EVALUATION OF PHYTOCHEMICAL DIVERSITY OF *CINNAMOMUM VERUM* J. PRESL IN SOUTH INDIA

by

ATHIRA S. NAIR

(2012-09-109)

THESIS

Submitted in partial fulfillment of the

requirement for the degree of

B. Sc. – M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2017

DECLARATION

I hereby declare that this thesis entitled **"EVALUATION OF PHYTOCHEMICAL DIVERSITY OF *CINNAMOMUM VERUM* J. PRESL IN SOUTH INDIA"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Athira

Place: Vellayani
Date: 28.11.17

ATHIRA S. NAIR
(2012-09-109)

JNTBGRI

Jawaharlal Nehru Tropical Botanic Garden and Research Institute

(An Institute of Kerala State Council for Science, Technology and Environment, Govt. of Kerala, National Centre of Excellence, Govt. of India)

Karimancode P. O., Palode, Thiruvananthapuram - 695 562, Kerala, India.

CERTIFICATE

This is to certify that this thesis entitled **" Evaluation of phytochemical diversity of *Cinnamomum verum* J. Presl in South India"** is a record of research work done by **Mrs. Athira S. Nair** (2012-09-109) under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

28/11/17

Place: Palode
Date: 28/11/2017

Dr. K. B. Rameshkumar
(Chairman, Advisory Committee)
Scientist C
Phytochemistry and
Phytopharmacology Division
JNTBGRI,Palode, Thiruvananthapuram- 695 562

CERTIFICATE

We, the undersigned members of the advisory committee of Mrs. Athira S. Nair (2012-09-109), a candidate for the degree of B. Sc. – M. Sc. (Integrated) Biotechnology, agree that the thesis entitled "Evaluation of phytochemical diversity of *Cinnamomum verum* J. Presl in South India" may be submitted by Mrs. Athira S. Nair in partial fulfillment of the requirement for the degree.

28/11/17

Dr. K. B. Rameshkumar
(Chairman, Advisory Committee)
Scientist C
Phytochemistry and
Phytopharmacology Division
JNTBGRI,Palode, Thiruvananthapuram- 695 562

Dr. Swapna Alex
(Member, Advisory Committee)
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram – 695 522

Dr. S. R Suja
Scientist and HOD (i/c)
Ethnomedicine and Ethnopharmacology
Division, JNTBGRI, Palode
Thiruvananthapuram- 695562

28/11/17

Dr. K. B. Soni
(Member, Advisory Committee)
Professor &Course Director
B. Sc. – M. Sc. (Integrated) Biotechnology
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram – 695 522

28/11/17

Dr. N. K. Leela
(External Examiner)
Principal Scientist
ICAR- Indian Institute of Spices Research
Kozhikode– 673012

4

ACKNOWLEDGEMENT

First and foremost, I must acknowledge and thank the God Almighty for blessing, protecting and guiding me throughout this period. This thesis is the end of my journey in obtaining my M. Sc. degree. I have not travelled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my guide, my parents, and my friends. At the end of my thesis I would like to thank all those people who made this thesis possible and an unforgettable experience for me.

It is with my heartfelt feelings, I wish to express my deep sense of gratitude and sincere thanks to Dr.K.B.Rameshkumar,Scientist C of Phytochemistry and Phytopharmacology Division , Jawaharlal Nehru Tropical Botanical Garden and Research Institute, for his valuable guidance, sincere help, patience, support, encouragement throughout the course of study and for giving me an opportunity to work at JNTBGRI for my thesis work.

My sincere gratitude to Dr. S. R. Suja, Scientist C, and Head of department (I/C) Division of Ethnomedicine and Ethno pharmacology, Jawaharlal Nehru Tropical Botanical Garden and Research Institute. I express my indebtedness to ma'am for her guidance, incessant inspiration, untiring attention and patience towards my lab experiments while devoting her precious time in the midst of her busy schedule.

I am thankful to the members of the advisory committee, Dr. Swapna Alex,, Professor and Head, Department of Plant Biotechnology, College of Agriculture, Vellayani, Dr.K.B.Soni Course Director, Integrated Biotechnology Course, for their valuable counselling and constructive suggestions that were much helpful throughout my research progress.

I take immense pleasure to express my thanks to every member of PPD Division for being there for me always especially Ananthakrishnan R, who have rendered whatever help I needed during my all experiments and for providing me suggestions and unwavering encouragement throughout my research work.

I express my heartfelt thanks to Dr. Anilkumar A, dean of College of Agriculture, Vellayani, for all the facilities provided.

I thankfully remember my friends who have rendered helping hands at all times and support during my research work.

I thank all my beloved folks Neethu, Gayathri, Vidya,,Anjali and my senior Vipinmohan,shahsad, and Meera Mohan.

I owe this achievement to my dear parents and husband for their supports for completing this course and research work and my special thanks to them.

I acknowledge the favour of numerous persons who, though not been individually mentioned here, who have all directly or indirectly contributed to this thesis work.

ATHIRA S. NAIR

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µg	Microgram
µL	Micro litre
µM	Micro Molar
AFLP	Amplified Fragment Length Polymorphism
Bp	base pair
Cm	Centi Metre
cM	CentiMorgan
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
et al.	And other co workers
Etc	Etcetera
g	Gram
g-1	Per gram
Hrs	Hours
Kb	Kilo base

M	Molar
mg	Milli gram
Min	Minutes
mL	Millilitre
mM	millimolar
nm	Nanometre
°C	Degree Celsius
OD	Optical Density
PCR	Polymerase Chain Reaction

INTRODUCTION

INTRODUCTION

Plants serve as a source of food, clothing and shelter for mankind. All the plant parts are useful to flourish human life. Plants form the basis for medicines, flavours, perfumes, timber, firewood, gums and so on. One of the important properties of plants is making use of its capability in imparting flavor. Flavors have had a noteworthy effect over the span of history and human advance. The flavor trade started in the Asiatic tropics and they were among the essential objects of business between the east and the west. Flavor has increased more significance because of its solution for the treatment of different sorts of disorders in the ayurvedic and other traditional medicinal systems in India from the time of Charaka Samhita. So great was the value of flavors in the medieval times that they are routinely compared with gold and other significant stones (Manosi *et al.* 2013).

Many of the flavors used by humans to season food are yielded by spice crops. Spice can be defined as an 'aromatic or pungent vegetable substance'. As spices, cinnamon stands first as essential commodity and cinnamon is one of the finest sweet flavors. It is indigenous in Sri Lanka, which still produces biggest amount and best quality, mostly as quills. But now it has become common spice crop all over the world. In India, this crop was introduced by the spice traders. Earlier studies on diversity of *Cinnamomum verum* in North and Central India have shown an extreme degree of variability with respect to growth habitat. Reports on wild distribution of cinnamon in south India are scarce and it is believed that the plant is naturalized in south India and no extensive study is so for available for this species in this region.

The present study reports the diversity study of *Cinnamomum verum* in south India by morphological, phytochemical and molecular evaluations. Study of physical form and external structure of plants helps to check diversity of plants. Plant populations may show morphological variations as adaptation to different selection pressures which may result from phenotypic plasticity or genetic differentiation due

to natural selection and evolutionary forces (Morrison and Weston, 1985). Morphological characters include both quantitative and qualitative parameters, such as leaf and floral characteristics (Azad *et al.*, 2016). Morphological characteristics are the earliest markers used for assessment of variation and are still of greater importance. Usually, these characters are simple to score. There are several sets of physical character assessment for different plants at different developmental stages such as juvenile, vegetative, flowering and fruiting. However, these sets of characters lack adequate coverage of the genome and are strongly influenced by environmental features (Wang and Tanksley, 1989).

Phytochemical profiling is yet another criterion in diversity checking. The accumulation of phytochemicals may vary depending on plant part, season, biogeography *etc.* (Costa, *et al.,* 1999, Harborne and Baxter, 1995). For the diversity study, secondary metabolites are selected as they are specific to plant groups, compared to the ubiquitous primary metabolites. The secondary metabolites are not important for the growth and plant development but they are required for the interaction of plants with the environment (Croteau, 2000). Chemical profiling establishes a characteristic chemical pattern for a plant material. This could be elaborated through volatile as well as non volatile chemical profiling. Volatile chemical profiling involves hydro distillation followed by GC/GC-MS analysis. Chromatographic tools like Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC) are used for qualitative and quantitative determination of non volatile chemical compounds.

Genetic diversity is the basis for development of elite varieties with desirable characteristics (Govindaraj *et al.*, 2015). In recent years, limitations of morphological and biochemical markers has been overcome by molecular markers. Molecular markers offer consistent results despite the prevailing environmental circumstances. Among the widely used markers, inter simple sequence repeat (ISSR) marker is a

PCR based molecular marker in which a DNA region situated between two similar microsatellite motifs aligned in opposite directions got amplified (Mohammad *et al.*, 2015). Knowledge on DNA sequence of study organism is not needed for ISSR marker study and can be undertaken for any plant species (Prakashkumar *et al.* 2015).

The present investigation has been undertaken with the major objective of 'mapping of the phytochemical diversity of *Cinnamomum verum* J. Presl in South India through secondary metabolite profiling'. The diversity has also been studied throughinterdisciplinary approaches including morphologicaland molecular evaluation. An attempt has also been made to discover high flavor quality elite lines of *Cinnamomum verum* in south India, that can be highlighted for large scale propagation.

Plant Profile

***Cinnamomum verum* J. Presl**

Cinnamomum belongs to family Lauraceae.The name *Cinnamomum zeylanicum* means "true cinnamon" in Latin and really is 'true cinnamon' as opposed to other species in the genus *Cinnamomum.*

Common name: Ceylon cinnamon

Habit: Tree

Habitat: Subtropical to tropical area with full tropical sunshine and moist soil

Flowering and Fruiting: October to February and May to June respectively

Distribution: Sri Lanka, India and Australia

Systematic position

Domain	:	Eukarya
Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Laurales
Family	:	Lauraceae
Genus	:	Cinnamomum
Species	:	*Cinnamomum zeylanicum*

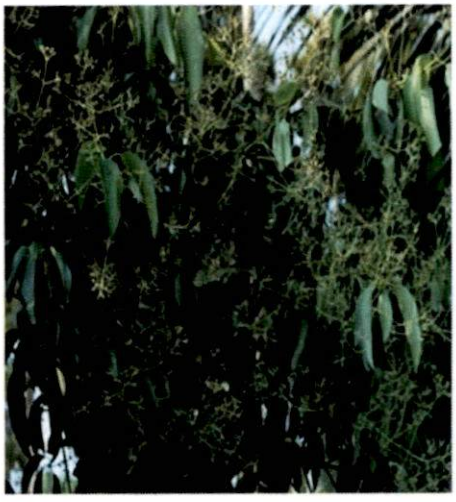
Plant description

The plants are ten to fifteen meters in height. The leaves are ovate-oblong in shape and seven to eighteen centimeters in length. The flowers, which are set in panicles, have a greenish shade and a distinct aroma. The plant can be effectively identified by its venation, as the noticeable element is petiole with three ribs.

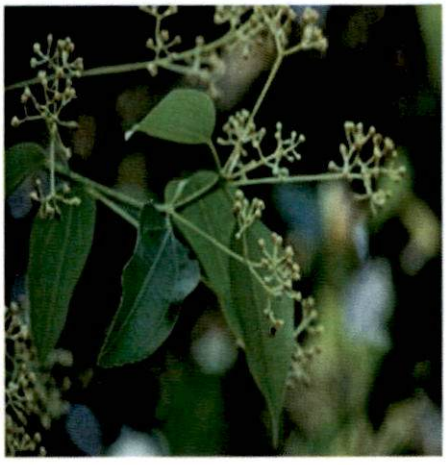
Cinnamomum verum,called true cinnamon tree or Ceylon cinnamon tree, is a little evergreen tree belonging to the family Lauraceae. The old natural equivalent word for

the tree *Cinnamomum zeylanicum* has evolvedfrom Sri Lanka's previous name, Ceylon. Sri Lanka still delivers 80-90% of the world's supply of *Cinnamomum verum*.

(a) *C. verum* plant habitat



(a) *C. verum* plant



(a) *C. verum* flower

(a) *C. verum* fruit

Fig 1: *Cinnamomum verum* J. Presl

17

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

Diversity of plants in south India

Geographical area of the India represents about 2.4% of the world's total landmass; it harbours a total of 47,513 plant species out of about 0.4 million hitherto known plant species in the world, representing as much as 11.4% of world flora (Arisdason and Lakshminarasimhan, 2016). About 28% of plants that occur in India are endemic to the country. South India is the area encompassing the Indian states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana, occupying 19.31% of India's area (635,780 km^2). South India is a peninsula in the shape of an inverted triangle bound by the Arabian Sea on the west, by the Bay of Bengal on the east and Vindhya and Satpura ranges on the north.There is a wide diversity of plants and animals in South India, resulting from its varied climates and geography. The Western Ghats is one of the eight hottest biodiversity hotspots in the world and a UNESCO World Heritage Site and it is a chain of mountains of 1600 km in length running parallel to West coast of Peninsular India from the river Tapthi to Kanyakumari. Status shows that southern Western Ghats is the richest area in context to floristic composition with 1286 species of endemic taxa (Nayar, 1996; Muthuramkumar, 2002) has reported that the Western Ghats is a reservoir of 4780 plant species of which 2180 species are endemic which is 45.6% of the Western Ghats and 0.7 % of the earth's endemic plants. South India is a centre of origin and diversity of several important food and spice crops. The genus *Cinnamomum* belonging to the family Lauraceae is one such important group of plants distributed widely in south India. Though the important species *Cinnamomum verum* is native to Sri Lanka, nearly 23 *Cinnamomum* species were naturally distributed in south India.

Distribution of *Cinnamomum*species (Family: Lauraceae)

Members of the Lauraceae family are nearly all woody trees and shrubs comprising 30 to 50 genera and 2,000 species. The name 'Lauraceae' was derived from the well-known member, the Grecian laurel, *Laurus nobilis* L. and is characterized by plants which have prominent oil cells in the leaves, wood and fruits. *Lauraceae* Consists of genus *Cinnamomum (* species: *aromaticum, iners, glanduliferum, camphora, cassia, culilawan,kanahirai, loureirii, micranthum, obtusifolium, xanthoneuron, zeylanicum,tamala); Ocotea (*species: *caudata, cymbarum, parviflora, pretiosa, sassafras)Sassafras (*species: *albidum); Cryptocaria (*species: *massoia); Laurus (*species: *nobilis); Umbellularia (*species: *californica)* and*Aniba (*species: *parviflora, rosaeodora)*(Joy*et al.*, 1995).

Economically, the family is very important and it is major source for medicine, timber, fruits, spices and perfumes. Maridass and Victor (2008) suggested that the *Cinnamomum* species namely, *C. walaiwarense*, *C. travancoricum* and *C. malabatrum* were used for the treatment for stomach pain. Likewise, *C. riparium*, *C. sulphuratum*, *C. filipedicellatum* and *C. wightii* were used for treating wounds, fever, intestinal worms, headaches and menstrual problems. The extracts from *C. cassia* have been claimed to reduce inflammation and to decrease serum glucose, total cholesterol and platelet counts (Khan *et al.*, 2003). *Cinnamomum tamala* leaves are used extensively in the cuisines of India, Nepal and Bhutan, particularly in the Moghul cuisine of North India and Nepal and in Tsheringma herbal tea in Bhutan (Qin *et al.*, 2003). The genus *Litsea* has (*Litsea glutinosa* and *L. quinqueflora*) ethereal oils, they form important sources for spices and perfumes. The alkaloids like linderalactone, linderane, neolinderane, zeylanine, zeylanicine and zeylanidine were isolated from the roots of *Neolitseazeylanica* reported the advanced flavonoids from some other members of lauraceae such as *Lindera*. Dried leaves of the *Laurus nobilis* L. (bay laurel) are used as a flavor for meat and fish dishes. A fat extracted from the seeds is used for making soaps. The spice cinnamon, is obtained from the inner bark

of *Cinnamomum verum* in India and Sri Lanka. The dried bark contains volatile oils, settled oil, tannin, sap, proteins, cellulose, pentosans, starch and minerals. Eugenol oil, distilled from the green leaves is widely used as a substitute for clove oil, as an ingredient in some perfumes, and as a flavouring agent for sweets, foods, and toothpaste. Camphor oil and safrole oil, are obtained from *Cinnamomum camphora* a local tree in China, Japan and Taiwan is utilized as a part of scents, for flavorings and furthermore utilized as a part of pharamaceuticals industry.Safrole, a segment in the camphor oil utilized as a part of fragrances, for flavorings and furthermore utilized in pharamaceutical industry. Numerous *Cinnamomum*species are developed locally for wood or medicine around the world. In Asia, a few *Litsea* species are developed for timber and essential oil extraction for the fragrance business, and for sustainable development of natural resources (Nath *et al.* 1996).

