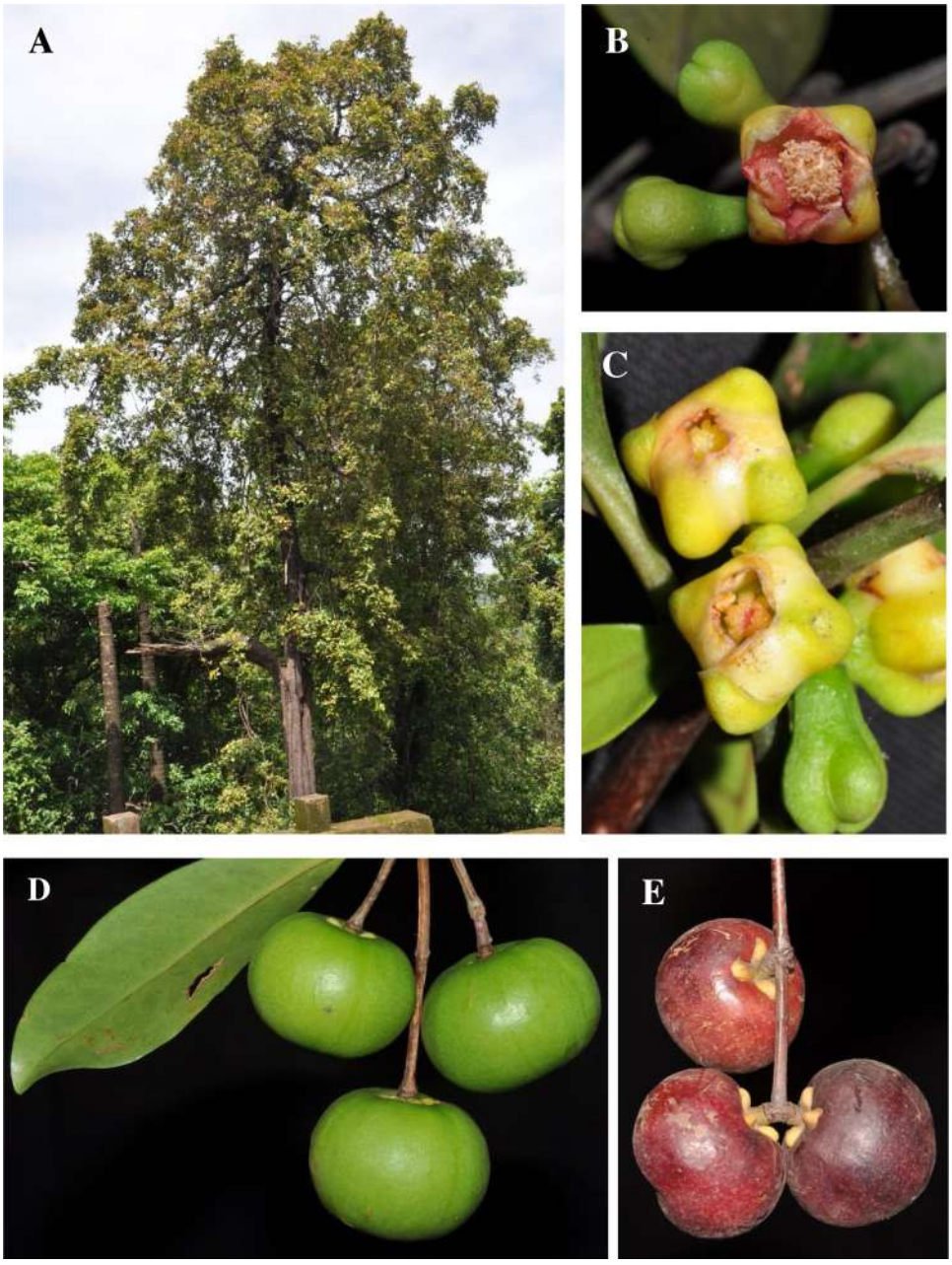


Fig. 1. *Garcinia indica*: A. Habit, B. Male flower, C. Female flowers, D. Immature fruits, E. Mature fruits.

Table 1
Details of accessions of *G. indica* collected from different populations of Goa and Maharashtra, India.

Population	State	Latitude	Longitude	Altitude (m)
Dhamapur	Maharashtra	N 16°01'59.00"	E 73°35'34.03"	44
Insuli	Maharashtra	N 15°51'35.06"	E 73°50'18.90"	29
Ukshi	Maharashtra	N 17°08'00.85"	E 73°26'32.31"	199
Dahibav	Maharashtra	N 16°18'42.39"	E 73°27'12.12"	11
Dabholi	Maharashtra	N 15°52'01.82"	E 73°37'47.49"	66
Narve	Goa	N 15°33'22.19"	E 73°55'29.17"	36
Bhattiwadi	Maharashtra	N 16°32'05.43"	E 73°48'33.98"	209
Sanvordem	Goa	N 15°31'48.89"	E 74°10'25.88"	52
Sangameshwar	Maharashtra	N 17°10'25.07"	E 73°32'34.04"	110
Konem Priol	Goa	N 15°25'19.99"	E 73°59'08.26"	66
Kinner Katta	Goa	N 15°01'02.33"	E 74°08'16.74"	73

Table 2
Details of different accessions of *G. indica* collected from natural population in Amboli, Maharashtra, India.

Accession No.	Latitude	Longitude	Altitude (m)
1	N 15°55'58.92"	E 73°56'27.96"	175
2	N 15°55'58.69"	E 73°56'26.79"	184
3	N 15°55'58.71"	E 73°56'25.48"	181
4	N 15°55'59.26"	E 73°57'36.96"	348
5	N 15°56'16.06"	E 73°57'46.60"	427
6	N 15°56'03.44"	E 73°57'59.45"	463
7	N 15°56'14.80"	E 73°58'16.50"	499

0.7666 with the average value 0.3196 for different populations (Table 5). Other genetic diversity parameters recorded such as observed number of alleles (na) ranged from 1.50 to 2.00, effective number of alleles (ne) ranged from 1.22 to 1.52, Shannon's Information index (*I*) ranged from 0.22 to 0.56, total heterozygosity (Ht) ranged from 0.15 to