Cinnamomum species areindustrially importantsource of camphor, cinnamaldehyde and safrole oil. *Cinnamomum* is the greatest assortment in the Lauraceae family which includes 250 species and among that *Cinnamomum verum* is the most important.It is the second most important spiceall over the world.

Cinnamon is well documented in ***Horthus Malabaricus***(1678), as *Carua*, and by *Babena*, by another name *Tiqui*, in the language of Brahmins. It is a tree rising to the height of four or five men. Stem, is with branches covered with green bark which then with the age turns red and surrounds the wood with a thin membrane and is covered with ash coloured crust, and with hard wood and its thin bark in its time peeled and dried in the sun is the very cinnamon. Leaves arise in row, broadly grooved petioles and are oblong- round in shape,with the length almost twice the width. From the petiole, three light green and yellow ribs come out with the midrib which is somewhat thicker, one on both sides. Flowers arise collectively or umbels light green petioles, which come out from above the origin of leaves and are star shaped and small with pleasing smell; in the middle is a core consisting of two series of stamens in the outer series six bright green stamens, standing above the other light

green stamens. Berries are oblong- round in shape. This tree bears flowers once a year in January.

Cinnamomum verum is common in south India and are cultivated in different parts of the region. However, the market samples of cinnamon in India originate from *Cinnamomum verum*plantations from Sri Lanka. In addition, barks of several other *Cinnamomum* species such as *Cinnamomum cassia* and*Cinnamomum tamala* were also being used as substitute or adulterant to true cinnamon, due to the cheap price of the substitutes in the spice and food flavouring market. However, consuming high amounts of substitutes like*C. cassia* is not recommended due to the high content of the toxic compound coumarin.

History of Cinnamon

Cinnamon has been used as a spice for thousands of years. In Egypt, cinnamon was a spice used in embalming fluids for preserving cadavers. In Ayurvedic medicine, the world's oldest system, cinnamon bark was used as an antiemetic, anti-diarrheal, anti-flatulent and also as a general stimulant. Moreover, The Portuguese found cinnamon trees growing in Sri Lanka (Ceylon) in the early 16th century; they imported cinnamon to Europe during the 16th and 17th centuries afterwards. The Dutch occupied Sri Lanka in the mid-17th century until the British captured the island in 1796. The East India Company then became the main exporter of cinnamon to Europe. The Dutch cultivated cinnamon in Java and the exports of Ceylon cinnamon decreased as a result of heavy export duties. In spite of that, Sri Lanka is the only regular supplier of cinnamon bark and leaf oils. The food industry chooses Ceylon cinnamon, but pharmaceutical manufacturers use both, oils from Ceylon cinnamon (cinnamon oil) and from Chinese cinnamon (cassia oil) interchangeably. China is the foremost exporter of cassia cinnamon. (Wijesekera,1978).

Cinnamon belongs to the herbal substances with one of the longest medicinal traditions.Conversely, There is no real reference to Ceylon as it is original source.

From the 8th century, cinnamon was introduced in Europe as an expensive spice that only could be used by kings and popes. There is a written report on the use of cinnamon in an Arabic source from around 1275. In 1310, Johannes of Montevino confirmed the existence of cinnamon trees in Ceylon. About 100 years later, Nicolo Conit accommodated a real prescription of the cinnamon tree.

Introduced by the Portuguese, cinnamon of Ceylon remained the preferred substance in Europe. It has been described, that in 1536 cinnamon of Ceylon would have cost 40 times more than the cinnamon from Java or the Philippines. The herbal substance was first accumulated from wild growing trees. The farming of cinnamon began around 1765, when Holland took the lead in the origin area. The manufacturing was streamlined in the way that a better quality was obtained and Holland could contribute enough cinnamon to cover the European needs. When the English took over in 1796, the cinnamon cultivation and trading became a dominance of the English Eastern-Indian Company. Around 1833, when Holland developed the cultivation of cinnamon in Java and Sumatra, the herb and the essential oil were introduced into medicine as a stimulant. The oil was contrived for the first time by St. Amando of Doornyk at the end of the 15th century.

Morphological diversity in *Cinnamomum verum*

Forty seven representative cinnamon accessions were collected from Matara District of Sri Lanka to analyze the morphological variation of *Cinnamomum verum* germplasm. Morphological characters *viz*. leaf length, leaf width, leaf length-width ratio, petiole length, leaf arrangement, leaf shape, leaf apex, leaf base, leaf texture, upper surface leaf color, flush color, bark color, bark surface, and bark fragrant were recorded. Principal component analysis (PCA) using four quantitative morphological characteristics, indicated that the first two principal components (PCs) with Eigen values of more than one and accounted for 88.88% of the total variance (Azad *et al.*, 2016).

According to a previous study leaf shape of cinnamon varies from oval or elliptic to lanceolate-oval or narrowly elliptic, 3 × 7 – 8 × 25 cm, leaf apex shortly or broadly acuminate and leafbase acutish or cuneate (Ravindran *et al.*, 2004). Cinnamon flower exhibits protogynous dichogamy and it is cross pollinated (Joseph, 1981). Thus, vegetative propagation is necessary for producing uniformly high yielding populations and for propagating elite lines. A core collection is a powerful material for evaluation of germplasm, identification of trait-specific accessions, gene discovery, allele mining, genomic study, marker development, and molecular breeding (Qui*et al.*, 2013). Cluster analysis followed by Principal component analysis had been used to cluster *Cinnamomum* spp. into groups and to show relationship among the species on the basis of morphological characters (Ravindran *et al.*, 1991).

Morphological variation of a crop indicates the genetic diversity and effect from environment. Both environmental and genetic effects contribute to phenotypic variation within and among populations (Allard and Bradshaw, 1964; Andrew *et al.*, 2010).

Karyology

Okada and Tanaka, (1975) provided a summary on the cytogenetics of South American Lauraceae. The base number is x = 12 (2n=24), with very little variation in the karyotype throughout the family. Polyploidy has however been observed in Cassytha, Laurus, Litsea and Sassafras (2n=48), and in species of Laurus and Neolitsea aciculata (2n=72). Metaphase chromosomes are 1–3 mm long (5- 7 mm in Cassytha), the position of centromere varies from median to sub terminal and heterochromatin is found only in the proximal regions of both arms.

Traditional uses of *Cinnamomum verum*

Asolkar *et al.*, (1981) suggests the inner bark of*C. verum*is acrid, bitter, sweet and aromatic and it is reported to be used in treating bronchitis, asthma, cephalagia, uropathy, nausea, vomiting, flatulence, fever, for preparing flu-preventive,

indigestion and for restoring normal skin colour. It was reported thateating cinnamon has been shown to reduce inflammation, improve memory,increase attention and enhance cognitive processing. It is a great spice for improving our immunity, with all the necessary minerals, vitamins and fibers all packed into one. Also it plays a great detoxification role.

Cinnamon oil is stomachic and carminative.It relieves stomach upset and gas, diarrhea, and various other ailments. It is also applied in the treatment of dyspeptic conditions of the gastro intestinal tract, fullness and loss of appetite and to cure abdominal pain with diarrhoea, amenorrhoea and dysmenorrhoea. Reduce pain of minor cuts and abrasions. Cinnamon aids in digestion by stimulating salivation, its volatile oils break down fats in the digestive tract, stimulate movement in the gastrointestinal tract, and has carminative (gas-reducing) and astringent properties. Also, cinnamonrefresh flavor in mouth. Also it has antiseptic properties that kill bacteria whichleadto tooth decay and gum disease.

Pharmacological Properties

Cinnamomum zeylanicum have beneficial effects both *in vitro* and *in vivo* as a potential therapeutic agent for diabetes (Ranasinghe *et al.*, 2013). The strong antimicrobial, anti-carcinogenic properties of the essential oil of *Cinnamomum zeylanicum* bark, indicating the possibilities to treatment of infections and neoplasms (Unlu *et al.*, 2010).Balijepalli*et al.*, 2017, conducted a study on biological activities (*in vitro* and *in vivo*) of extracts and constituents from *Cinnamomum*. Crude extracts and constituents from about 30 species of *Cinnamomum* displayed significant antibacterial, antifungal, antiseptic, antiviral, anti-inflammatory, antipyretic, antioxidant, chemopreventive, cytotoxic, antidiabetic, hypolipidemic, antispasmodic, antiulcer, antiplatelet, anodyne, choleretic, immunostimulant, anaesthetic and sedative activities. Essential oil, aqueous/alcoholic extracts, cinnamaldehyde and proanthocyanidins were reported to be mainly responsible for biological activities displayed by most of the plants. Regarding sugar metabolization, researches found

that cinnamon is bioactive in directly stimulating cellular glucose metabolism i.e., the ability of cells to utilize sugar, the effects of cinnamon for potentiating insulin.

i)Anti-oxidant potential of *Cinnamomum verum*

Antioxidant activities of the methanolic concentrates of *Cinnamomum verum* barks (CBE) was determined and contrasted with mixes like butylated hydroxyl anisole, trolox and ascorbic corrosive. By goodness of their hydrogen giving capacity, the greater part of the tried mixes and CBE showed reducing power.They were found to be potent in free radical scavenging activity especially against DPPH. radicals and ABTS radical cations. The hydroxyl (.OH) and superoxide radicals were also scavenged by the tested compounds. CBE also exhibited metal chelating activity. The peroxidation inhibiting activity of CBE recorded using a linoleic acid emulsion system, showed very good antioxidant activity(Mathew and Emilia, 2004).

The antioxidant activities of the leaves and stem bark methanol and ethyl acetate extracts of *C. verum*from the Democratic Republic of Congo were assayed using total phenolic content, diphenylpicrylhydrazyl radical (DPPH), ferric reducing, iron chelating and thiobarbituric acid (TBA) assays. The methanol solvent extracted high content of phenolics from the leaves and stem bark. The bark methanol extracts exhibited good DPPH activity (IC_{50}= 76.5 µg/mL) than the leaves extract (IC_{50}= 100.2 µg/mL). The methanol extracts of leaves (IC_{50}= 98.5 µg/mL) and stem bark (IC_{50}= 72.3 µg/mL) exhibited a good metal chelating capacity. In the TBA assay the leaf and bark methanol extracts displayed high activity (81-85 % inhibition)(Mazimba *et al.*, 2015).

ii) Antimicrobial activity of *Cinnamomum verum*

Studies evaluating *invitro*anti-microbial properties of *C. verum*showed potentialanti-microbial action against a wide variety ofbacteria (*Acinetobacter baumannii, Acinetobacter lwoffii,Bacillus cereus, Bacillus coaguiaris, Bacillus subtilis,Brucella melitensis, Clostridium difficile, Clostridiumperfringens, Enterobacter aerogenes,*

Enterobacter cloacae,Enterococcus faecalis, Enterococcus faecium, Escherichiacoli, Haemophilus influenza, Helicobacter pylori, Klebsiellapneumonia, Listeria ivanovii, Listeria monocytogenes,Mycobacterium smegmatis, Mycobacterium tuberculosis,Proteus mirabilis, Pseudomonas aeruginosa, Saccharomycescerevisiae, Salmonella typhii, Salmonella typhimurium,Staphylococcus albus, Staphylococcus aureus, Streptococcusagalactiae, Streptococcus pneumoniae, Streptococcuspyogenes and *Yersinia enterocolitica*). In addition, they showed activity against numerous fungi (*Aspergillusfiavus, Aspergillus fumigatus.Aspergillus nididans, Aspergillus niger, Aspergillusochraceus, Aspergillus parasiticus, Aspergillus terreus,Candida albicans, Candida glabrata,* and *Candida krusei*. CZ extract also demonstrated activity against human rota virus(Ranasinghe, *et al*., 2013).

iii) Anti-diabetic activity of *Cinnamomum verum*

A recent analysis on the effects of *C. verum* extractson diabetes demonstrates numerous beneficial effects both*in-vitro* and *in-vivo*. *C. verum*has demonstrated*in-vitro* potential for; a) reducing post-prandial intestinal glucoseabsorption by inhibiting the activity of enzymes involvedin carbohydrate metabolism (pancreatic α–amylase andα–glucosidase), b) stimulating cellular glucose uptake bymembrane translocation of GLUT-4, c) stimulating glucosemetabolism and glycogen synthesis, d) inhibitinggluconeogenesis by effects on key regulatory enzymes andf) stimulating insulin release and potentiating insulin receptor activity. Cinnamtannin B1 was identifiedas the potential active compound responsible for these effects.. The beneficial *in-vivo*effects of *C. verum*includes;a) attenuation of weight loss associated with diabetes,b) reduction of Fasting Blood Glucose, c) reducing LDLand increasing HDL cholesterol, d) reducing HbA1c ande) increasing circulating insulin levels. In addition *C. verum*also showed beneficial effects against diabetic neuropathy and nephropathy (Ranasinghe, *et al*.,2012 and Bandara *et al*., 2012).

Other uses of *Cinnamomum verum*

An aqueous extract of *C. verum* is known to inhibit tau aggregation and filament formation,which are hallmarks of Alzheimers disease(Peterson *et al.*,2009). *C. verum* extracts have strong inhibitory effects on osteoclastogenesis(Tsuji-Naito,2008). A study conducted by Takasao *et al.*,2012 shows that the plant extract has remarkable effect in treatment of anti-aging of skin. Also plant extracts exhibited activity against mosquito larvae.

Phytochemistry of *Cinnamomum verum*

Apart from the primary metabolites, plants particularly those of certain genera and families synthesize a number of organic compounds which are not in the mainstream of metabolism and appear to have no direct function in the growth and development of plants . These compounds are extremely numerous and chemically diverse in nature and are called as secondary metabolites. Secondary metabolite is derived from primary metabolite through various bio-synthetic pathways. Secondary metabolites are in principle non-essential to plant life but they definitely contribute to the survival of plant species. The secondary metabolite helps to communicate or respond to environment as in the case of plant –plant microorganism and plant animal interaction in order to combats the external factors such as temperature, seasonal change, light, wind, flooding etc. Plant produces a series of secondary metabolites and stores them in various organs like leaf, root, stem, flowers, and fruit for immediate and future use.Presently, 100,000 such compounds have been isolated from higher plants. The biosynthesis of secondary metabolites varies among plants, even in different organs of plants, and their biosynthesis depends on the environmental factors in which they grow (Khan *et al.*, 2003).

Intra-specific variation in phytoconstituents has been documented extensively among the plants. Many environmental factors such as precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period, and the intensity of radiation under clear sky

conditions have been reported to differ between low and high altitude sites (Korner 1999). Moreover, study on phytochemicals of wild populations of plants at different altitudes were performed, and it is not conclusive whether the observed variations are a response of individual plants to environmental factors related to altitude or a genetic adaptation of the populations growing at different altitudes to their specific environment (Zidorn *et al.*, 2005).

Genus*Cinnamomum* produces various types of secondary metabolites.Based on previous studies, the most common secondary metabolites compound found in the *Cinnamomum* species are phenyl propanoids, alkaloids, proantocyanidin, flavonoids, and terpenoids.

i)Volatilechemical profiling

The essential oil of cinnamon bark growing in south-west Ethiopia was obtained by hydrodistillation andanalysed through gas chromatography-mass spectrometry. GC-MS and FTIR spectra, combined with microscopy were used for the detection of essential oil composition in various *Cinnamomum* species (Adinew, 2014).

Several*Cinnamomum* species are distilled on a much smaller scale and the oils used either locally or exported. Mallavarapu *et al.*, 1995has identified 53 constituents along with the major component eugenol in *Cinnamomum* leaf oil. Sandigawad and Patil (2011) identified camphor, β- terpineol, heptanal, safrole, and trans-cinnamaldehyde from *C. verum* oil.

Wong*et al.*, 2014 conducted a study on extraction of essential oil from cinnamon (*Cinnamomum zeylanicum*). The essential oil from plant *C. verum*was extracted using two methods which were steam distillation and Soxhlet extraction. Steam distillation produced high quality essential oil extraction using separatingfunnel. Soxhlet extraction produced essential oil in crude form, using rotatory evaporator the extracted productwere purified. Cinnamon essential oil contains high cinnamaldehyde content which is the main component in cinnamon.

The percentage of cinnamaldehyde in essential oil from steam distillation was about 90% and 62-73% from Soxhlet extraction.

The essential oil from the bark of *Cinnamomum zeylanicum*are (E)-Cinnamaldehyde (68.95%), benzaldehyde (9.94%) and (E)-cinnamyl acetate (7.44%) (Unlu *et al.*, 2010). The bark and fruits of *C. zeylanicum* were found to contain proanthocyandins with doubly linked bis-flavan-3-ol unitsin the molecule.(Jayaprakasha & Rao, 2011).The essential oil profiles of leaf, bark and fruit stalks of *C. verum* through GC-MS and eugenol was the major compound in leaf, cinnamaldehyde in bark, cinnamyl acetate and caryophyllene as the principal components of fruit stalks (Jayaprakasha *et al.*, 1997).

Patel et al., 2007analysedleaf essential oils of *C. verum*of Fijian origin by GC and GCMS. The paper reports the characterization of thirty one components of the Fijian *Cinnamomum verum* leaf essential oil, of which eugenol (86.02 %), (E)-caryophyllene (5.70 %) and linalool (2.30 %) were the major constituents. From the Indian *Cinnamomum verum* leaf essential oils, only nine components were previously reported. The differences in the Fijian *Cinnamomum verum* leaf essential oils, when compared with the Indian *Cinnamomum verum*leaf essential oils could be due to the location of the plant collected, the time of the plant collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of components identified could be due to the type of instrument used and the parameters used for analyzing the essential oil.

Leaf oil is heavier than water, yellow to yellowish brown in colour with a slight camphoraceous odour resembling that of clove oil due to the presence of 70-95% of eugenol. Leaf oil has specific gravity (15.5°C) 1.065, refractive index (20°C) 1.530 to 1.545, optical rotation (20°C) -1° to +3°, acid value 14.0-15.7, ester value 4.7-16.7 and its solubility in 70%alcohol is 10 volumes. Leaf oil contains approximately 0.2% β-pinene, 1.65% 1, 8-cineole,0.35% p-cymene, 0.25% a-ylangene, 1.5% linalool, 1.85% caryophyllene, 0.2%a-humulene, 0.15% a-terpineol,

0.1% piperitone, 0.65% safrole, 1.3% cinnamaldehyde,0.8% cinnamyl acetate, 87% eugenol, 1.0% acetoeugenol, 0.60% cinnamyl alcohol, 2.68%benzyl benzoate and traces of over 15 compounds (Gulathi, 1982).

Cinnamaldehyde is an oily yellow liquid at room temperature with a boiling point of 246°C. It is mainly used as a flavoring agent or as a scent of candles. It is also non-toxic but may irritate the skin if in contact for too long. Moreover, there are various biological activities shown by cinnamaldehyde such as antioxidant, antiviral, antifungal and antibacterial. Cinnamaldehyde is a natural antioxidant and the animal studies suggest that the extracted cinnamon bark may help to prevent stomach ulcer.In addition, it can completely inhibit both sensitive and resistant strain of *Helicobacter* (Joshi *et al.*, 2009).

Leaf oil is a yellow to brownish yellow liquid with a warm, spicy and somewhat harsh odour, lacking in the richness of bark oil. Its taste is slightly bitter, burning, very spicy and powerful. Eugenol content of Sri Lankan leaf oil ranges 60-85% depending on the place of origin. Seychelles leaf oil is a valued source of eugenol usually above 90% with phenols 78-95% and aldehydes 5%. Major uses of leaf oil are in food processing and pharmaceutical industries.

Leela and Rema (2008) conducted Chemical characterization of *Cinnamomum* germplasm at IISR, Calicut. They listed Cinnamon germplasm in terms of its chemical components and identified the lines with desirable quality traits for further improvement. 215 Accessions of *C.verum* were analysed for bark oil and bark oleoresin contents and bark oil constituents; and the accessions with high bark oil and bark oleoresin contents were identified. Chemical constituents of bark oil of five *Cinnamomum* species were determined. 213 Accessions of *Cinnamomum* which included 198 *C. verum* accessions and 15 *C. cassia* accessions were analysed for leaf oil content and composition. In *C. verum,* accessions with high eugenol content and in *C. cassia* those with high cinnamaldehyde content were identified. Based on leaf oil constituents, 3 chemotypes of *Cinnamomum verum* were identified. Chemical finger printing of essential oil of leaf of eleven species of *Cinnamomum,*

were carried out. Variation in essential oil composition of aerial parts of *Cinnamomum* species was studied. In four *Cinnamomum* species, optimum period of harvest for obtaining high leaf oil yield was determined. From leaf extracts of *C. tamala* and *C. malabatrum* two crystalline constituents were isolated and characterized.

ii) Non-Volatile chemical profiling

Phytochemical screening of cinnamon barkextractsshowed the presence of secondary metabolites. The extract of plant was evaluatedby standard screening method. Qualitative phytochemical analysis of this plant confirms presence of various phytochemicals like alkaloids, flavanoids, tannins, steroids and glycosides in their solventextracts (Pandey *et al.*, 2014).

Quantification of marker compounds in different accessions of the plant collected from different locations can be carried out with thin layer chromatography, which is a powerful and simple technique used for this purpose and high-performance thin layer chromatography (HPTLC) method. The qualitative as well as quantitative HPTLC analysis under standard conditions provides chromatograms, which are very useful for assessing the quality of phytopharmaceuticals(Singh *et al.*, 2007).

Abraham *et al.*, (2009) conducted a study on toxic compound coumarin present in *Cinnamomum* species. Coumarin is a secondary phytochemical with hepatotoxic and carcinogenic properties. European Food Safety Authority in 2004 suggested a tolerable daily intake (TDI) for the first time, and a value of 0.1 mg/kg body weight was arrived at based on animal hepatotoxicity data. Using the human data, a TDI of 0.1 mg/kg body weight was derived, confirming that of the European Food Safety Authority. Nutritional exposure may be considerably, and is mainly due to use of cassia cinnamon, which is a popular spice especially, used for cookies and sweet dishes.

Coumarin as an additive or as a constituent of tonka beans or tonka extracts is banned from food in the United States due to its potentially adverse side effects.

However, coumarin in food from other natural ingredients is not regulated. "True Cinnamon" refers to the dried inner bark of *Cinnamomum verum*. Other cinnamon species, *C. cassia*, *C. loureiri*, and *C. burmannii*, commonly known as cassia, are also sold in the U.S. as cinnamon. In the present study, coumarin and other marker compounds were analyzed in authenticated cinnamon bark samples as well as locally bought cinnamon samples, cinnamon-flavored foods, and cinnamon-based food supplements using a validated UPLC-UV/MS method. The experimental results indicated that *C. verum* bark contained only traces of coumarin, whereas barks from all three cassia species, especially *C. loureiroi* and *C. burmannii*, contained substantial amounts of coumarin. These species could be potential sources of coumarin in cinnamon-flavored food in the U.S. Coumarin was detected in all locally bought cinnamon, cinnamon-flavored foods, and cinnamon food supplements. Their chemical profiles indicated that the cinnamon samples and the cinnamon in food supplements and flavored foods were probably Indonesian cassia, *C. burannii*.(Yan-hong wang *et al.*,2013).

Wang *et al.*, 2013 analysed thecoumarin and other marker compounds in authenticated cinnamon barksamplesas well as locally bought cinnamon samples, cinnamon flavored foods, and cinnamon basedfood supplements using a validated UPLC-UV/MS method. The experimental results indicated that *C. verum*bark contained only traces of coumarin, whereas barks from all three cassia species, especially *C. loureiroi* and *C. burmannii* contained substantial amounts of coumarin.

Genetic diversity in *Cinnamomum verum*

During the last decade, several novel DNA markers (RAPD, RFLP, SSR, ISSR, etc.) have been rapidly integrated into the molecular tools available for genome analysis (Salimath *et al.*, 1995). DNA bands are treated as unit characters and its presence or absence in the amplicon may be used to study genetic relationship (Sang and Soren, 2000) and inter-and intra-specific genetic variations (M' Ribu and Hilu, 1994).

Sandigawadand Patil (2011) assessed genetic diversity in *Cinnamomum zeylanicum* Blume using random amplified polymorphic DNA (RAPD) markers. RAPD-PCR analysis involving 11 decamer random primers was used to assess the genetic variation within *C. zeylanicum* in Western Ghats of southern India. Some primers showed appreciable intra-species variation or molecular polymorphism at amplicon levels. Despite morphological similarity, a great deal of genetic polymorphism was observed among the accessions. In this study, unweighed pair group method with arithmetic averages (UPGMA) analysis showed up to 89% genetic variation among these accessions, which is further supported by principle co-ordinate analysis (PCA).

An investigation study on "Identification ofSri Lankan *Cinnamomum* species" using fourteen RAPD primers and 20 sets of SRAP primer combinations gave amplification products. However, one set of SRAP primer combinations produced more markers for *Cinnamomum* species than the RAPD markers. Amplification products from both techniques could be categorized as genus specific, species specific and intra-species specific markers. Both polymerase chain reaction (PCR) based techniques employed in this study identified the species, estimated the genetic diversity of *Cinnamomum* spp. and detected polymorphism, in the accessions of germplasm collected at the Department of Export Agriculture, Sri Lanka (Abeysinghe*et al.*, 2014).

A previous study suggests that, different species of *Cinnamomum* are rich in polysaccharides and secondarymetabolites, which hinder the process of DNA extraction. High quality DNA is the prerequisite for any molecular biology study.This paper had reportedmodified method for high quality and quantity of DNA extraction from both lyophilized and non-lyophilized leaf Samples. Protocol reported differs from the CTAB procedure by addition of higherconcentration of salt and activated charcoal to remove the polysaccharides and polyphenols.Wide utility of the modified protocol was proved by DNA extraction from different woody species and 4 *Cinnamomum* species. Therefore, this protocol has also been validated in different

species of plants containing high levels of polyphenols and polysaccharides. The extracted DNA showed perfect amplification when subjected to RAPD, restrictiondigestionand amplification with DNA barcoding primers. The DNA extraction protocol is reproducible and can be applied for any plant molecular biology study (Bhau *et al.*, 2015).

Some molecular study has been conducted to evaluate genetic differences on *Cinnamomum* species (Sandigawad and Patil, 2011; Kuo *et al.*, 2010). A work was carried out to identify *Cinnamomum* species using reliable approach like RAPD and SRAP techniques. Some primers gave highly polymorphic banding patterns using these techniques. The study showed that using these molecular markers, it is possible to identify the *Cinnamomum* species (genus specific and species specific) and intra-species variations (Abeysinghe *et al.*, 2014).

Major Diseases found in *Cinnamomum* species

Studies during 1940 reported that nymphs and adults of Pauropsyila depressa Crawford (Homoptera: Triozidae) produced galls on leaves and shoots of cinnamon in India for the first time (Ayyar, 1940). Subsequent empirical study on plant galls reported various types of plant galls viz., globose or irregular swellings of the inflorescence axis, pit gall, pouch or epiphyllus, hypophyllus, biconvex green, conical and rugose pouch gall on the various species are *Cinnamomum zeylanicum, C. camphora, C. nitidum, C. macrocarpum, Machilus gamblei, M. macrantha, M. odoratissima, Phoebe lanceolata, Lindera assamica, L. pulcherrima, Litsea polyantha, L. glabrata, L. ligustrina, Neolitsea zeylanica, Beilschmiedia sikkimensis* (Mani, 1965). In addition this study also prepared a dichotomous key for the identification of the plant galls. Rajapakse and Kulasekera (1982) have listed the pest fauna associated with cinnamon in Sri Lanka for the first time in Sri Lanka. Devashayam *et al.* (1997) reported that the major insect pests of cinnamon, which include cinnamon butterfly Chilasa ciyua and leaf miner *Conopomorpha civica* in India. Rajeev and Dinesh (2005) reported the leaf spot and die back disease

(*Colletotrichum gloeosporioides*), seedling blight caused by Diplodia sp. and Grey blight caused by *Pestalotia palmarum*. They also recognized the attack of leaf miner infestation by the Conopomorpha civica and cinnamon butterfly (*Chilasa clytia*), a most serious pest in younger plantations of *Cinnamomum verum*.

It is also reported the occurrence of White Root disease in cinnamon, caused by a fungus known as Fomes noxis (Anonymous, 2013). White colour fungal mycelia growths can be observed on roots of infected plants. Other minor diseases are leaf blight, black powdery mildew algae growth on leaves. They also recognized the attack of Pink Stem Borer (*Ichneumoniptera* cf. xanthosoma). Rough bark disease is the most common disease of cinnamon which affects on young bark of immature shoots as brown spots and spread gradually throughout the bark. Leaves of the infested plants show chlorosis.

Propagation

The plant is propagated mainly by seed. The major drawback of seed propagation is that, cinnamon being a cross-pollinated plant, exhibits wide variability in yield,quality of produce and oil content and other morphological characteristics. Hence, seed propagation is not advisable while clonal propagation is recommended in cinnamon. Air layering is also a successful method of vegetative propagation. But seasonal variation in rooting pattern has also been observed in air layers.

Joy *et al.*,(1998) reported cinnamon selections for crop improvement. Lack of genetic variability for resistance to major diseases and pests and poor availability of quality seeds make conventional breeding programme ineffective and hence micro propagation is needed in the production of large number of elite lines within a short time span.

Somatic embryogenesis has reported in *Cinnmomum camphora* and also has reported tissue culture works of *Cinnamomum verum* and *Cinnmomum cassia*. Deb *et al.* (2013) made a successful attempt for callus mediated plant regeneration of the species from leaf and zygotic embryos. The cultured foliar explants developed callus

on MS medium fortified with sucrose (3%, w/v), PVP (100 mgl-1) and BA + NAA (6 and 3 μM respectively in combination) responded positively.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled 'Evaluation of Phytochemical diversity of *Cinnamomum verum* in south India' was carried out at Phytochemistry and Phytopharmacology Division of Jawaharlal Nehru Tropical BotanicGarden and Research Institute, Palode, during 2016-2017 academic year.

3.1 Chemicals and Equipments

All chemicals were of analytical grade. Chemicals used were Magnesium turnings, Sodium hydroxide, Ethanol, Sulphuric acid, Chloroform, Ethyl acetate, HCl, Acetic acid, Acetone, n-Hexane, Methanol, Fehling's solution A and B, Ammonium sulphate, Aluminium chloride, Potassium acetate, Acetic Acid, Acetylsalicylic acid,Acetone, PVP (Polyvinylpyrollidone),Agar, TBE, Ethidium bromide. Equipments like Clevenger apparatus, Soxhlet, Rotavapour, Weighing balance, Centrifuge,spectrophotometer,HPTLC, GC-MS Gel document system,.

3.2 Sample collection

The leaves, barks, tender leaves,flowers and fruitsof*Cinnamomum verum* were collected from various locations of South India and JNTBGRI, Palode.The specimens of the plant material were deposited in the herbarium collection of the JNTBGRI.

3.3 Morphological study

Morphological examinations of the plant in its habitat were carried out. Morphological data on characters (qualitative and quantitative), were noted for collected samples in each locations. The character states of qualitative characters were identified visually and the character states of quantitative characters were determined based on mean value of 3 scorings in each.

Quantitative parameters like leaf length, leaf width, leaf length-width ratio, petiole length and qualitative characters like leaf shape, leaf apex shape, leaf base shape, tender leaf texture, leaf margin, venation, number of veins, bark color and bark fragrant were recorded.Dendrogram was constructed using SPSS software.

3.4 Phytochemical studies

3.4.1. Volatile chemical profiling

a)Isolation of essential oil

100 g leaves, bark and fruitwere cut into small pieces and hydrodistilledseparately in a Clevenger-type apparatus for 3 hr.The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator till further analysis.

b)Essential oil analysis

i) GC- MS analysis

GC-MS analysis of essential oil was carried out by 1µL injection of diluted essential oil on a ShimadzuTQ (Shimadzu, Japan) fitted with an Rx sil-5 capillary column, coupled with a model 5973 series mass selective detector. GC-MSoperation conditions.

Injection mode : Split (1:1 ratio v/v)
Injector temperature : 220^0C
Transfer Line temperature : 240^0 C
Detector temperature : 250^0 C
Oven temperature programme: 60-246^0C (3^0C/min)
Carrier gas : Helium at 1.4mL/min

ii) Determination of RRI

Relative retention indices (RRI) of essential oil constituents were determined on the HP-5column using C_8-C_{30} straight chain alkanes as standards. Individual constituents in the leaf oil were identified by WILEY database matching, comparison of mass spectra with literature data and comparison of their RRIs (Adams. 2007).

$$RRI=100[(Ex-Hn)/(HN+1-Hn)]+100n$$

Where Ex is the retention time of the compound (oil constituents),H_N and H_{N+i} are retention times of reference hydrocarbons with n and n+1 carbon items respectively.

iii) Identification of the components

The oil components were identified by MS library search (WILEY & NIST), relative retention indices (RRI) calculated with respect to homologous of n-alkanes and compared with literature data and by comparison with published mass spectra (Adams,2007).

iv) Dendrogram construction

GC-MS data was compiled in excel and using SPSS software dendrogram was plotted separately for bark and leaf samples.