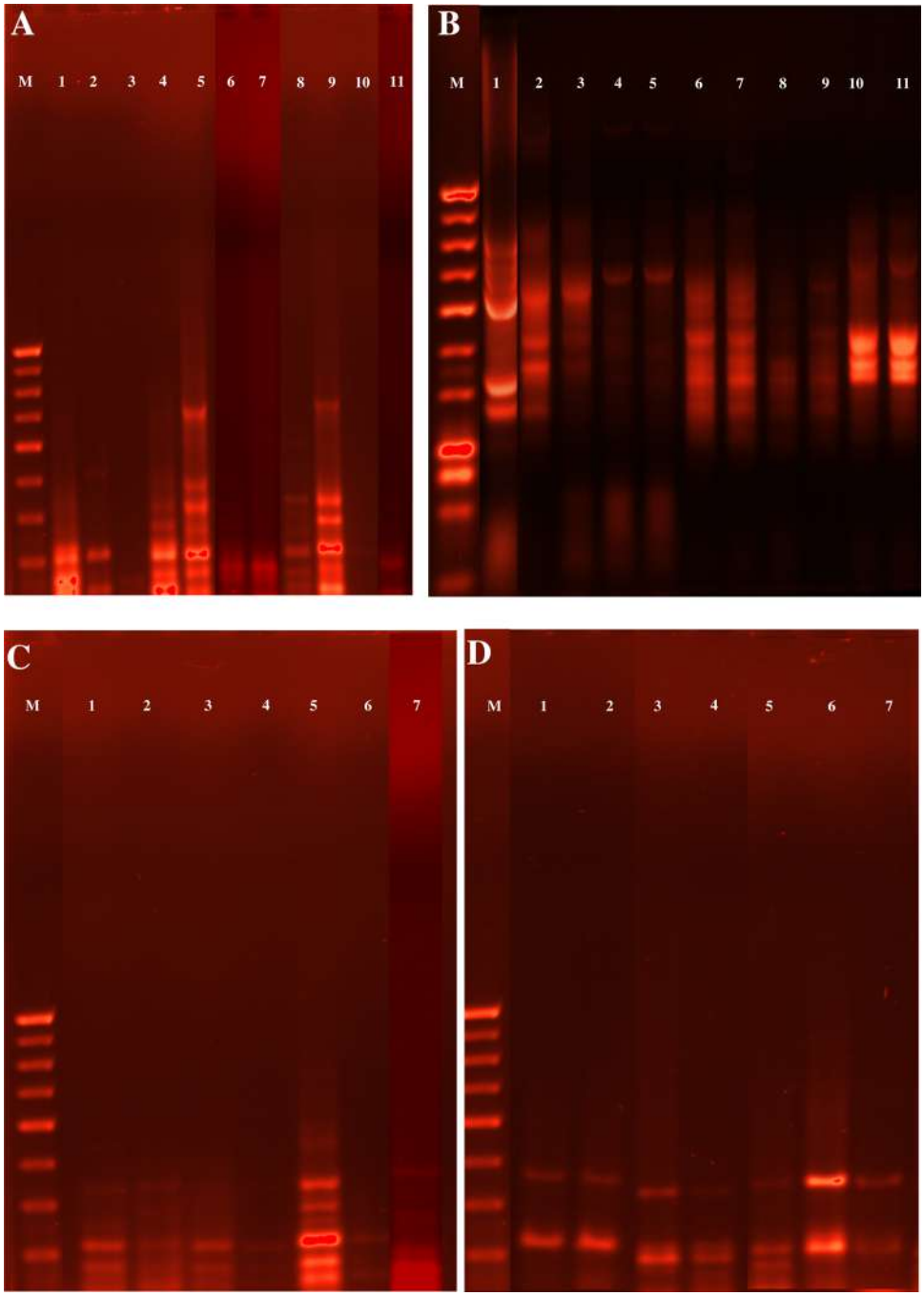


Fig. 2. ISSR amplification profile between and within the populations of *G. indica* using different primers. Between populations: A. Primer 857a; B. Primer ISSR-7 (Lanes: M. Gene Ruler™ 100 bp DNA ladder; 1-11. Different populations: 1. Dhamapur, 2. Insuli, 3. Ukshi, 4. Dahibav, 5. Dabholi, 6. Narve, 7. Bhattiwadi, 8. Sanvordem, 9. Sangameshwar, 10. Konem Priol, 11. Kinner Katta). Within population: C. Primer 857a; D. Primer PKBT-7 (Lanes: M. Gene Ruler™ 100 bp DNA ladder; 1-7. Different accessions: 1. ACCN 1, 2. ACCN 2, 3. ACCN 3, 4. ACCN 4, 5. ACCN 5, 6. ACCN 6, 7. ACCN 7).

0.32. The mean genetic differentiation coefficient (G_{ST}) was 0.81 which indicated high genetic variation was distributed between populations of *G. indica* (Table 7). The Mantel test showed significant correlation (r = 0.44) between geographical distance and genetic distance. The dendrogram constructed using UPGMA method based on Nei's genetic distance is presented in Fig. 3. The dendrogram showed two main clusters of all 11 natural populations. First cluster is divided into two sub-clusters (Fig. 3I-a, I-b) with nine populations while in second cluster with two different populations namely Sanvordem and Konem Priol grouped together (Fig. 3-II).

3.2. Genetic diversity within population

To understand the genetic diversity within the population, leaf samples from seven different plants at various locations (accessions) spread over the single natural population at Amboli region of Maharashtra were generated total number of 85 amplified bands using 12 primers, 05 to 12 (average 7.08) bands per primer. Out of them 74 bands were polymorphic found among all samples and number of bands varied from 03 to 12 (average 6.16) per primer. The average percent polymorphism found to be 84.80%. Polymorphism varied from 60% to 100% per primer. Highest polymorphism (100%) was shown by ISSR-7, ISSR-2, PKBT-4 and PKBT-5 while lowest polymorphism (60%) was

Table 3
Primer details and molecular profile characteristics between the populations of *G. indica*.

Primer name	Sequence details	Annealing temperature (°C)	Amplified bands	Polymorphic bands	Percent polymorphism (%)
PKBT-7	(GA)9A	55	10	10	100
ISSR-7	(GGC)5TA	67	6	6	100
ISSR-3	(AAG)5TG	45	10	8	80.0
857a	(AC)8TG	54	11	11	100
ISSR-2	(AAG)5GC	48	14	14	100
852a	(TC)8AA	52	8	7	87.5
PKBT-4	(AG)8AA	52	13	12	92.31
PKBT-5	(AG)8TA	52	10	9	90.0
PKBT-8	(GA)9C	57	6	6	100
PKBT-11	(GT)9C	57	14	14	100
ISSR-834	(AG)8YT	53	10	9	90.0
835b	(AG)8TC	54	15	13	86.66
Total			127	119	
Mean			10.58	9.91	93.87

shown by PKBT-11 and 835b primers (Table 4). The Nei's genetic diversity value (h) varied from 0.2995 to 1.0415 with the average value 0.3659 for different accessions (Table 6). Other genetic diversity parameters recorded such as observed number of alleles (na) ranged from 1.50 to 2.00, effective number of alleles (ne) ranged from 1.40 to 1.76, Shannon's Information index (*I*) ranged from 0.25 to 0.61, total heterozygosity (Ht) ranged from 0.17 to 0.42. The mean coefficient of differentiation (G_{ST}) was 0.86 which indicated population of the species is highly diverse (Table 7). The Mantel test performed significant correlation between geographical distance and genetic distance with r = 0.59 value. The dendrogram constructed using UPGMA method based on Nei's genetic distance is provided in Fig. 4. The dendrogram showed two main clusters of all seven accessions. First cluster is divided into two sub-clusters (Fig. 4I-a, I-b) with five accessions while in second cluster two accessions were grouped together (Fig. 4-II).

4. Discussion

In the present study, assessment of genetic diversity between the natural populations of *G. indica* from the Northern Western Ghats of India using ISSR molecular markers were carried out. Also assessment of genetic diversity within a natural population from Amboli, Maharashtra region was performed. Earlier studies were carried out to standardize DNA extraction protocol using *G. indica* for RAPD analysis (Sahasrabudhe and Deodhar, 2010). The genetic relationship among the twelve accessions of *G. cambogia* from different geographical accessions of Kerala was evaluated using RAPD markers (Tharachand et al., 2015).