3.4.2. Non-volatile chemical profiling

a) Preparation of extract

For phytochemical analysis, the leaves of *Cinnamomum verum* were cleaned with water and dried in oven until they become breakable and powdered using an electric blender. The powdered leaves of *Cinnamomumverum were* kept in a sealed polyethylene container until use. For extraction, powdered leaves of *Cinnamomum verum* (1g each) were extracted by Soxhlet apparatus using n-hexane solvent. Each filtrate was collected, concentrated and dried under vacuum at 50^0C using a rotary evaporator (Buchi, Rotavapor, R-3) and kept in desiccators at room temperature until analysed.

b)Phytochemical screening of methanol and hexane extracts

A small quantity of methanolic extracts and hexane extracts prepared from Soxhlet extraction was taken for the following chemical tests.

i) **Steroids and Terpenoids**

Leibermann-Burchard Test:10 mg of extract is dissolved in dry chloroform, into this acetic anhydride is added followed by a few drops conc.H_2SO_4.Green colour shoes the presence of steroids and pink colour shows the presence of terpenoids.

ii) Alkaloids

a) Dragendorff's test

10 mg of extract is dissolved in MeOH and few drops Dragendorff's reagent is added. An orange red precipitate shows the presence of alkaloids.

b) Wagner's test

To the extract add few drops of Wagner's reagent (dissolved 2 g of iodine 6 g of potassium iodide in 100 ml of water) gives reddish brown precipitate.

iii) Flavanoids

Shinoda's test:10 mg of extract is dissolved in MeOH. Mg turnings are added in to this, and then conc.HCl is added in drops. A pink colour shows the presence of flavoids.

iv) Coumarins

10 mg extract was dissolved in methanol and alcoholic KOH was added. Appearance of yellow colour, which decolourizes while adding conc. Hydrochloric acid, shows the presence of coumarins.

v) Saponins

Extract is dissolved in water and shaken well. Froth formation, which lasts for a long time, shows the presence of saponins.

vi)Carbohydrates

Molisch's test:About 1 g of the extract was dissolved in 1 ml water .Two drops of 1% alcoholic solution of α-naphthol was added.Then,1 ml conc. H_2SO_4 is carefully added along the sides of the test tube so that it forms a heavy layer at the bottom. Deep violet colour at the junction of two liquids indicates the presence of carbohydrates.

3.5. Thin Layer Chromatography (TLC) of extracts

TLC of hexane extracts of *Cinnamomum verum* leaves and barks were done using different solvent systems. The solvent system which gave best resolution for compounds observed was chosen for its column chromatographic separation of compounds. Varying polarities of hexane:ethylacetate combinations were chosen as solvent system.

Solvent System : Hexane: Ethyl acetate (9.5: 0.5)

Standard compounds: Eugenolandcinnamaldehyde(concentration- 1mg/mL in n-Hexane)

Using Thin Layer Chromatography 10µl of extracts (test samples) and 2µl of standard solution was spotted on a precoated silica gel 60 F_{254} TLC plate of uniform thickness of 0.2mm. The spotted TLC plate was kept in a beaker containing the solvent system. Kept the plate for few minutes till the solvent reaches the maxima. The developed chromatograms were air dried and derivetized using anisaldehyde-sulphuric acid reagent and heated at 110^0C for 5min.The $R_f$values were calculated using formula,

R_f= Distance travelled by the solute/ Distance travelled by the solvent.

Visualisation:

a) Iodine chamber: The plate was kept in iodine chamber for few minutes.

b) UV chamber : Observed under UV 254 nm and 365 nm.

c) Derivatization : Dervetized using anisaldehyde sulphuric acid reagent.

3.6 High Performance Thin layer chromatography

Chromatographic conditions

Stationary phase	: Pre-coated silica gel plate
Mobile phase	: Hexane: Ethyl acetate (95: 5)
Samples	: Hexane extracts of *Cinnamomum verum*
Marker compound	: Eugenol,cinnamaldehyde(1mg/mL in Hexane)

HPTLC was carried out using CAMAG HPTLC system. All the n-hexane extracts of *Cinnamomum verum*prepared were analyzed using a pre-validated HPTLC method. 2µL of standards and 10 µL of n-Hexane extracts (Soxhlet extraction) of *Cinnamomum verum* leaf and bark were loaded on a pre-coated silica gel 60 F_{254} TLC plate (E-Merck) of 0.2mm thickness using sample applicator of HPTLC. The plates were developed in the above solvent system in a twin trough chamber to a distance of

8cm. The developed chromatograms were air dried, derivatised using anisaldehyde-sulphuric acid reagent and heated at 110^0C for 5min. After development, the plate was removed and dried and spots were visualized in CAMAG UV chamber at 580 nm.

3.7 Estimation of coumarin content

Extraction

1g of dried powdered bark wasextracted in cold with 10 mL acetone for 24 hours.Each filtrate was concentrated and dried under vacuum at 50^0C using a rotary evaporator (Buchi, rotavapor, R-3).GC-MS was performed using 1mg/ml sample of each extract.

GC-MS estimation of coumarin

Acetone extracts and standard coumarin at different concentrations were injected to GC-MS. From the standard graph obtained, the content of coumarin in extracts were calculated.

3.8 Genetic study

3.8.1.Isolation of Genomic DNA from leaf samples of *Cinnamomum verum*

DNA of collected plant samples were extracted using readymade DNeasy plant mini kit of QIAGEN.

Procedure

100mg of tender leaves were disrupted in the presence of liquid nitrogen using a mortar and pestle.20mg of polyvinylpyrrolidone (PVP) was added while grinding,to reduce polyphenol contamination. After that 400 µL AP1 buffer and 4 µL of RNase enzyme was added to it.The lysate was incubated for 10 minutes at 65^0C in a pre-set water bath.Added 130 µL of P3 buffer and incubated on ice for 5 minutes.The lysate was centrifuged for 5 minutes at 20,000g (14000 rpm). The lysate was transferred in to a QlAshredder spin column placed in a 2ml collection tube and again centrifuged for 20,000g.The flow- through waspipetted in to a new tube without disturbing the

pellet present. Using a pipette, 1.5 times the volumes of AW1 buffer was mixed to it. From that 650ul of mixture was transferred in to a DNeasy Mini spin column placed in a 2ml collection tube and centrifuged for 1 minute at 6000g.Repeated the same step with remaining sample and flow through was discarded. Again 500ul of AW2 buffer was added to spin column placed in a collection tube and centrifuged at 20,000g. Spin column was placed in a new 1.5 ml micro centrifuge tube and added 100ul of AE for elution. Then it was incubated at room temperature for 5 minutes and centrifuged for 1 minute.And the tubes were stored at -20^{0}C.

3.8.2. Gel Electrophoresis

0.8% gel was used for electrophoresis.

Procedure

0.8 g of agarose was weighed and dissolved in 100ml 1X TBE buffer and casted in a gel tray after adding 5 µL EtBr. 5 µL of each sample was mixed with 2 µL of tracking dye and loaded in an electrophoresis unit using.Voltage was set and kept undisturbed for 1 hour.And DNA was visualized using Gel Document system (UVP).

3.8.3. PCR amplification

Amplification of genomic DNA was performed with ISSR Primers (Table: 1). Each reaction was performed in 25 µL reaction volume, which contains buffer, dNTps, distilled water, primer, DNA polymerase and DNA (Table: 2).After mixing the PCR components, the reaction was carried out in an Applied Biosystems Thermal Cycler using the conditions mentioned in the Table:3.

Table 1: Details of ISSR primers used to analyze genetic diversity in 12 Cinnamon accessions

Sl. No	ISSR Primer	T_a (^{0}C)
1	46	47.6
2	816	52.6
3	824	55.0
4	856	55.0

45

Table 2: Reaction mixture

Sl. No	Reagents	Volume(μL)
1	Distilled water	10.59
2	Buffer	1.25
3	dNTps	0.03
4	Primer	0.07
5	DNA Polymerase	0.05
6	DNA	0.50

Table 3: Reaction condition

Sl. No	Stages	Temperature(°C)	Time
1	Denaturation	94°C	30s
2	Annealing	52°C	1min
3	Elongation	72°C	2min

The PCR condition above mentioned was repeated for 30cycles to produce the amplified PCR products. The amplified fragments were electrophoresed in 1.4% agarose gel. Then the gel was documented using a gel documentation system (UVP).

3.8.4. Data analysis and Dendrogram construction

Data from the ISSR marker analysis was scored for presence (1) and absence (0) of bands. Faint and unclear bands were not counted. The POPGEN ver 1.31 software used to measure the following parameters:observed number of alleles (na), effective number of polymorphic loci and percentage of polymorphism(p%). A dendrogram was constructed based on the genetic distance.

RESULTS

4. RESULTS

This diversity study was carried out by analyzing morphological, phytochemical and genetic variations of plant materials from different locations of south India.16 accessions of *Cinnamomum verum* were used for the study. The results were tabulated and are given below.

4.1. Morphological study

Morphological examination of *Cinnamomum verum* in its habitat was carried out. The plant grows up to 10-15m height, leaves are ovate to oblong in shape, flowering and fruiting during October to February. Detailed morphological data of 12 characters (qualitative and quantitative), were recorded (Figure: 2, Table: 4).

Bark characteristics were studied using parameters like barks color, bark fragrance and bark flavour.

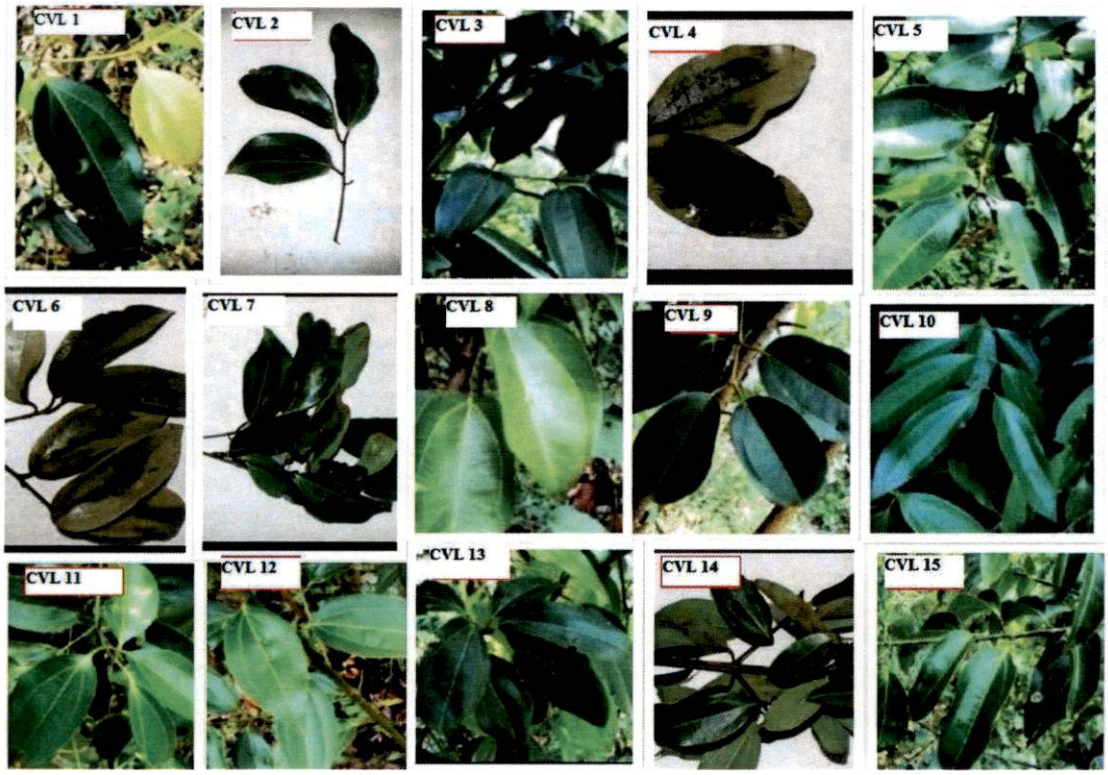


Fig 2: Leaves of *Cinnamomum verum*

SL No	Accession Name & location	Shape	Length (cm)	Breadth (cm)	L/B ratio	Petiole length(cm)	Leaf base shape	Leaf tip shape	Margin	venation	Bark fragrant	Bark taste	Bark color
1	JNTBGRI , Palode	Ovate	13.5	6.1	2.2	1.4 cms	obtuse	acuminate	Entire	tripariate,Laterally parallel	Intermediate fragrant aroma	lightly sweet in nature	brown
2	KAU , Trichur	Elliptical	13.4	6.9	1.9	1.5 cms	rounded	obtuse	Entire	tripariate,Laterally parallel	strongfragrant aroma	spicy	brown
3	CoA , Vellayani	Ovate	12.5	5.1	2.4	1.3 cms	obtuse	obtuse	Entire	tripariate,Laterally parallel	Intermediate fragrant aroma	spicy	brown whitish
4	Pilicode, Kasargod	Ovate	15.0	6.2	2.4	1.5 cms	cuneate	acute	Entire	tripariate,Laterally parallel	strongfragrant aroma	spicy	brown
5	Andaman	Ovate	14.2	6.0	2.4	1.4 cms	cuneate	acute	Entire	tripariate,Laterally parallel	strongfragrant aroma	hot spicy	brown
6	YCD1, Yercadu,salem	Ovate	13.5	6.1	2.2	1.3 cms	obtuse	attenuate	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	light brown
7	PP1, Pechiparai	Elliptical	13.3	6.6	2.0	1.4 cms	rounded	acute	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	light brown
8	Vazhachal	Ovate	11.1	5.5	2.0	1.4 cms	cuneate	acuminate	Entire	tripariate,Laterally parallel	Intermediate fragrant aroma	spicy	brown
9	Sugandhini-ODC130,AMPRS, Odakkali	Ovate	13.2	5.7	2.3	0.8cms	cuneate	attenuate	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	light brown
10	Navasree, IISR,Calicut	Lanceolate	13.3	5.6	2.4	1.5 cms	obtuse	acuminate	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	brown
11	Nithyasree, IISR, Calicut	Ovate	16.0	7.6	2.1	1.7cms	rounded	acute	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	brown
12	IISR, Calicut	Ovate	10.1	5.8	1.7	1.1 cms	rounded	acute	Entire	tripariate,Laterally parallel	Intermediate fragrant aroma	hot spicy	brown whitish
13	Calicut University	Ovate	11.4	6.2	1.8	2 cms	rounded	acute	Entire	tripariate,Laterally parallel	Intermediate fragrant aroma	spicy	brown
14	Anjarakandy Kannur	Ovate	11.0	5.2	2.1	1.3 cms	cuneate	acute	Entire	tripariate,Laterally parallel	good fragrant aroma	spicy	brown
15	I&B Seeds Pvt Ltd, Banglore	Elliptical	14.2	7.1	2.0	1.5 cms	cuneate	acute	Entire	tripariate,Laterally parallel	strongfragrant aroma	spicy	brown
16	YCDI progeny	Ovate	14.0	6.5	2.1	1.4 cms	Rounded	Acuminate	Entire	tripariate,Laterally parallel	good fragrant aroma	sweet	light brown

Table 4: Morphological characteristics of leaves of *Cinnamomum verum*

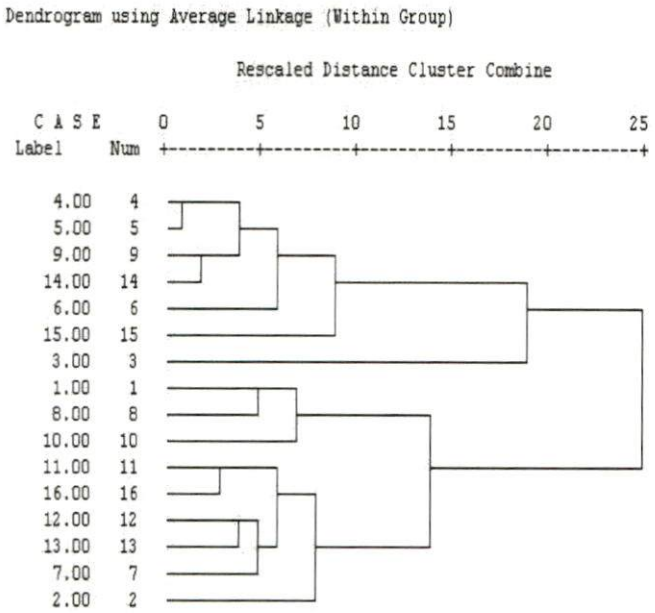


Fig 3: Dendrogram of *Cinnamomum verum* accessions derived through cluster analysis based onquantitative and qualitative characters of leaves

4.2 Phytochemical analysis

Chemical profiles of collected samples were performed through various phytochemical methods and are tabulated.

4.2.1. Volatilechemical profiling

4.2.1.1 Essential oil yield and characterstics

Cinnamomum verum leaf oil was denser than water and showed characteristic pale green colour with fragrant aroma.Whereas bark oil wassweet smelling, yellow and lighter than water. Bark oil yield was lesser than leaf oil yield.

Table 5: Oil yield and characteristics of *C. verum* bark and leaf collected from south India

Sl. No	Sample Location	Parts	Sample code	Yield (% v/w%) of leaves	Yield (% v/w%) of bark
1	JNTBGRI	Leaf and Bark	CVL1,CVB1	2.0	0.5
2	Kerala Agriculture University, Thrissur	Leaf and Bark	CVL2,CVB2	2.0	0.5
3	College of Agriculture, Vellayani	Leaf and Bark	CVL3,CVB3	2.0	0.4
4	Pilicode,Kasargode	Leaf and Bark	CVL4,CVB4	2.2	0.3
5	HRS,Yercaud,Salem, YCDI mother plant	Leaf and Bark	CVL6,CVB6	2.3	0.6
6	HRS,Pechiparai, PP1variety	Leaf and Bark	CVL7,CVB7	2.2	0.4
7	Vazhachal	Leaf	CVL8	0.7	-
8	Aromatic and Medicinal Plant Research Station, Odakkali, Sugandhini *ODC130*	Leaf and Bark	CVL9,CVB9	2.3	0.4
9	Indian Institute of Spice Research, Calicut, Navasree	Leaf and Bark	CVL10,CVB10	2.2	0.5
10	Indian Institute of Spice Research, Calicut, Nithyasree	Leaf and Bark	CVL11,CVB11	2.5	0.5
11	Indian Institute of Spice Research, Calicut	Leaf and Bark	CVL12,CVB12	2.1	0.5
12	Calicut University	Leaf and Bark	CVL13,CVB13	2.0	0.5
13	HRS, Yercaud, Salem, YCDI progeny	Leaf	CVL16	2.1	-
14	Calicut University	Leaf and Bark	CVL17,CVB17	2.1	0.5
15	Krishi bhavan Mannamkonam, TVM	Leaf	CVL18	1.5	-
16	Wayanad	Leaf	CVL19	1.5	-
17	Indian Institute of Spice Research, Calicut, *Cinnamomum cassia*	Leaf and Bark	CCAIL,CCAIB	0.5	0.4

CVB - *Cinnamomum verum*bark , CVL - *Cinnamomum verum* leaf, CCA- *Cinnamomum cassia*)

4.2.1.2 Analysis of essential oil

GC-MS analysis of oils showed that, phenylpropanoids were the major class of compounds present in leaf and bark samples.

Table 6: Volatile compounds identified in *C. verum* bark oil

Compound	RRI lit	RRI com	CNBM	CVB14	CVB4	CVB7	CVB8	CVB9	CVB10	CVB12	CVB1	CVB6
α-Pinene	932	929	-	-	-	-	-	0.50	1.10	-	-	-
Camphene	946	945	-	-	-	-	-	0.10	0.30	-	-	-
Benzaldehyde	952	956	-	-	-	-	2.10	0.40	33.3	-	-	-
β-Pinene	974	973	-	-	-	-	-	0.20	0.50	-	-	-
α-Phellandrene	1002	1006	0.70	-	-	-	-	2.10	0.50	-	-	2.90
α-Terpinene	1014	1016	-	-	-	-	-	-	1.60	-	-	-
p-Cymene	1020	1023	3.00	-	-	-	2.10	3.40	3.10	1.60	-	13.2
Limonene	1024	1025	-	-	-	-	-	0.70	1.70	-	-	-
β-Phellandrene	1025	1029	1.90	-	0.20	-	2.40	0.20	13.6	-	-	-
1,8-Cineole	1026	1028	-	-	-	-	-	5.80	-	-	-	-
Terpinolene	1086	1081	-	-	-	-	-	0.50	-	-	-	-
Linalool	1095	1101	6.30	5.40	-	-	13.5	0.20	4.70	37.2	4.80	16.3
3- Methyl-3-butenyl-3-methyl butanoate	1112	1112	-	-	-	-	-	-	-	-	-	-
Borneol	1165	1166	-	-	-	-	-	1.10	-	-	-	-
Terpinen-4-ol	1174	1176	-	2.40	-	-	-	0.50	2.40	0.50	-	-
α-Terpineol	1186	1191	-	2.30	-	3.10	1.10	1.10	-	2.40	1.80	2.60
Z-Cinnamaldehyde	1217	1213	-	-	-	-	-	0.50	-	-	-	-

E-Cinnamaldehyde	1267	1276	56.5	30.6	50.1	10.5	40.5	45.1	20.1	6.10	41.1	22.2
Thymol	1289	1294	-	-	-	-	-	-	-	-	-	1.10
Eugenol	1356	1353	10.2	3.70	-	-	-	-	-	-	-	1.10
α-Copaene	1374	1370	-	-	-	5.20	1.50	-	-	0.60	3.70	-
E-Caryophyllene	1417	1416	9.70	10.5	4.90	11.7	3.20	11.7	8.50	7.70	9.10	7.40
β-Humulene	1436	1416	-	2.00	-	-	-	-	-	-	-	-
E-cinnamylacetate	1443	1444	6.10	11.8	6.40	58.9	10.1	7.40	-	-	21.2	10.6
α-Humulene	1452	1452	1.70	-	5.80	6.50	4.30	0.50	1.80	1.70	-	1.80
β-Bisabolene	1505	1510	-	-	-	-	-	-	-	0.40	-	-
Caryophyllenyl alcohol	1570	1531	-	1.40	-	-	-	-	-	-	-	-
Spathulenol	1577	1569	-	-	-	-	3.40	-	-	0.90	-	-
Caryophyllene oxide	1582	1578	2.10	5.80	1.50	-	1.10	1.10	-	11.1	12.1	10.6
Guaiol	1600	1610	-	-	-	-	-	-	-	0.40	-	-
Humulene epoxide(ii)	1608	1601	-	1.70	-	-	3.40	0.40	-	-	3.10	3.00
Tetradecanal	1611	1608	-	-	-	-	4.70	0.60	-	9.20	-	-
Muurola-4,10(14)-dien-1630-1-β-ol	1630	1636	-	-	-	-	-	-	-	0.50	-	-
Caryophylla-4(12),8(13)-dien-5β-ol	1639	1629	-	-	-	-	1.10	-	-	-	-	-
Caryophylla-4(12),8(13)-dien-5α-ol	1639	1628	-	0.70	-	-	-	-	-	1.20	-	-
α-Cadinol	1652	1651	-	-	2.30	-	-	-	-	-	-	-
14-hydroxy(z)-Caryophyllene	1666	1662	-	-	3.80	-	4.30	1.00	-	10.5	-	-
14-hydroxy-9-epi-(E)-Caryophyllene	1668	1675	-	-	9.10	-	-	-	-	2.30	-	1.30
14-hydroxy-4,5-dihydro-Caryophyllene	1706	1692	-	-	5.30	-	-	-	-	-	-	-
Benzyl benzoate	1759	1786	-	14.3	8.10	-	-	4.90	-	3.30	-	2.30

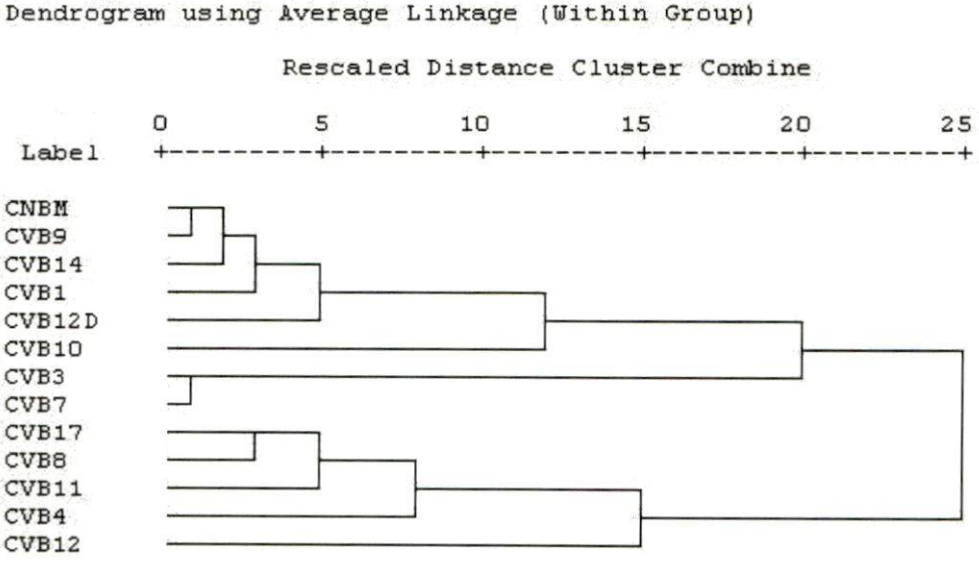


Fig 4: Dendrogram (Bark oil of *Cinnamomum verum)*

Table 7 :Volatile compounds identified in *C. verum* leaf oil

COMPOUND	RRI lit	RRI comp	CNLM	CVL1	CVL4	CVL6	CVL7	CVL8	CVL9	CVL10	CVL12	CVL14
α-Thujene	924	922	-	-	-	0.10	-	-	-	-	-	-
Tricyclene	925	921	-	-	-	-	-	-	-	-	-	-
α-Pinene	932	929	-	0.30	-	-	-	0.30	0.10	0.70	-	-
camphene	946	945	-	0.10	-	0.10	0.10	0.10	-	0.40	-	-
β-Pinene	974	973	-	0.10	-	-	0.10	-	-	0.30	-	-
Myrcene	988	985	-	-	-	0.10	0.10	0.10	-	-	-	-
α-Phellandrene	1002	1003	-	0.70	-	0.50	-	0.50	0.20	-	-	-
S-3-Carene	1008	1006	-	0.10	-	-	0.70	-	-	-	-	-
α-Terpinene	1014	1017	-	-	-	0.70	-	-	-	-	-	-
p-Cymene	1020	1020	-	0.90	-	4.50	-	-	-	-	0.20	0.30
Limonene	1024	1025	-	0.20	-	0.20	0.30	0.20	-	-	-	-
Sylvestrene	1025	1025	-	-	-	-	-	-	-	-	-	-
β-Phellandrene	1025	1026	-	-	-	-	0.10	0.50	-	-	-	-
1,8-Cineole	1026	1028	-	0.10	-	0.20	-	-	-	-	-	-
(E)-β-Ocimene	1044	1042	-	-	0.10	0.10	-	-	-	-	-	-
γ-Terpinene	1054	1054	-	-	-	-	-	-	-	-	-	-
Terpinolene	1086	1087	-	-	-	-	0.10	-	-	-	-	-
Linalool	1100	1095	2.60	1.10	-	0.10	-	1.30	-	0.50	1.50	1.10
12-propenyal phenol	1146	1145	-	-	-	-	-	-	0.30	-	-	-
Benzyl acetate	1152	1158	-	-	-	-	-	-	0.10	-	-	-
α-Terpineol	1186	1191	-	0.20	-	0.10	2.70	-	-	-	-	-
Z-Cinnamaldehyde	1217	1214	-	-	-	-	-	-	0.70	-	-	-
Chavicol	1247	1251	-	-	-	-	-v	-	0.10	-	-	-

E-Cinnamaldehyde	1271	1267	0.70	1.60	4.80	-	0.30	0.40	-	1.10	-	0.40
Safrole	1285	1287	1.10	-	-	-	-	-	-	-	-	-
Eugenol	1356	1352	82.7	80.1	15.1	82.8	80.4	86.2	81.2	88.2	91.3	87.1
α-Copaene	1374	1370	-	-	-	-	-	0.20	-	-	-	-
Z-Iso esugenol	1406	1384	-	-	-	-	3.90	-	0.10	-	-	-
Z-Caryophyllene	1408	1393	-	-	-	-	-	-	-	-	-	1.80
E-Caryopyllene	1417	1416	3.30	-	1.20	-	-	3.70	-	2.40	0.70	-
E-Caryopyllene acetate	1443	1443	1.20	-	-	-	-	-	-	-	-	-
E-Cinnamylacetate	1443	1439	-	-	2.50	-	0.30	1.30	-	0.80	-	0.10
α-Humulene	1452	1451	0.50	1.40	-	-	-	0.60	-	-	0.10	0.50
δ-selinene	1492	1488	-	0.10	-	-	-	-	-	-	-	-
Bicyclogermacrene	1500	1488	-	-	-	-	3.10	0.40	2.30	-	-	-
Pentadecane	1500	1497	-	-	-	-	-	-	-	-	-	-
Eugenol acetate	1521	1513	1.90	0.30	-	2.50	0.80	-	0.90	2.30	0.40	-
Chavibetol acetate	1524	1510	-	-	-	-	-	-	-	-	-	1.90
Spathulenol	1577	1569	-	0.10	-	0.30	-	0.40	-	-	0.10	-
Caryophyllene oxide	1582	1578	0.40	4.50	-	0.70	0.60	0.60	4.20	0.70	0.80	0.90
Humulene epoxide (ii)	1608	1606	-	0.20	0.50	-	-	-	-	-	0.10	-
α-Cadinol	1652	1648	-	0.70	-	-	-	-	-	-	-	-
Benzyl benzoate	1759	1763	3.10	0.10	-	-	0.10	-	0.10	-	2.30	4.70
Benzoin	1803	1844	-	-	-	-	-	-	0.30	-	-	-

The present study shows that Eugenol (77.5 ± 22.2) was the major compound in all the accessions of *Cinnamomum verum* leaf oil.