In another report, standardization of protocol for DNA isolation and PCR parameters for different *Garcinia* species has been done using RAPD markers (Ashish et al., 2010). Priya Devi et al. (2010) evaluated genetic diversity and relationship among *Garcinia* species such as *G. indica*, *G. mangostana*, *G. hombroniana* and *G. tinctoria* in the region of Goa using RAPD and ISSR markers. It suggested the use of above mentioned molecular markers in genetic fingerprinting and development of species specific markers as well as to develop markers linked with the sex trait. Supporting the later work, sex determination in micro propagated plants and natural population of *G. indica* was carried out by Dias and Krishnan (2015) using ISSR and RAPD markers which can be used as male or female specific markers.

ISSR primers are dominant like RAPD. However they are more stable, reproducible, simplest and highly polymorphic in closely related varieties over RAPD (Vijayan, 2005). ISSR markers showed higher level of polymorphism than RAPD in finger millet (Salimath et al., 1995), sorghum (Yang et al., 1996) and RFLP markers in maize (Kantety et al., 1995), finger millet (Salimath et al., 1995). Hence, ISSR markers proved more favorable than RAPD and RFLP for DNA fingerprinting, genetic diversity analysis and germplasm management in crop plants. It is also proved useful in assessment of genetic diversity in medicinal plants *G. mangostana* (Mansyah et al., 2010). For development of new germplasm using breeding programmes in fruit crops *Carica papaya* (Sabara and Vakharia, 2018) and in floriculture *Gladiolus* (Singh et al., 2018) ISSR markers were demonstrated useful.

In the present study, it is observed that primer 857a exhibited 100% polymorphism along with five other primers such as PKBT-7, ISSR-7, ISSR-2, PKBT-8 and PKBT-11 which were screened for genetic diversity between the natural populations (Table 3). In earlier reports the same primer proved good polymorphism in Indian *Garcinia* species (Parthasarathy et al., 2013). Hence, above mentioned primer can be used for genus specific studies.

Table 4
Primer details and molecular profile characteristics within the population of *G. indica*.

Primer name	Sequence details	Annealing temperature (°C)	Amplified bands	Polymorphic bands	Percent polymorphism (%)
PKBT-7	(GA)9A	55	6	5	83.33
ISSR-7	(GGC)5TA	67	6	6	100
ISSR-3	(AAG)5TG	45	7	6	85.71
857a	(AC)8TG	54	9	7	77.77
ISSR-2	(AAG)5GC	48	12	12	100
852a	(TC)8AA	52	6	5	83.33
PKBT-4	(AG)8AA	52	6	6	100
PKBT-5	(AG)8TA	52	10	10	100
PKBT-8	(GA)9C	57	5	4	80.0
PKBT-11	(GT)9C	57	5	3	60.0
ISSR-834	(AG)8YT	53	8	7	87.5
835b	(AG)8TC	54	5	3	60.0
Total			85	74	
Mean			7.08	6.16	84.80

Table 5
Nei's measures of genetic identity and genetic distance between the populations of *G. indica.*

Pop ID	Dhamapur	Insuli	Ukshi	Dahibav	Dabholi	Narve	Bhattiwadi	Sanvordem	Sangameshwar	Konem Priol	Kinner Katta
Dhamapur	****	0.6614	0.7953	0.6772	0.6614	0.6772	0.6299	0.4646	0.6142	0.5906	0.7244
Insuli	0.4134	****	0.6929	0.6378	0.5433	0.5748	0.5118	0.6142	0.4961	0.6929	0.6693
Ukshi	0.2291	0.3669	****	0.7244	0.5984	0.6457	0.6299	0.4646	0.5984	0.5906	0.6929
Dahibav	0.3898	0.4497	0.3224	****	0.7323	0.7165	0.6220	0.5669	0.6378	0.6457	0.7480
Dabholi	0.4134	0.6101	0.5135	0.3116	****	0.8268	0.7008	0.5984	0.6535	0.6299	0.7953
Narve	0.3898	0.5537	0.4375	0.3333	0.1902	****	0.7165	0.6142	0.7165	0.6614	0.8110
Bhattiwadi	0.4622	0.6698	0.4622	0.4747	0.3556	0.3333	****	0.5039	0.6693	0.5512	0.7323
Sanvordem	0.7666	0.4875	0.7666	0.5675	0.5135	0.4875	0.6853	****	0.4724	0.8268	0.6457
Sangameshwar	0.4875	0.7011	0.5135	0.4497	0.4253	0.3333	0.4015	0.7498	****	0.5512	0.7008
Konem Priol	0.5267	0.3669	0.5267	0.4375	0.4622	0.4134	0.5957	0.1902	0.5957	****	0.7402
Kinner Katta	0.3224	0.4015	0.3669	0.2903	0.2291	0.2095	0.3116	0.4375	0.3556	0.3009	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

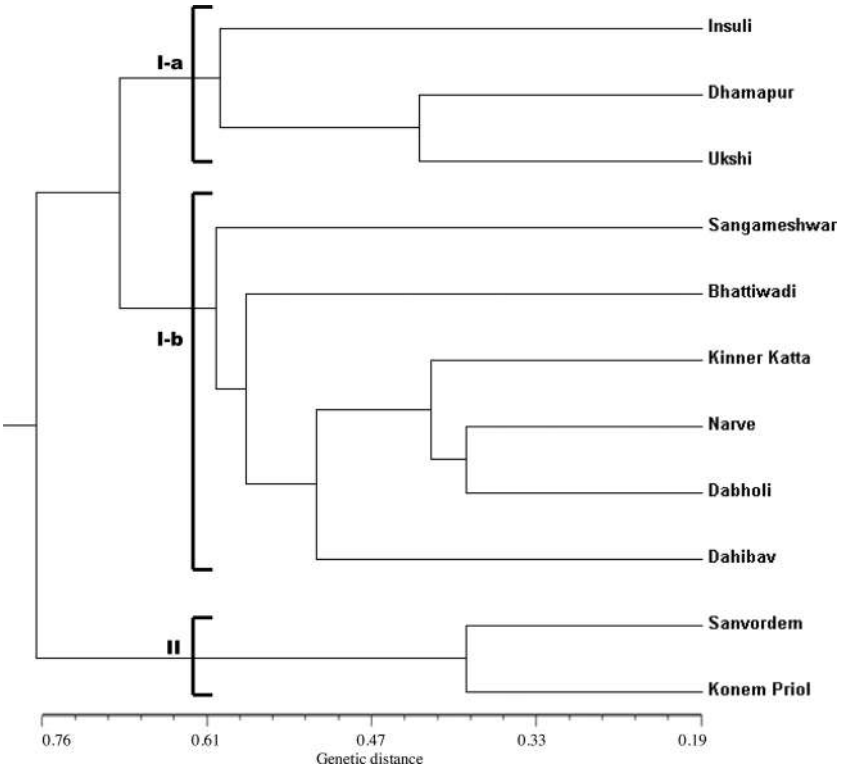


Fig. 3. Dendrogram based on Nei's genetic distance between the natural populations of *G. indica* using ISSR markers.