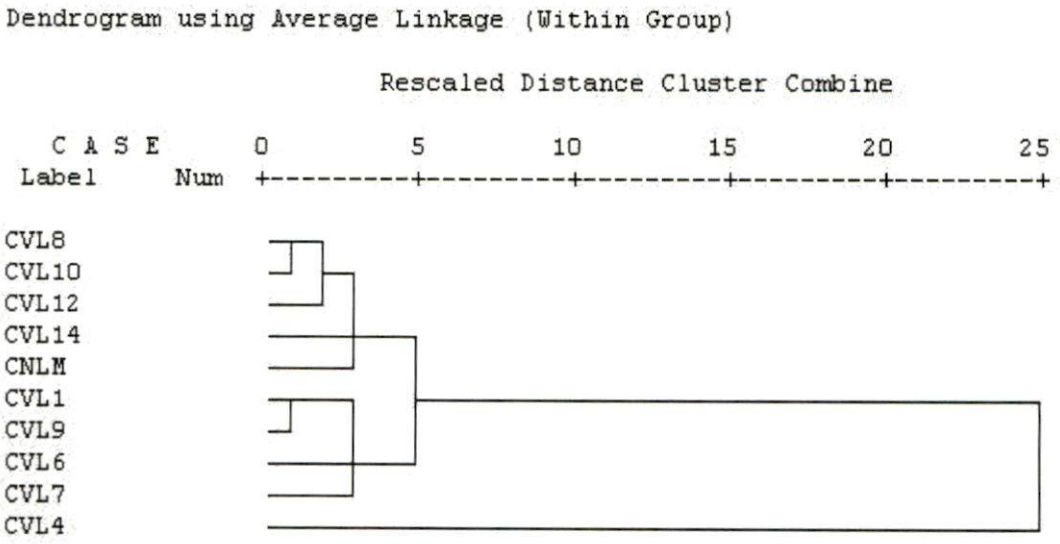


Fig 5: Dendrogram (*Cinnamomum verum*leaf oil)

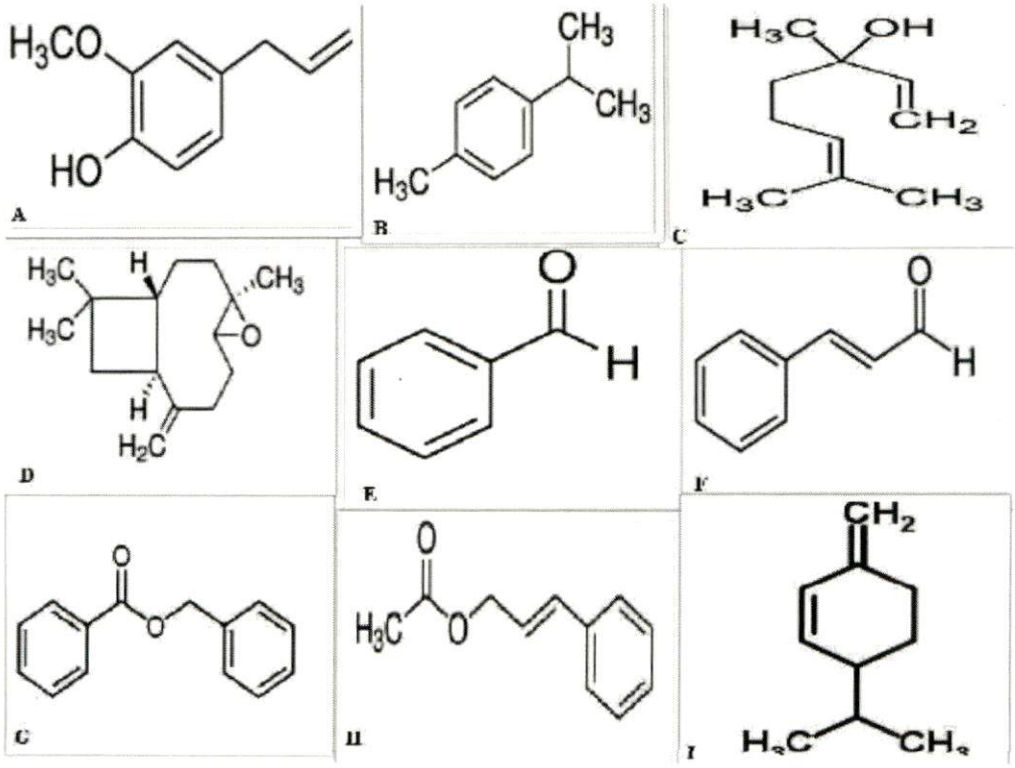


Fig 6: Structure of major compounds identified in *C. vreum* leaf and bark

(A) Eugenol B)p-Cymene C) Linalool D) Caryophyllene oxideE)Benzaldehyde F) Cinnamaldehyde G) Benzyl benzoate H) Cinnamyl acetate I) β- Phellandrene

4.2.2. Non-volatile chemical profiling

4.2.2.1 Preliminary phytochemical screening

Qualitative phytochemical screening of hexane extracts of leaf and bark revealed the presence of steroids andterpenoids.While carbohydrates, steroids, terpenoid andsaponins were detected in methanol extract of leaf. In addition to these, flavanoids and coumarins were found in methanol extract of bark.

Table 8: Class of Phytochemicals present in n-Hexane and Methanolic leaf extracts of *Cinnamomum verum*

Sl.No	Compound	Hexane extract	Methanol extract
1.	Carbohydrates	-	+
2.	Steroids	+	+
3.	Alkaloids	-	-
4.	Terpenoid	+	+
5.	Coumarin	-	-
6.	Flavonoids	-	-
7.	Saponins	-	+

+ Presence - Absence

Table 9: Class of Phytochemicalspresent in n-Hexane and Methanolic bark extracts of *Cinnamomum verum*

Sl.No	Compound	Hexane extract	Methanol extract
1.	Carbohydrates	-	+
2.	Steroids	+	+
3.	Alkaloids	-	-
4.	Terpenoids	+	+
5.	Coumarin	-	+
6.	Flavonoids	-	+
7.	Saponins	-	+

+ Presence - Absence

4.2.2.2High Performance Thin layer chromatography

Figures(Fig 7 and Fig 8) below show the HPTLC profile of twelve accessions of *C. verum*and standards eugenol and cinnamaldehyde.

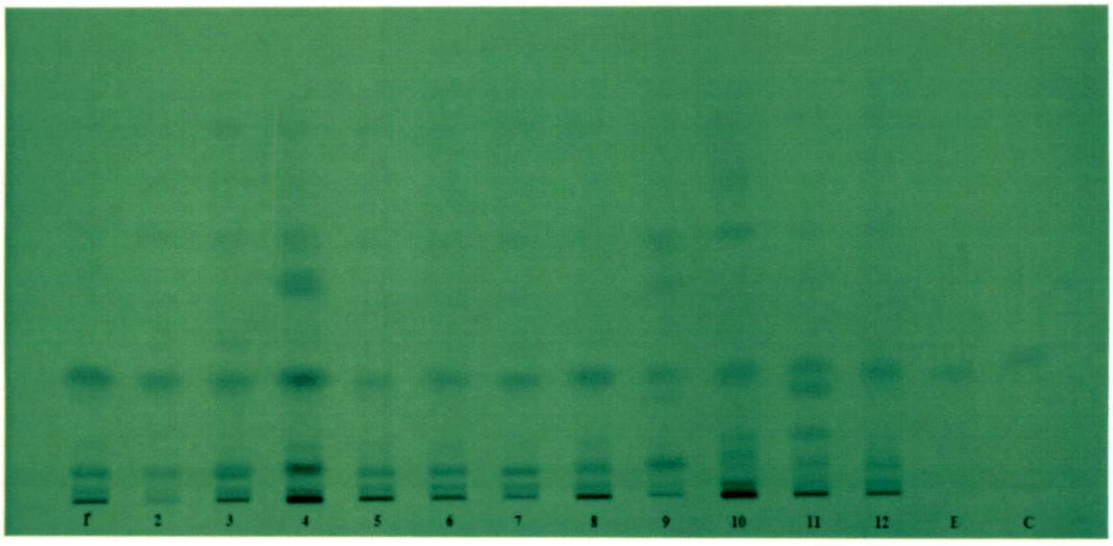


Fig 7: Developed leaf plate under UV 254 nm(CVL1 to CVL12)

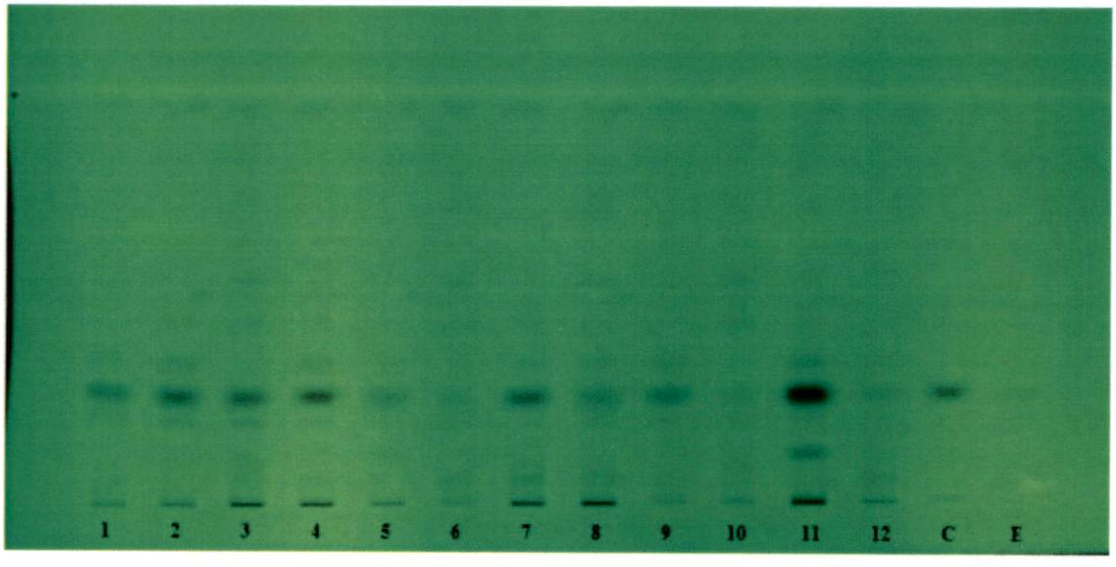


Fig 8: Developed bark plate under UV 254 nm(CVB1 to CVB12)

C- Cinnamaldehyde, **E-** Eugenol

4.3 Estimation of coumarin content

The result shows that content of coumarin in authentic *Cinnamomum verum* sample was negligible, while market sample contained high concentration which cannot be recommended for consumption.

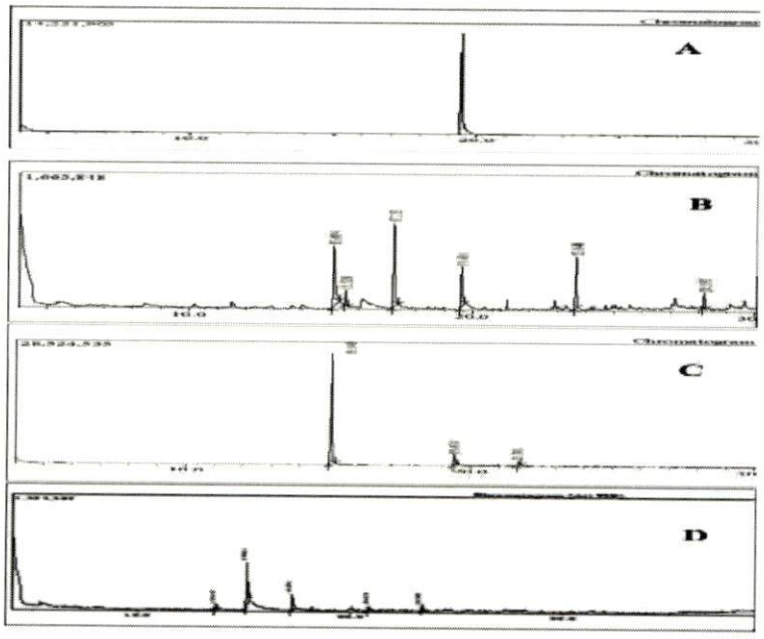


Fig 9: Chromatogram of A) coumarin standard, B)Commercial sample, C)*Cinnamomumcassia*, D)Authentic sample

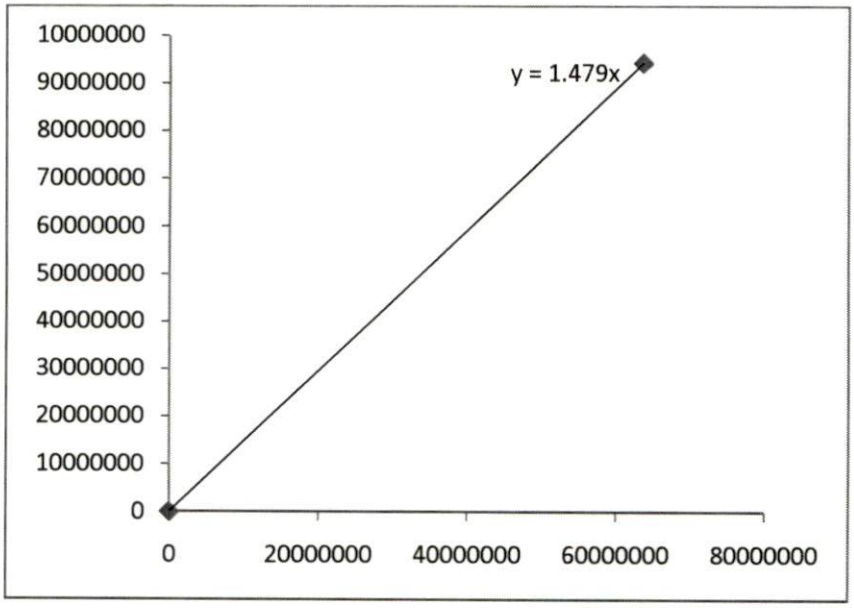


Fig10: Concentration versus area graph of coumarin standard

Table 10: Content of Coumarin in different samples of cinnamon

Sl.No	Sample code	Sample name	Coumarin content(µg/g)
1	CVBC15	Srilanka keya powder	0.31
2	CVBC17	Market sample Munnar	13.44
3	CVBC18	Madikkeri market sample	50.55
4	CVBC19	Garden fresh Kannur	7.58
5	CVBC20	Nilgiri spices sample	21.42
6	CVBC21	*C. verum* , Anjarakandy	0.0
7	CVBC22	*Cinnamomum cassia,* Calicut	1.30

4.4 Genetic study

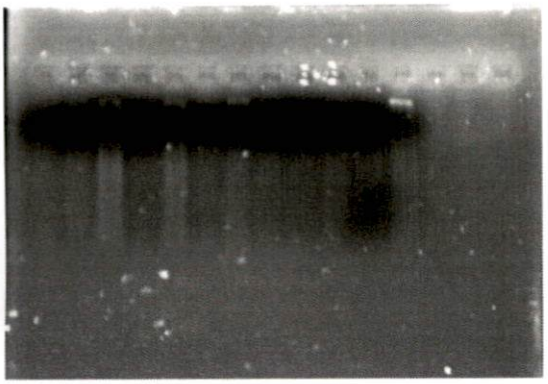


Fig 11:Genomic DNA isolated from plant leaves (CVL1 toCVL12) resolved under 0.8% agarose gel.

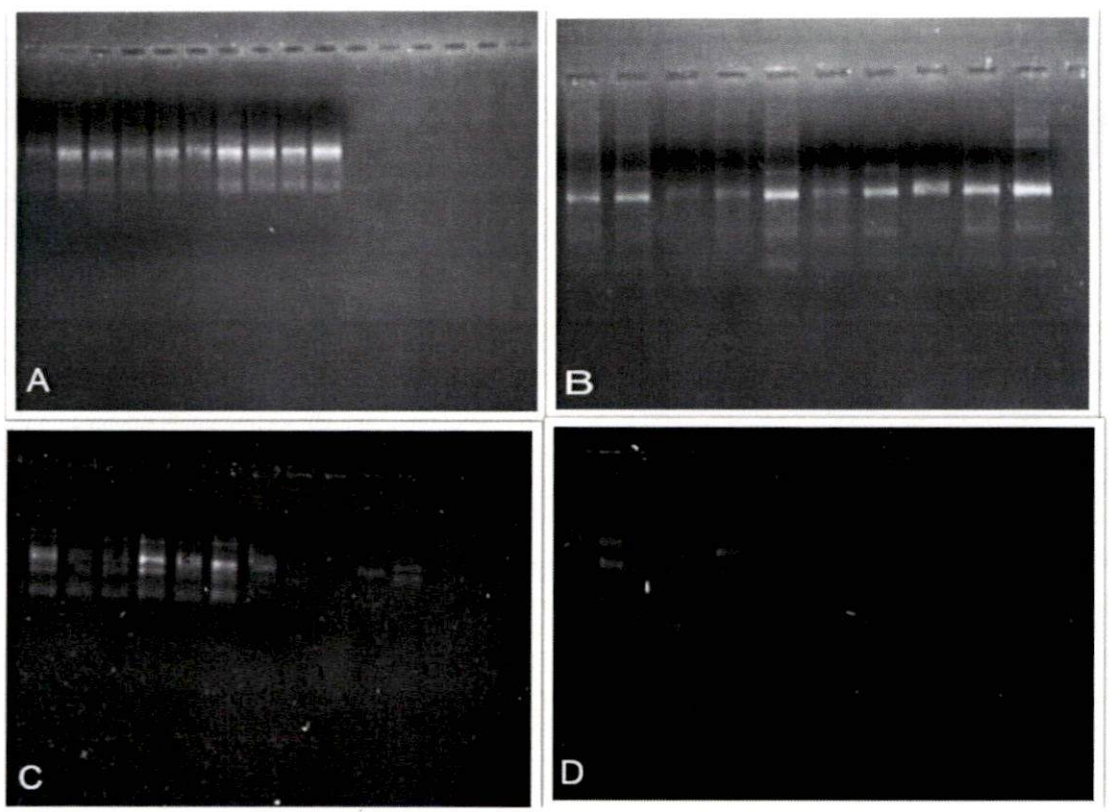


Fig 12: A) Amplified products of ISSR Primer No.46 resolved under 1.4% agarose gel, B)Amplified products of ISSR Primer No.816 resolved under 1.4% agarose gel, C Amplified products of ISSR Primer No.824 resolved under 1.4% agarose gel D) Amplified products of ISSR Primer No.856 resolved under 1.4% agarose gel.