Table 6
Nei's measures of genetic identity and genetic distance within the population of *G. indica.*

Accession	1	2	3	4	5	6	7
1	****	0.5882	0.6706	0.6000	0.5647	0.5176	0.5412
2	0.5306	****	0.5882	0.6588	0.6235	0.6000	0.4588
3	0.3996	0.5306	****	0.5765	0.6353	0.6118	0.5647
4	0.5108	0.4173	0.5508	****	0.5176	0.5412	0.3529
5	0.5715	0.4724	0.4537	0.6585	****	0.5765	0.5059
6	0.6585	0.5108	0.4914	0.6140	0.5508	****	0.7412
7	0.6140	0.7791	0.5715	1.0415	0.6815	0.2995	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

According to the results it has been found that high level of genetic diversity is present at both between and within population. The percent polymorphism ranged from 80 to 100% and 60 to 100% for between and within population respectively. However, earlier report of genetic diversity in *G. indica* at inter and intra-population levels in a small region which comprised only three districts of Maharashtra showed comparatively low level of diversity with percent polymorphism ranged from 4.70% to 11.37% (Thatte et al., 2012) and also during the study mainly genotypes under cultivation were used. While the present study covers inter-population genetic diversity of natural populations from the entire Northern Western Ghats of India mainly Konkan regions

Table 7
Genetic diversity of *G. indica* between and within the population using ISSR markers.

Population/ Accession	No. of genotypes	na	ne	*I*	Ht	G_{st}
Between	11	1.50-2.00	1.22-1.52	0.22-0.56	0.15-0.32	
Mean		1.82	1.37	0.41	0.26	0.81
Within	7	1.50-2.00	1.40-1.76	0.25-0.61	0.17-0.42	
Mean		1.88	1.56	0.48	0.32	0.86

Note: na represents Observed number of alleles; ne represents Effective number of alleles; *I* represents Shannon's Information index; Ht represents Total heterozygosity; G_{ST} represents coefficient of genetic differentiation.

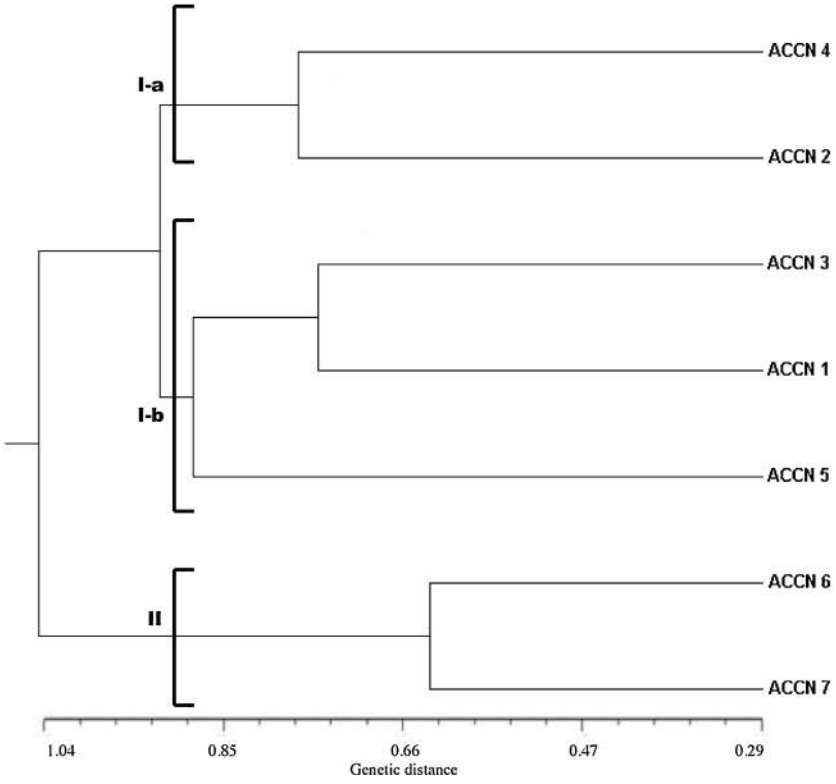


Fig. 4. Dendrogram based on Nei's genetic distance within single natural population of *G. indica* from Amboli using ISSR markers.

where plants are abundant and intra-population genetic diversity of single large population at Amboli, Sindhudurg district, Maharashtra.

The other genetic diversity parameters revealed the existence of high diversity and differentiation between populations with average values of h, na, ne, *I*, Ht and G_{ST} i.e. 0.3196, 1.82, 1.37, 0.41, 0.26 and 0.81 respectively and in case of within population average value of h was 0.3659, na was 1.88, ne was 1.56, *I* was 0.48, Ht was 0.32 and G_{ST} was 0.86 respectively. Fang et al. (2018) reported high genetic diversity and differentiation among 17 populations of *Callicarpa kwangtungensis* Chun. and Mantel test was employed to establish the correlation between morphological, biochemical and molecular markers. In the present study Mantel test was performed to show the presence of significant correlation between geographical distance and genetic distance for both between and within population.

The dendrogram constructed using UPGMA method with ISSR data matrix revealed correlation between altitudinal range and clustering pattern of different populations (Fig. 3). The altitudes of leaf samples collected from different natural populations for between populations studies were varied from 11 m to 209 m among different populations (Table 1). Population with same altitudinal level such as Sanvordem (52 m) and Konem Priol (66 m) were grouped together and formed separate cluster. However, high altitude populations formed different cluster which is separated again as sub-clusters according to altitude range. High altitude populations such as Sangameshwar (110 m), Bhattiwadi (209 m), Kinner Katta (73 m) and Dabholi (66 m) grouped together. But genotypes from same altitude range have different architecture or they are nesting in different clusters may be due to other factors such as geographical locations, direction of wind etc (Inasi et al., 2010). Above fact is supported by present study in which high altitude populations Ukshi (199 m) and Dabholi (66 m) showed close proximity with low altitude population Dhamapur (44 m) and Dahibav (11 m) respectively, since both are from same geographic location Maharashtra. Same pattern observed in populations from Goa too in which Kinner Katta (73 m) and Narve (36 m) populations are nested together. Sanvordem population showed highest genetic distance with Dhamapur and Ukshi population because both populations are from different geographic locations (Tables 1 and 5). However, it showed highest genetic identity with Konem Priol and Narve populations since, both are from same geographic locations (Tables 1 and 5). It indicates geographical isolation is one of the important factors for genetic distance as reported in earlier work (Parab and Krishnan, 2008).

Rajasekharan et al. (2017) reported that tropical trees show high levels of diversity in intraspecific populations. This fact is well supported in present study which showed high polymorphism in samples analyzed from different locations (accessions) within single large natural population (Table 4). The altitude range within the single population varied from 175 m to 499 m (Table 2). Dendrogram constructed showed two different clusters (Fig. 4). In first cluster, five accessions were grouped together and formed different sub-clusters according to altitude and other factors. In this cluster, accession 1 (175 m) and accession 3 (181 m) are grouped together due to same altitude range but accession 2 (184 m) and accession 4 (418 m) are showed close relationship which is not based on altitude, but may be due to other factors such as geographical locations, direction of wind etc., (Inasi et al., 2010) as later accession was collected on the slope region. Also the accessions 4 and 7 showed highest genetic distance while accessions 6 and 7 showed lowest genetic distance (Table 6). While in second cluster high altitude accessions such as accession 6 (463 m) and accession 7 (499 m) are grouped together and formed separate cluster.