63

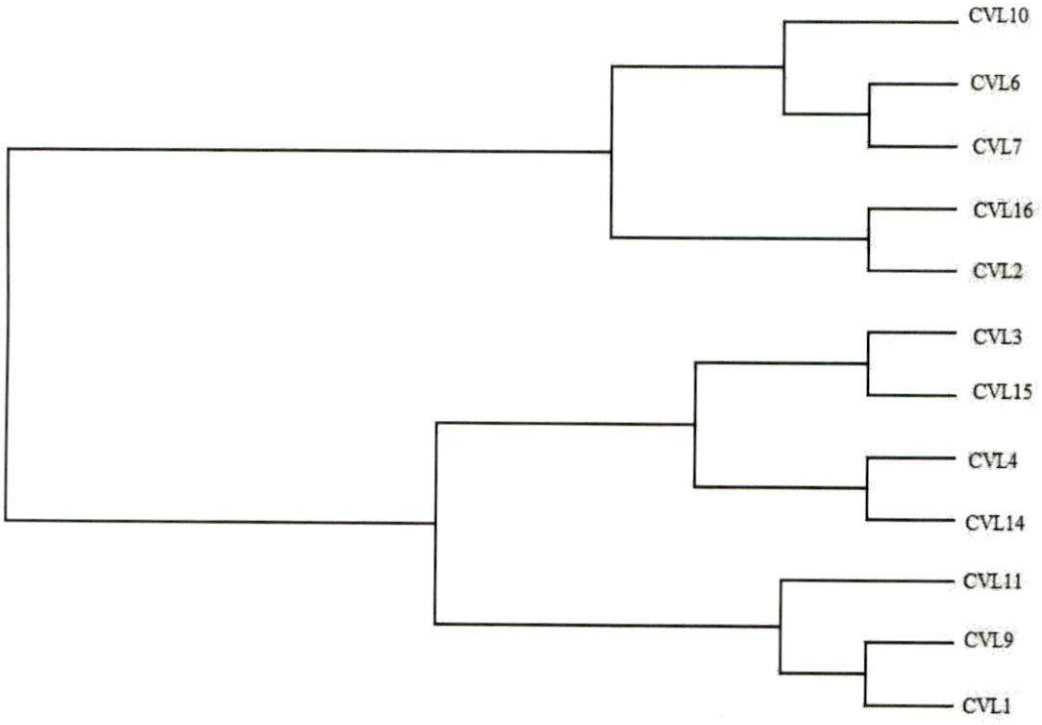


Fig13: **Dendrogram showing the genetic relationship between twelve studied *C. verum* accessions**.

Table 11: Analysis of genetic polymorphism obtained with ISSR primers in different *C. verum* accessions

SL.NO	SAMPLE CODE	na	h	No. of polymorphic loci	*P* (%)
1	CVL1	1.25	0.06	1	25%
2	CVL2	2.00	0.41	4	10%
3	CVL3	1.50	0.22	2	5%
4	CVL4	1.75	0.27	3	75%
5	CVL6	1.75	0.24	3	75%
6	CVL7	2.00	0.34	4	10%
7	CVL9	1.50	0.11	2	5%
8	CVL10	1.75	0.21	3	75%
9	CVL11	1.50	0.18	2	5%
10	CVL14	1.75	0.35	3	75%
11	CVL15	1.50	0.18	2	5%
12	CVL16	2.00	0.43	4	10%

na=Observed no. of alleles, *h* = Nei'sgene diversity, *P* (%) = percentage ofpolymorphic loci

64

Table 12: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

pop ID	CVL6	CVL16	CVL2	CVL3	CVL4	CVL15	CVL14	CVL7	CVL9	CVL1	CVL10	CVL 11
CVL6	****	0.8320	0.9037	0.7232	0.7975	0.7980	0.7865	0.9744	0.8784	0.8908	0.9809	0.8408
CVL16	0.1839	****	0.9378	0.8687	0.8420	0.8583	0.8254	0.9168	0.7681	0.7495	0.8588	0.7778
CVL2	0.1012	0.0642	****	0.6897	0.7262	0.7105	0.7269	0.9361	0.7050	0.6995	0.8928	0.6996
CVL3	0.3240	0.1408	0.3714	****	0.9315	0.9856	0.9303	0.8153	0.8983	0.8753	0.7783	0.9157
CVL4	0.2262	0.1720	0.3200	0.0709	****	0.9563	0.9592	0.8188	0.8974	0.8906	0.7824	0.8813
CVL15	0.2257	0.1527	0.3417	0.0145	0.0447	****	0.9339	0.8579	0.9435	0.9313	0.8374	0.9396
CVL14	0.2402	0.1919	0.3190	0.0722	0.0417	0.0684	****	0.8226	0.9230	0.9057	0.7821	0.9390
CVL7	0.0259	0.0869	0.0660	0.2042	0.1999	0.1533	0.1952	****	0.8914	0.8907	0.9906	0.8767
CVL9	0.1297	0.2639	0.3496	0.1073	0.1083	0.0582	0.0801	0.1149	****	0.9978	0.9004	0.9900
CVL1	0.1156	0.2883	0.3574	0.1332	0.1159	0.0712	0.0990	0.1157	0.0022	****	0.9061	0.9789
CV10	0.0193	0.1522	0.1133	0.2507	0.2454	0.1775	0.2458	0.0094	0.1049	0.0986	****	0.8754
CVL11	0.1734	0.2513	0.3572	0.0881	0.1263	0.0623	0.0630	0.1316	0.0101	0.0213	0.1330	****

DISCUSSION

5. DISCUSSION

Cinnamomum verum belonging to the familyLauraceae,is a very useful tree spice, and every part of the tree is used for differentpurposes like culinary, pharmaceutical and perfumery. It is native to Sri Lanka and in India and cultivated in lower elevations of Western Ghats in Kerala, Tamil Nadu andKarnataka.

5.1 Field study

Field study was conducted across south India and majority of the samples were from Kerala. Itrevealed that, like other spices cinnamon became naturalized, especially in homesteads of Kerala. The plant could reach to a height of 15 meters and wide distribution was found at low land, middle and high land regions. Several accessions including released varieties like *PP1*, *YCD1*, *Navasree*, *Nithyasree*and *Sugandhini* were collected.Samples were also collected from Anjarakandy Cinnamon plantation,Kannur which was established by the Britishduring their colonial period.It was the largest cinnamon plantation in Asia, but now its remnants only can be seen. If properly maintained, large quantity of good quality cinnamon can be produced incommercial scale.

5.2 Morphological study

Twelve morphological characters wererecorded from 16 accessions. Among twelve morphological characters, four quantitative characters; leaf length, leaf width, leaf length-width ratio, petiole length, and eight qualitative characters,leaf shape, leaf apex, leaf base, bark color, bark flavor and bark fragrant were recorded.Tripariate,laterally parallel venation with entire margin type was the specific feature found in all the accessions.Most of the accessions have ovate leaf shape except those of CVL3, CVL7, and CVL15 which were found in elliptic and CVL10 in lanceolate shape.*C.verum* has a leaf length in the range of 10.1 cm to 16 cm, breadth range of 5.1 cm to 7.6 cm. Three type of leaf base shape was found, which

includes rounded, cuneate and obtuse. Leaf tip was another criteria, in which most of them have acute shape and remaining were accuminte and a few obtuse. Small sized leaves having ovate shape, cuneate base and acute tip was the morphological features of Anjarakandy accession. Nithyasree, a cultivar released by IISR, Calicut has the largest leaf size with a length of 16 cm, 7.6 cm breadth, petiole of about 1.7cm and rounded leaf base. Significant variation was found in bark features like bark color, bark flavor and bark fragrance.Bark color varies from whitish brown to dark brown. Sweet taste of bark was the special featureof released cultivarslike YCDI, PP1, navasree, nithyasree and sugandhini.*C.verum* bark collected from JNTBGRI also has light sweet taste.

Analysis was performed on the basis of morphological characters revealed, one of the accessions (CVL3)belongs to cluster No.2 have obtuse leaf base and tip shape which is different from the other accessions. Inaddition, accessions CVL4, CVL5, CVL9, CVL14, CVL6 and CVL15 were groupedin to cluster No.1, which showedovate leaf shape and acute leaf tip.Accessions(CVL1,CVL8 and CVL10) having acuminate leaf tip were grouped in to cluster No.3 .Cluster No.4 consists of CVL11,CVL16,CVL12,CVL13,CVL7 and CVL2 in which all of them have rounded leaf base.

The present study demonstrates a considerable diversity of morphological characters among the accessions.Primary causes of these variations are cross pollination, positional effects, environmental effects, and juvenility as previously studied by(Ibanez *et al.*, 2015) in*Cosmos bipinnatus.*

5.3 Phytochemical study

5.3.1 Volatile chemical profiling

5.3.1.1 Essential oil yield and characteristics

Leaf and bark oil of all the accessions, isolated by Clevenger apparatus revealed that the leaf has greater oilyield than the bark. Leaf oils were pale green and denser with fragrant aroma,whereas bark oilswere yellow and less denser with sweet aroma. Essential oilyield of *Cinnamomum cassia* was less than oil yield of *C. verum*.Essential oil yield of *C.verum* barks investigated were varied between 0.4%v/w to 0.6%v/w. However higher oil yields up to 1%v/w was reported for Cinnamon barks (Hema *et al.*, 2010, Jayaprakasha and Rao. 2011). Leaves of *C. verum*upon hydrodistillation yielded 1.5% to 2.5%v/w essential oil,which was higher compared to earlier studies which reported only up to 1.5%v/w oil from leaves (Chakraborthy, *et al.*, 2015, Jayaprakasha and Rao, 2011). Variety*Nithyasree* has higher oilyield than other accessions as it produces2.3% v/woil from leaves and 0.5% v/wfrom bark. The remaining samples have produced an average range of 2.0%v/w oil from leaf and 0.45%v/w bark oil.

Wijesinghe and Gunarathna, (2001) has conducted a study on leaf size and oil yield and suggests that variations in leaf size are comparable with oil yield. According to their observation trees with large round leaves and big leaves had high bark yield. Variety *Nithyasree* has large leaf size and oil yield was high, which is comparable to the above study. So this variety can be recommended for farmers cultivating cinnamon in large scale.

5.3.1.2 Analysis of bark oil

Forty compounds were identified from GC analysis of essential oils of ten accessions of *C. verum* barks. Essential oil composition of the *C. verum* bark oils ranged from 92.6% to 98.2%. Previous study shows that phenyl propanoids were the major constituents of cinnamon bark and leaf oil (Paranagama *et al.*, 2001). In this study despite of monoterpenoids and sesquiterpenoids, phenyl propanoids were the major class of compounds distributed in the essential oils of *C. verum* barks. Phenyl propanoid composition widely varied from 6.1% to 72.4% in the oils analyzed.

Among phenyl propanoids E- cinnamaldehyde was the major component in *C. verum* barks ranging from 6.1% to 56.5%. Highest cinnamaldehyde content was found for the samples CNBM, CVB9 and CVB1 (56.5%, 45.1% and 41.1% resp.).Various study reported (E)-cinnamaldehyde as the major component in the *C. verum* bark essential oils (Baruah *et al.*, 2010). Apart from cinnamaldehyde, E-cinnamyl acetate was also found in higher quantities from *C. verum* bark oils, ranging from 6.1% to 58.9%. The average content of cinnamaldehyde in bark of 10 accessions was32.3± 17.1.The highest value of standard deviation shows the diversity in distribution of major constituents studied. Earlier studies also revealed wide variation in cinnamaldehyde content in *C. verum* barks from 24.2% to 91.8 % (Pooja et al., 2013, Kazemi and Mokhtariniya 2016).From these results it is clear that CVB9 and CVB1 are good quality cinnamon as the cinnamaldehyde content are close to that in market sample CNBM, while CVB3 and CVB7 are the chemical variants of *C. verum* which is evident from their high cinnamyl acetate content. The present study was in consonance with above findings and results showed that the *C. verum* bark is a good source of Cinnamaldehyde and Cinnamyl acetate.

5.3.1.3 Chemotaxonomy studies based on Dendrogram

Hierarchichal cluster analysis was performed using the essential oil compositions of thirteen accessions of *C. verum* bark. CNBM, CVB9 and CVB 14 falls under a clad by their similarity in cinnamaldehyde and cinnamyl acetate content, while CVB 1 as well as CVB6 stands separate by their high content of caryophyllene and caryophyllene oxide compared to the former clad. It is interesting to note that CVB8, CVB4 and CVB12 forms the, second clad are marked by their more caryophyllene and caryophyllene oxide content compared very low cinnamaldehyde content.Contrary to previous reports essential oil analysis and cluster analysis shows that there is considerable variation in volatile composition of *C. verum* barks in south India.

5.3.1.4 Analysis of leaf oil

Fourty four compounds were identified from GC analysis of essential oilfrom ten accessions of *C. verum* leaves. Essential oil composition of the *C. verum* leafoils ranged from 95.9% to 98.8%.Despite of monoterpenoids and sesquiterpenoids, phenyl propanoids were the major class of compounds distributed in the essential oils of *C. verum*leaves as in bark oil. Phenyl propanoid composition widely varied from 0.4 % to 91.3% in the oils analyzed.

Among phenyl propanoids eugenol was the major component in *C. verum*leaves ranging from 15.1 % to 91.3 %. Highest eugenol content was found for the sampleCVL12 (91.3%).Previous study had showed eugenol (92.7%) as the predominant compound identified in *C. verum* leaf oil from North-East India (Barauh *et al.*, 2010).Except CVL4, all other accessions have more than 80% eugenol content. Apart from eugenol, eugenol acetate (0.3% o 2.5%) and caryophyllene oxide (0.4% to 4.2%) were also found inmost of the *C. verum*leaf oils. The average content of eugenol in leaf oil of 10 accessions was 77.5 ± 22.2%.From these studies it is clear that CVL12proved to be the good quality cinnamon as the eugenol content was higher than that in market sample CNLM (82.7%). The present results corroborates with earlier studies on leaf essential oil composition of *C. verum* with 85.5% to 92.3% of eugenol. (Kirthi et al., 2013; Mallavurupu and Rao., 2007; Jayaprakasha et al., 2011). The present study showed that *C. verum* leaf oil composition varies in accordance with pedoclimatic conditions.

5.3.1.5 Chemotaxonomy studies based on Dendrogram

Hierarchical cluster analysis was performed using the essential oil compositions of ten accessions of *C. verum*leaf. CNLM, CVL8, CVL10, CVL12 and CVL14 falls under a clad by their high eugenol content while CVB4 stands separate by its lesscontent of eugenol. CVL1, CVL9,CVL6 and CVL7 forms the second clad are marked by their low eugenol content compared to former clad andpresence ofeugenol acetate content. Contrary to previous reports essential oil analysis and cluster analysis

shows that there is considerable variation in volatile composition of *C. verum*leaves in south India.

5.3.1.6 Comparative study of leaf and bark oil based on dendrogram

Comparative study of *Cinnamomum verum* bark and leaf oil show that phenyl propanoids were the major class of compounds in both oils. Barks having high cinnnamaldehyde content was distributed infirst clad and leaves containing high eugenol in the first clad of leaf dendrogram.High cinnamldehyde content was found in CVB9 and eugenol content in CVL12. No relation was found between percentage of cinnamaldehyde and eugenol content in accessions. CVB7 has high cinnamyl acetate content than cinnamaldehyde, despite of all other accessions CVL7 has slight amount of cinnamyl acetate. Analysis of the phytochemical variability between accessions will help to identify them in terms of their differences in phytochemical constitution. Moreover, phytochemical differences are good indicators of their genotypic distances. Studies on phytochemical variability have been attempted by early workers in different crops like cinnamon (Ravindran et al., 1992), pepper (Ravindran and Nirmal Babu, 1994), tea (Ramasubramanian, 2005) and medicinal plants (Raghu et al., 2007). The present study shows that all the accessions are phytochemicallyinconsistent, that represents high diversity.

5.3.2.1 Preliminary phytochemical screening

The results obtained in the present study indicates *C. verum* leaf and bark have the potential to act as a source of useful drugs because of the presence of various phytochemical components such as carbohydrates, steroids, terpenoid, flavonoids,saponinsand coumarins.A previous study shows that plants containing carbohydrates, glycosides and coumarins are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. Coumarins can be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects (Theis

andLerdau, 2003). In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana and Ata, 2008). Bark is supposed to be of maximum medicinal value out of the investigated leaf as it possesses majority of identified phytoconstituents. According to the above findings cinnamon contribute significantly towards the biological activities such as hypoglycemia, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic activities.