5. Conclusions

Genetic diversity study is an important part of conservation biology which can be used to assess species existence status. The present study revealed the occurrence of high genetic diversity and variation for both between and within population which indicates the species long term persistence by increasing ability to adapt to changing environmental conditions. The genetic diversity was mainly due to the altitudinal ranges, geographic distribution of the species and other environmental factors such as direction of wind etc. Considering the above, following

conservation strategies are proposed: Existence of high genetic diversity in *G. indica* indicates that the natural populations are considerably stable. Some of the germplasms identified in the natural populations are high yielding, high acid taste, large fruit size and early ripening genotypes. As this natural populations are highly exploited for various culinary preparations, pharmaceutical applications, these germplasms to be conserved *in situ* in their natural habitats. During the previous study, we have developed high frequency *in vitro* plant regeneration system for the production of high yielding elite clones of *G. indica* via direct organogenesis (Baskaran and Krishnan, 2011). As *ex situ* conservation measure, these *in vitro* regenerated plants could be used by farmers for large scale cultivation. Further, this species is also highly exploited for the presence of bioactive compounds *viz*. hydroxycitric acid (anti-obesity compound) and garcinol (anti-cancerous properties) by various small and large scale industries, the potential germplasm which are high yielding and highly genetically diverse could be utilized for breeding programme to develop economically and pharmaceutically important new varieties that could be used by farmers for large scale cultivation.

Funding

This work was supported by the Council of Scientific and Industrial Research (CSIR) [No.38 (1348)/13/EMR-II], New Delhi, India.

Declaration of Competing Interest

None.

Acknowledgements

Authors are grateful to Dr. V. Arunachalam, Principal Scientist, ICAR-Central Coastal Agricultural Research Institute, Goa, for his help in statistical analysis.

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Natl. Acad. Sci. Lett. (November 2020) 43(6):585–591
https://doi.org/10.1007/s40009-020-00918-y

SHORT COMMUNICATION

Prediction of Potential Distribution and Climatic Factors Influencing *Garcinia indica* in the Western Ghats of India Using Ecological Niche Modeling

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Received: 1 May 2018 / Revised: 20 November 2018 / Accepted: 21 January 2020 / Published online: 7 February 2020
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Abstract *Garcinia indica* is endemic to Western Ghats of India and belongs to the family Clusiaceae. It has anti-obesity, antioxidant, anticancer and other high potential medicinal values. Considering the importance and threat of overexploitation of *G. indica*, the prediction of potential distribution and understanding the climatic factors responsible for the niche of the species in wild including areas for its cultivation have been worked out based on primary occurrence data. Ecological niche modeling has been carried out using MaxEnt and BIOCLIM algorithms. Among them, MaxEnt performed better as compared to BIOCLIM model with high AUC value. However, predictions by both models were found to be almost similar. The prediction revealed that the species habitat suitability is in the Konkan regions of Maharashtra, Goa and Karnataka. The precipitation and temperature are the major factors defining the distribution of the species. As this species is commercially exploited, the outcome of present study will help in large-scale cultivation and sustainable utilization of the species.

Keywords DIVA-GIS · ENM · *Garcinia indica* · MaxEnt

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The genus *Garcinia* L. belonging to the family Clusiaceae contains about 260 species worldwide predominantly distributed in tropics of the Old World especially in Asia and Africa [1]. *Garcinia indica* is one of the most important species in the genus because of its medicinal and economic value. It is a slender, dioecious tree with blackish tree trunk showing drooping branches (Fig. 1) distributed in forests of Western Ghats of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. It is also cultivated predominantly in the states of Goa, Karnataka and Maharashtra covering the entire Konkan region for commercial purpose and plant is popularly known as "kokum" in the region. The fruits and leaves of the species contain hydroxycitric acid (HCA), garcinol, polyphenols, several acids and other phytochemical compounds [2, 3]. Due to the presence of these compounds, it has pharmaceutical values. These compounds possess many biological activities such as anti-obesity (fat-reducing agent), anti-cancerous, antioxidant, antibacterial, antifungal, anti-HIV, anti-inflammatory, antipyretic and anti-Alzheimer's activity [4]. The seeds of kokum fruit yield edible fats known as "kokum butter" which is used in the manufacture of soaps, candles, ointments and several cosmetics. Fruit is popularly used as condiments for flavoring vegetarian as well as non-vegetarian food preparations; several traditional and modern preparations such as sherbet, syrups, jams, squashes and wine are made of it. It contains pigments such as anthocyanins which are used as food colorants and as a natural dye [5]. As the threat status of *G. indica* is categorized as vulnerable [6] and due to the overexploitation of the species, the present study on its potential distribution in the Western Ghats of India in wild population has been worked out based on the species occurrence data and environmental variables using predictive modeling approaches in MaxEnt and BIOCLIM implemented in DIVA-GIS.



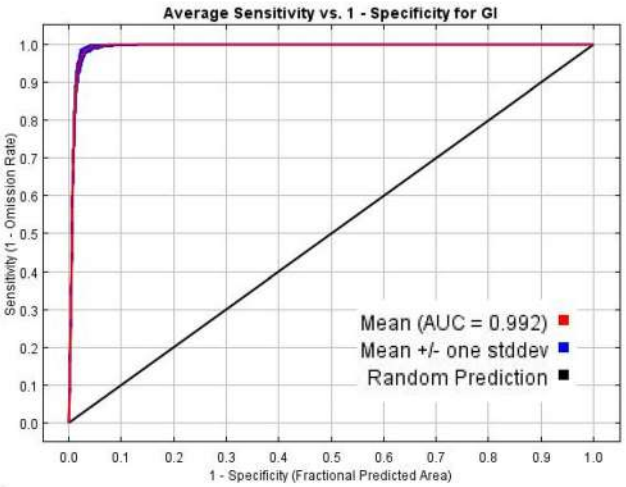
Fig. 1 Habit of *G. indica.* Inset is mature fruits

The study area covers the entire Western Ghats of India. The study was conducted from August 2013 to July 2016. Based on herbarium data, floras and previous literature, field survey was carried out at different locations to collect primary occurrence data of *G. indica* from wild populations. During intensive field survey, the latitude, longitude and altitude of 156 accessions were recorded using handheld GPS (Model: GARMIN MONTANA 650). Environmental data are key input for modeling which includes 19 default temperature and precipitation layers downloaded from the WorldClim data portal (http://www.worldclim.org). Data analysis was carried out by using two ecological niche modeling algorithms, namely maximum entropy (MaxEnt Version 3.4.1) [7, 8] and BIOCLIM implemented in DIVA-GIS version 7.5 [9] freely available online. The default settings were used for performing modeling experiments with each algorithm. The MaxEnt algorithm was performed using random seed with random test percentage 30, bootstrap replicate run type with 15 replicates, 500 maximum iterations, cumulative output format and .asc output file type. To attain best fit model, quality was evaluated based on area under the receiver operating characteristic (ROC) curve or AUC values. The jackknife procedure was used to assess the importance of the variables. The final potential distributional area at different levels of probability and actual occurrence of the species was generated using DIVA-GIS for both algorithms. Results of the analysis have been interpreted based on jackknife evaluation, percent contribution and permutation importance of each variable and prediction maps [10, 11]. Sample bias has been attained by sample stratification to avoid overrepresentation of the training data set [12].

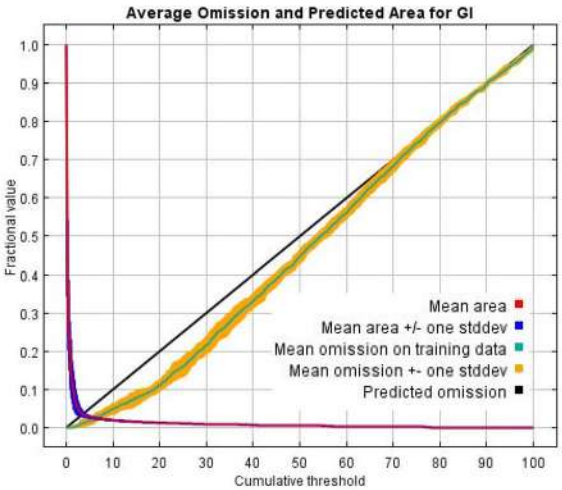
Potential distribution of *G. indica* has been predicted with the help of primary occurrence data using MaxEnt and BIOCLIM models. The prediction of habitat suitability by both algorithms was found to be almost similar (Figs. 3, 4). However, the model performance was evaluated based on AUC values which showed MaxEnt model with better performance of 0.99 AUC value compared with BIOCLIM model with 0.91 AUC value. High AUC value obtained during this study by using MaxEnt model indicates the significant prediction that is discrimination between the presence and absence areas for the species distribution. Hence, among the two algorithms used the best fit model was found to be MaxEnt and considered for elaborate interpretation in this paper.

In addition to threshold-independent AUC value (ROC curve), the MaxEnt algorithm has been validated with omission/commission rate which is a threshold-dependent binomial test based on omission and predicted area [7, 13]. The analysis of omission/commission revealed the lines of average omission and predicted area are close to each other which indicates that the model performed better than random and showed that the model is more accurate (Fig. 2a, b).

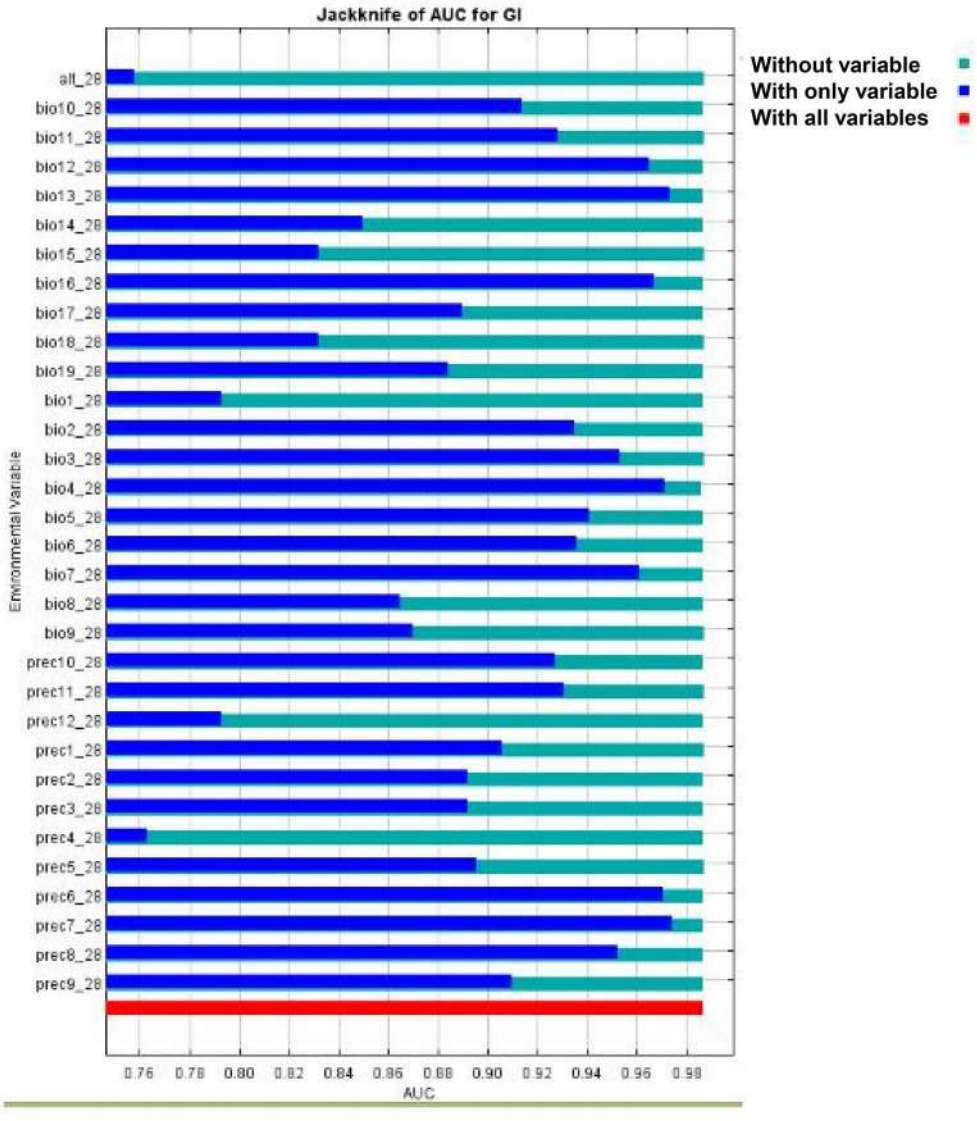
The prediction maps obtained using both algorithms MaxEnt and BIOCLIM depict the potential geographical distribution, range of richness and density of *G. indica* in the Western Ghats of India (Figs. 3, 4). The MaxEnt prediction revealed the potential range for richness of the species and habitat suitability is excellent at the junction of Northern-Central Western Ghats mainly comprising Konkan region of Maharashtra, Goa and Karnataka as indicated in the map as 80–100%. This part comprises Sindhudurg and Ratnagiri districts of Maharashtra; North and South Goa districts of Goa; and Uttara Kannada and Udupi districts of Karnataka. Also, these regions are popular for various kokum fruit processing industries and utilization of kokum products for culinary applications [2]. Additionally, small part of Northern Kollam district of Kerala state also found to be excellent for the niche of the species. The range of high (40–60%) and very high (60–80%) habitat suitability was found to occur in the regions of Raigad district of Maharashtra; Dakshina Kannada district of Karnataka; and Kasargod, Alappuzha, Pathanamthitta and Idukki districts of Kerala in a lesser extent. The suitability for niche of the species was found to be medium (20–40%) in the regions of Palghar, Thane and Northern part of Raigad districts of Maharashtra; Kodagu district of Karnataka; and Kannur, Wayanad and Northern part of


Average Sensitivity vs. 1 - Specificity for GI
Sensitivity (1 - Omission Rate)
1 - Specificity (Fractional Predicted Area)
Mean (AUC = 0.992)
Mean +/- one stddev
Random Prediction

a


Average Omission and Predicted Area for GI
Fractional value
Cumulative threshold
Mean area
Mean area +/- one stddev
Mean omission on training data
Mean omission +- one stddev
Predicted omission

b


Jackknife of AUC for GI
Environmental Variable
AUC
Without variable
With only variable
With all variables
alt_28
bio10_28
bio11_28
bio12_28
bio13_28
bio14_28
bio15_28
bio16_28
bio17_28
bio18_28
bio19_28
bio1_28
bio2_28
bio3_28
bio4_28
bio5_28
bio6_28
bio7_28
bio8_28
bio9_28
prec10_28
prec11_28
prec12_28
prec1_28
prec2_28
prec3_28
prec4_28
prec5_28
prec6_28
prec7_28
prec8_28
prec9_28

c

◄**Fig. 2** **a** The receiver operating characteristic (ROC) curve; **b** omission versus predicted area; **c** jackknife test, using AUC showing importance of different environmental variables

Thiruvananthapuram district of Kerala. According to MaxEnt prediction, niche of the species prefers western

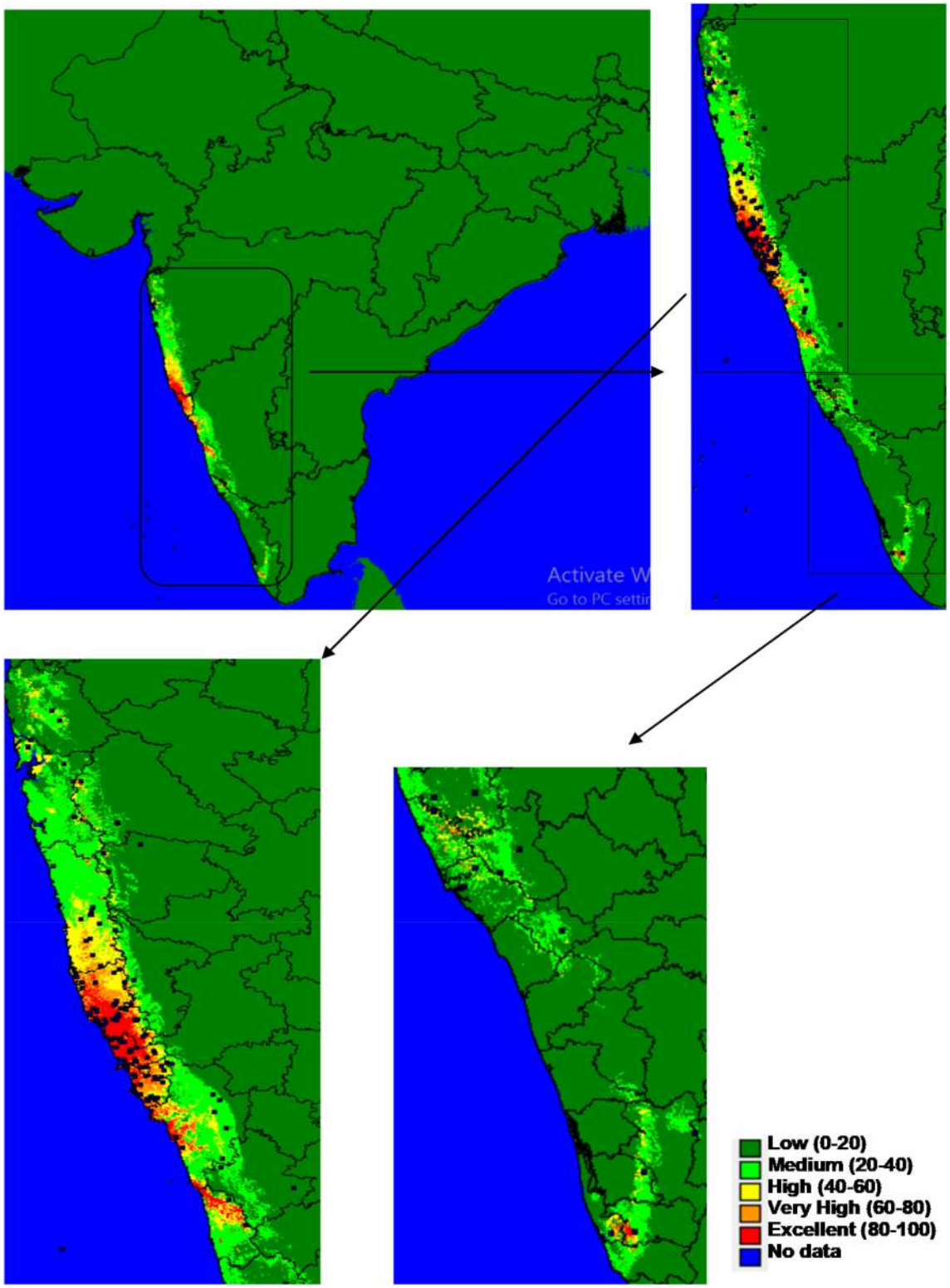


Fig. 3 Predicted (maximum) potential distributional area of the Western Ghats of India which signifies different levels of probability of occurrence and actual occurrences of the *G. indica* under current climatic conditions with MaxEnt

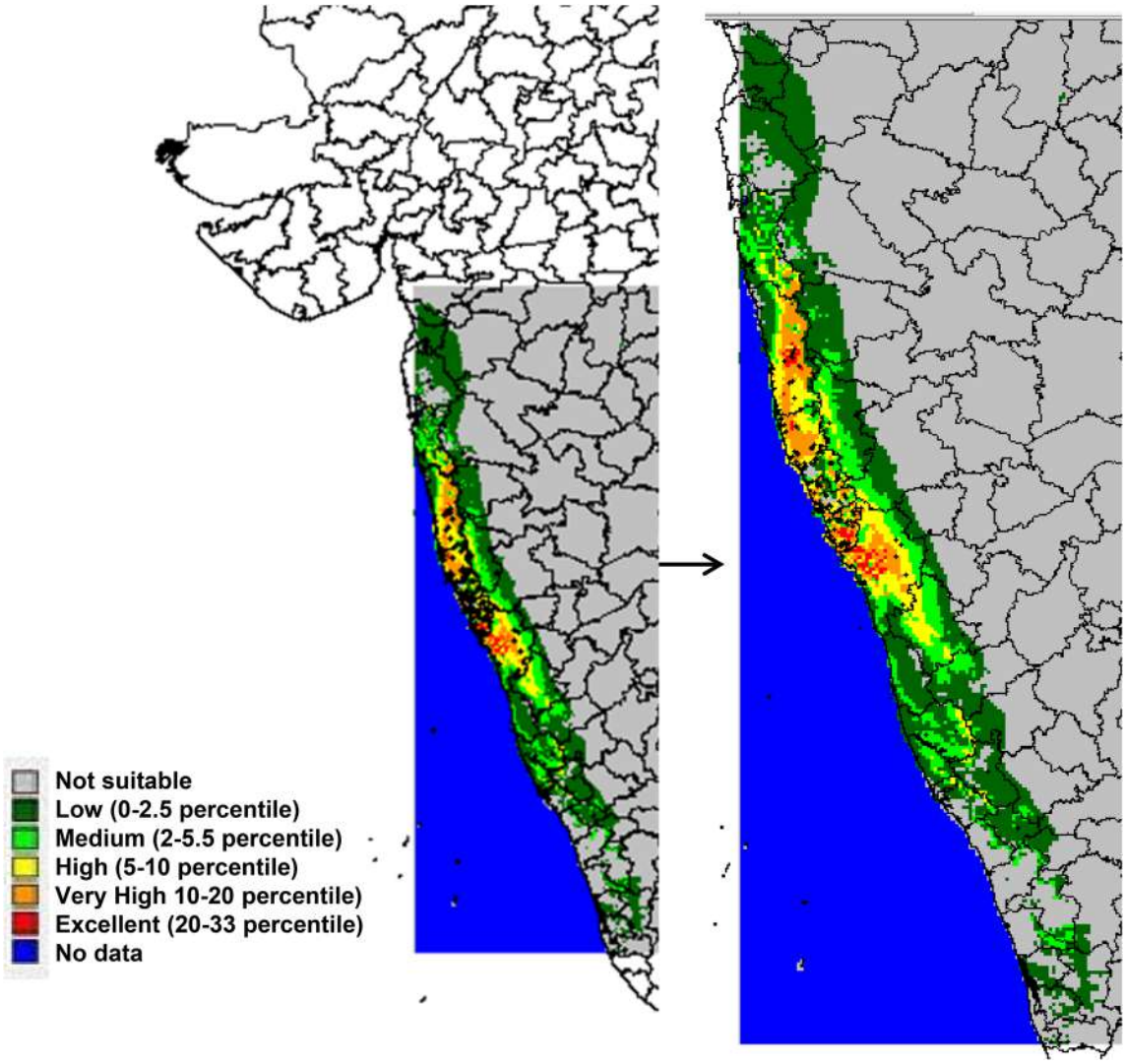


Fig. 4 Predicted (maximum) potential distributional area of the Western Ghats of India which signifies different levels of probability of occurrence and actual occurrences of the *G. indica* under current climatic conditions with BIOCLIM

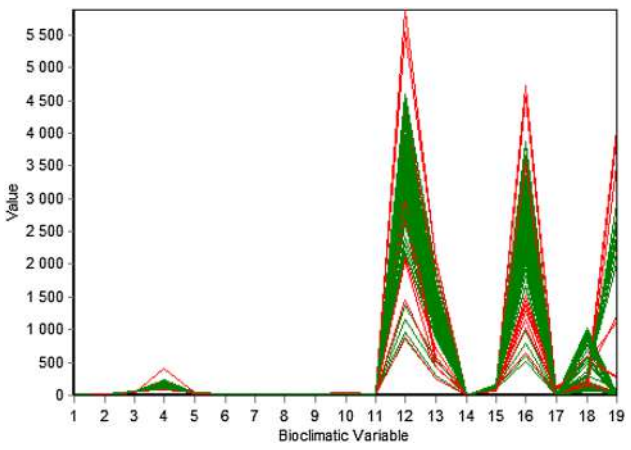


Fig. 5 Relative contribution of the environmental variables with BIOCLIM model

part of Western Ghats, but entire stretch of Western Ghats is not suitable for the growth of *G. indica.* However, earlier study carried out using grid sampling method showed the presence of some *Garcinia* species such as *G. gummi-gutta*, *G. morella* and *G. talbotii* in the entire Western Ghats including *G. indica* [14]. BIOCLIM algorithm has also depicted similar prediction to MaxEnt and high habitat suitability was found to occur in the regions of Raigad, Ratnagiri and Sindhudurg districts of Maharashtra; North and South Goa districts; and Uttar Kannada district of Karnataka.

The richness and density of *G. indica* have been significantly influenced by few environmental variables. The major environmental factors responsible for the species distribution and habitat suitability predicted by MaxEnt and BIOCLIM (Fig. 5) models are precipitation and temperature. The estimates of percent contribution and

Table 1 BIOCLIM layers used in the study with their percent contribution and permutation importance

Code	Environmental variable	Percent contribution	Permutation importance
bio13	Precipitation of wettest month	34.2	0.1
bio4	Temperature seasonality (standard deviation × 100)	16.2	32.7
prec6	Precipitation in the month of June	13.4	6.4
bio12	Annual precipitation	10.8	0.2
prec7	Precipitation in the month of July	5	19.5
bio19	Precipitation of coldest quarter	3.4	2.2
bio16	Precipitation of wettest quarter	3.2	0.3
bio7	Temperature annual range (bio5–bio6)	1.4	0.4
bio11	Mean temperature of coldest quarter	1	3.5
bio9	Mean temperature of driest quarter	1	3
bio17	Precipitation of driest quarter	0.9	0.1
bio6	Minimum temperature of coldest month	0.9	0.2
prec5	Precipitation in the month of May	0.8	1.8
bio8	Mean temperature of wettest quarter	0.8	0.8
bio3	Isothermality [(bio2/bio7) × 100]	0.8	0.5
prec1	Precipitation in the month of January	0.7	0
prec2	Precipitation in the month of February	0.7	0.5
prec11	Precipitation in the month of November	0.6	4.9
bio15	Precipitation seasonality (CV)	0.6	1.4
bio10	Mean temperature of warmest quarter	0.6	0.6
bio5	Maximum temperature of warmest month	0.5	0.7
bio1	Annual mean temperature	0.5	0.7
prec4	Precipitation in the month of April	0.4	1.8
prec9	Precipitation in the month of September	0.4	2.4
prec10	Precipitation in the month of November	0.3	4.2
prec3	Precipitation in the month of March	0.3	0.9
bio2	Mean diurnal range (mean of monthly max. and min. temp.)	0.2	0.9
prec8	Precipitation in the month of August	0.2	0.2
bio18	Precipitation of warmest quarter	0.2	2.3
Alt	Altitude	0.1	4.3
prec12	Precipitation in the month of December	0.1	1.4
bio14	Precipitation of driest month	0.1	0.8

permutation importance of each variable are shown in Table 1. These results are consistent with jackknife evaluation (Fig. 2c). Considering the percent contribution, eight environmental variables out of 19 were contributed to the model output. Among them, precipitation of wettest month (BIO 13) (34.2%) and temperature seasonality (BIO 4) (16.2%) have maximum influence on the habitat suitability followed by precipitation in the month of June (PREC 6) (13.4%) and annual precipitation (BIO 12) (10.8%). However, the permutation importance revealed that the temperature seasonality (BIO 4) (32.7) was found to be major factor followed by precipitation in the month of July (PREC 7) (19.5), precipitation in the month of June (PREC 6) (6.4) and altitude influences the suitability in a lesser extent. Along with the above parameters, other parameters are also responsible in a lesser extent for species distribution pattern. The data support that the species prefers specific environmental conditions such as precipitation and temperature. Earlier study was carried out using Kira's warmth index, coldness index, Xu's humidity index and other climate indexes revealed that *G. pedunculata* Roxb. species richness was due to humidity and thermal factors [15]. The species distribution modeling has been used to estimate the probability of occurrence of species with climate data based on presence-only (or absence) data for many plant species [16, 17].

Both MaxEnt and BIOCLIM algorithms predicted that niche of the species is predominantly found to be Konkan

regions of Maharashtra, Goa and Karnataka. The study revealed that the precipitation and temperature were the major factors which influence the habitat suitability for *G. indica.* As the species is medicinally important and major industries are involved in processing, developing and marketing various kokum products, the large-scale cultivation is essential. Further, the output of the study may help in conservation and sustainable utilization of the species by using appropriate management programs.

Acknowledgements The authors gratefully acknowledge financial support provided by the Council of Scientific and Industrial Research (No. 38 (1348)/13/EMR-II), New Delhi, India, to carry out the above research work.

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