5.3.2.2 High Performance Thin layer chromatography

Cinnamomum verum contains active components like cinnamaldehyde, eugenol, cinnamic acid, cinnamyl acetate etc. Among all the active components cinnamaldehyde present in bark is responsible for distinct flavor and odor. Previous studies suggested that the authentic samples of *C. verum*contain 91.5 % of cinnamldehyde in its bark (Hossein *et al.*, 2013) and81.7% of eugenol in leaf (Chakraborthy *et al.*, 2015) In this study bark and leaf of twelve accessions of *C. verum* were checkedto assess the presence of marker compound using HPTLC.Cinnamaldehyde and eugenol were detected inall the n-hexane extracts ofbark and leaf respectively. HPTLC plate visualised under UV 254nm differentiated phenyl propanoid markers in the extracts corresponding to the standards spotted. All the leaf samples showed bands parallel to eugenol.CVL11, CVL2, CVL17, CVL5 and CVL6 exhibited bright band and GC-MS analysis of these samples reported more than 80% eugenol.Similarly all the bark samples except CVB8, CVB9 andCVB5 have clear band analogous to cinnamaldehyde. GC-MS analysis of these accessions showed less cinnamldehyde content in the range of 0.49% to 1.98%.The study revealed that the concentration of marker compounds varies noticeablyin *C. verum* from south India which indicates considerable diversity in chemical profile of accessions. The variations are due to changes in location of plant, time of harvest, environmental stresses and part of plant used.

5.3.2.2Estimation of coumarin content

The experimental results indicated that *C. verum* bark contain only negligible amount of coumarin, whereas barks from cassia and market samples have substantial amount of coumarin.Ample quantity of coumarin was found inCinnamon barks sold atMadikkeri(50.55 µg/g), nilgiri spices (21.42 µg/g) , Munnar market (13.44 µg/g) and Kannur garden fresh (7.58 µg/g) while, *C. cassia* contains 1.3 µg/g coumarin, which was higher than authentic *C. verum* which contains LOD amount of coumarin. Previous studies revealed that, high coumarin contents were in*C.burmannii* or *C. loureiri* as they are least expensive than *C.verum* (UN Comtrade , 2011-*http://comtrade.un.org/db*). A study reported that the majority of ground cinnamon available in the retail trade was cassia cinnamon and cinnamon without specification of origin, with coumarin content ranging from 2880 to 4820 and 0 to 8790 $mg \cdot kg^{1}$, respectively and analyzed five samples of true cinnamon which were coumarin-free(Sproll *et al.*, 2008). 70% of the tested ground cinnamon samples marketed in Italy was originated from Cassia cinnamon(Lungarini et al.,2008).Reviewing accumulated human data, theGerman Federal Institute for Risk Assessment (BfR) reaffirmed the TDI of 0.1 mg/kg of coumarin in 2007(Abraham *et al.*, 2010).

Hence consuming market cinnamon other than authentic *C. verum* can cause harmful effects on body, as it is hepatotoxic.Because Cassia cinnamon contains high content of coumarin, heavy consumption of this spice may result in doses exceeding the tolerable daily intake. Most of the cinnamonsamples available in markets may beadulterated by inferior cassia species. So care should be taken while using cinnamon for flavouring.

5.4 Genetic study

Pure DNA is required for genetical study. As *Cinnamomum verum* contains high amount of polyphenols and polysaccharides, it is hard to obtain pure DNA from leaf

samples.Polyvinylpyrrolidone (PVP) is an important agent to remove the polyphenols byforming complex hydrogen bonding with polyphenols and efficiently separate it from DNA (Kit and Chandran, 2010). In the extraction buffer of QIAGEN, DNAeasy plant mini kit, all the components were kept constant andPVP concentration was changed to see its effect on the extracted DNA. In this experiment, 8mg, 16mg and 20 mg PVP were usedrespectively. Different concentrations of PVP were used previously for plants having high content of secondary metabolites like polyphenol and polysaccharides (Khanuja *et al.*, 1999). In the present investigation, addition of 20mg PVP yielded theoptimum quality and quantity of DNA. Approximately 84.7 ng/µL DNA having A260/A280 ratio 1.91was obtained when 20 mg PVP was used. Likewise 16mg PVP produced 55.7ng/ µL and A260/A280 ratio 1.50 and very less when 8mg was used. However the samples have showed poor banding pattern in 0.8% agarose gel. So modified protocol has to be adopted for obtaining high purity DNA (Bhau et al., 2015).

Four ISSR primers (primer no; 46,816,824 and 856) that generated polymorphic bandswere used for genetic diversity analysis of the twelve accessionsof cinnamon. The selected primers showed good, reliable, repetitive, and distinct bands which enabled effective scoring for genetic diversity study within the populations.Four primers generateddistinct bands and polymorphism was shown by all the samples.The results showed that within the 12 studied accessions, the number of observed alleles(na) ranged from 1.25 in CVL1 to 2.00 inCVL2,CVL7 and CVL16. Based on the (h) value, CVL1 showed a slightly lower genetic diversity, whereas results for the genetic diversity in CVL2 andCVL16 were convergent. A previous study conducted on the screening of the genetic relationships in *Cinnamomum zeylanicum* usingRAPD marker showed polymorphismof 89% (Sandigawad *et al*, 2011) in the selected individuals. In this study highest percentage of polymorphism (100%) and gene diversity were observed in CVL2 and CVL16 collected from KAU and Yercaud, Horticulture Research Station respectively. The lowest values were observed in CVL1 collected from JNTBGRI. So the samples CVL2 and CVL16

could be considered to possess a higher genetic variation as compared to other accessions.

The Nei's genetic distance (Table 12) revealed the genetic differentiation between the individuals from the different regions analysed in the study. This matrix showed that the highest genetic identity value and genetic distance (0.990, 0.0101) were observed among CVL9(SugandhinI) and CVL11 (Nithyasree), whereas the lowest values (0.6897, 0.3714) were observed between CVL2 (KAU, Thrissur) and CVL3(CoA,vellayani). This was also confirmed by dendrogram constructed based on the genetic distance. All the accessions were classified our main groups and seven sub groups.Accessions CVL10,CVL6 and CVL7 showed similarity as they belonged to same cluster. This can be correlated to their oil yield, morphological characters and chemical profile. Similarly CVL16 and CVL2, CVL3 and CVL15, CVL4 and CVL14, CVL11, CVL9 and CVL1 has shown convergence. High divergence was observed between samples, exhibiting high intraspecific genetic diversity. The above findings are in consonance with studyconducted by (Alansi *et al.*, 2016). Variation in the population's gene pool that allows them to adapt to the changing environmental conditions, habitat and human interventions that leads to diversity at various level.

SUMMARY

6. SUMMARY

The study entitled "Evaluation of Phytochemical diversity of *Cinnamomum verum* in South India" was carried out at Phytochemistry and Phytopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, during 2016-2017 academic years. The objective of the study was to scientifically enumerate distribution status of plant for the purpose of conservation and investigation of intra species diversity of *Cinnamomum verum* J. Presl distributed in south India, interdisciplinary by morphology studies, phytochemistry and molecular biology.*Cinnamomum verum* J. Presl were collected from various localities of Kerala, Tamilnadu and a few from Karnataka state, to carry out morphological, phytochemical and genetic diversity studies.

Field study helped to identify distribution pattern of plant in low, middle and high land. And the study revealed that cinnamon became naturalized in all levels of land.In the morphological investigation, qualitative and quantitative parameters of leaves and bark were carried out. Within the species, variation in leaf size, leaf shape, bark color, barkfragrance and bark flavor was found.The results discovered considerable variation among the 16 accessions.

Volatile as well as non volatile chemical profiling was carried out with leaves and bark of all the accessions. Preliminary phytochemical screening of leaves of*Cinnamomum verum*has shown the occurrence of secondary metabolites like carbohydrates, terpenoids, steroids, flavonoids, coumarins and saponins and these phytochemicals, may play a vital role in different kinds of biological activities.Hydro distillation was performed using Clevenger-type apparatus and oil was collected above water. Leaves yielded aromatic oil (2.0%v/w to 2.5%v/w) than bark (0.3%v/w to 0.5%v/w). Also leaf oil was much denser, whereas bark oil was lighter. Among the accessions, *Nithyasree* has high leaf and bark oil which was a promisingresult and is as comparable with its variation in morphology.

GC-MS analysis of leaf and bark oil identified monoterpenoids, sesquiterpinoids and phenyl propanoids.Despite monoterpenoids and sesquiterpenoids, phenyl propanoids were the major class of compounds distributed in the essential oils of *C. verum* barks and leaves. Cinnamaldehyde was the major compound identified in most bark accession and eugenol in leaves. However chemical polymorphism was identified in a few accessions.HPTLC profiling confirmed the same by identifying marker compounds in all the samples. Content oftoxiccoumarins, which is a benchmark in the authenticity of cinnamon, was also evaluated .The results showed that authentic samples contains negligible amount of coumarin but concentration was high in case of market samples and cassia. So the name true cinnamon is relevant.

Genetic study was carried out using four polymorphic ISSR markers. All the markers were reliable and showed considerable polymorphism. As *Cinnamomum verum* contains high amount of polyphenols and polysaccharides, pure DNA from leaf samples were obtained by adding 20mg PVP in extraction buffer of QIAGEN, DNAeasy plant mini kit. PCR amplification of ISSR marker products resolved under1.4 % agarose gel produced distinct bands. Each bandwas manually scored. The POPGEN 32 software used to measure the following parameters:observed number of alleles (na), effective number of polymorphic loci and percentage of polymorphism(p%). A dendrogram was constructed based on the genetic distance.Gene diversity(h) was observed and found considerable genetic diversity in all the accessions.The samples CVL2 and CVL16 could be considered to possess a higher genetic variation as compared to other accessions.

The present study revealed that *Cinnamomum verum* is a potential spice having anti-oxidant properties due to rich source of phytoconstituents. From the detailed field study distribution pattern was confirmed. The hypothesis of study was fulfilled as satisfactory diversity was found in morphology, chemical constituents and at genetic

level. High variability exhibited by the different accessions of cinnamon may be caused by cross pollination phenomenon, changes in altitude, different stages of development and changing climatic conditions.

REFERENCES

7. REFERENCES

Abeysekera, W. P. K. M., Premakumara, G.A.S., and Ratnasooriya, W.D. 2013.In vitro antioxidant properties of leaf and bark extracts of Ceylon cinnamon (*Cinnamomum zeylanicum* Blume). *Tropical Agricultural Res.* 24 (2): 128 – 138.

Abeysinghe, N. G. C. D., Samarajeewa, G. Li., and Wijesinghe,K. G. G.2014. Preliminary investigation for the identification of Sri Lankan *Cinnamomum* species using randomly amplified polymorphic DNA (RAPD) and sequence related amplified polymorphic (SRAP) markers. *J.Natn.Sci.Foundation Sri Lanka.* 42 (3): 201-208.

Abraham, K., Woehrlin, F., Lindtner, O., Heinemeyer, G. and Lampen, A. 2010. Toxicology and risk assessment of coumarin: Focus on human data. *Mol. Nutr. Food. Res.* 54: 228 239.

Adinew. B. 2014. GC-MS and FT-IR analysis of constituents of essential oil from cinnamon bark growing in south-west of Ethiopia. *Int. J. Herb. Med.* 1: 22-31.

Allard, R.W. and Bradshaw, A.D. 1964. Implication of genotype-Environmental interaction in applied plant breeding. *Crop Science*.85: 3-5.

Alansi, S., Tarroum, M., Al-Qurainy, F., Khan, S. and Nadeem, M. 2016. Use of ISSR markers to assess the genetic diversity in wild medicinal (*Ziziphusspina-christi* L.) Wild collected from different regions of Saudi Arabia. *Biotechnology and biotechnological equipment*. 30(5): 942-947.

Andrew, R. L., Wallis, I. R. Harwood, C. E., and Foley, W.J. 2010.Genetic and environmental contributions to variation and population divergence in a broad-spectrum foliar defence of Eucalyptus tricarpa.*Ann. Bot.* 105: 707-717.

Anonymous. 2013. Department of export Agriculture. Ministry of Agriculture, New Delhi.

Arisdason., W. and Lakshminarasimhan., P. 2016. Status of plant diversity in India: an overview. *Envis centre on floral diversity.*

Asolkar, L.V., Chopra, R.N. and Kakkar, K. K. 1981. Second supplement to glossary of Indian medicinal plants with active principles.Part 1 (A–K).

Ayyar, T.V.R. 1940. Hand Book of economic entomology for South India, Govt. Press, Madras, India. 528.

Azad, R., Ranawaka, R. A. A. K., Senanayake, G., Wasantha, Kumara K. L., Pushpakumara, D. K. N. G., Wijesingh, K. G. G. and Sudarshanee, Geekiyanage. 2016. Morphological variation of cinnamon (*Cinnamomumverum*Persl) germplasm in Matara District of Sri Lanka. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*. 2 (1):6 – 14.

Balijepalli, M. K., Buru, A. S., Sakirolla, R. and Pichika, M. R. 2017. *Cinnamomum* genus: A review on its biological activities. *Int J Pharm Pharm Sci*. 9: 2.

Bandara, T., Uluwaduge, I. and Jansz, E. R. 2012. Bioactivity of Cinnamon with special emphasis on diabetes mellitus: a review. *Int J Food Sci Nutr*. 63: 380–386.

Barauh, A., Nath, S. C., and Hazarika., A. K. 2010. Investigation of essential oils of *Cinnamomumverum*J.Presl grown at lower Brahmaputra valley of Assam.*Indian perfumer*.54: 21-23.

Bhau, B. S., Gogoi, G., Baruah, D. Ahmed, R., Hazarika, G., Borah, B., Gogoi, B., Sarmah, D.K., Nath, S. C., Wann, S. B. 2015. Development of an effective and efficient DNA isolation method for *Cinnamomum* species.*Food chem*.188: 264-270.

Chakraborty, A., Vishwam, S., Murugan, R. and David, R. C. 2015. Chemical analysis of leaf essential oil of *Cinnamomum verum* from Palni hills, Tamil Nadu. *J chem. Pharm sci*. 8(3): 476-479.

Costa, M. A., Zia, Z. Q., Davin, L. B and Lewis, N. G. 1999.Toward engineering the metabolic Pathways of cancer-preventing lignans in cereal grains and other crops.*Recent AdvPhytochem*. 33: 67-87.

Croteau, R., Kutchan,T.M.,and Lewis, N.G. 2000. Natural products (secondary metabolites), in Biochemistry and molecular biology of plants (eds B. Buchanan, W. Gruissem and R. Jones). American Society of Plant Physiologists, Rockville.1250-1318.

Deb, C.R., Deb, M.S and Jamir, N.S. 2013. Callus mediated organogenesis and plant regeneration of *Cinnamomum tamala* Nees. (Lauraceae) from leaf and zygotic embryos.*Int. J. Pharma & Bio Sc*. 4(1): 614.

Devashayam, S., Koya, K.M.A. and Reddy, P.P. 1997. IPM in spices- challenges for the future. Proc.First Nat Symp. on pest management of horticultural crops, (Eds.) N. K. K. Kumar and A. Verghese, Indian Ins. Spices Res. Bangalore, India. 157-168.

Govindaraj, M., Vetriventhan, M. and Srinivasan, M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet. Res. Int.* 2015: 14.

Gulathi, B. C., Atal, C.K. and Kapur, B. M. 1982. Essential oils of Cinnamomumspecies In: Cultivation and utilization of aromatic plants. Regional research laboratory (CSIR).607-619.

Harborne, J. B. and Baxter, H. 1995. Phytochemical dictionary: a handbook of bioactive compounds from plants. 4 John St. London: Taylor & Francis Ltd.

Hema, R., Kumaravel, S. and Devi Martina T. 2010.Chromatograph interfaced to a Mass Spectrometer Analysis of *Cinnamomumverum*. *Nat Sci.* 8(11): 152-155.

Ho, K. Y., and Hung, T. Y. 2011. Cladistic relationships within the genus *Cinnamomum* (Lauraceae) in Taiwan based on analysis of leaf morphology and inter-simple sequence repeat (ISSR) and internal transcribed spacer (ITS) molecular markers. *Afr. J. Biotechnol.* 10(24): 4802-4815.

Hossein, N., Zahra, Z., Abolfazl, M., Mahdi, S. and Ali, K. 2013. Effect of *Cinnamon zeylanicum* essence and distillate on the clotting time.*J. Med. Plants Res.* 7: 1339–1343

Ibanez, P. and Maribel. 2015. Morphological variation of *Cosmos bipinnatus* (Asteraceae) and its relation to abiotic variables in central Mexico. *Historia natural.* 18. 88(1): 14-14.

Jayaprakasha, G. K., Jaganmohan, R. L. and Sakariah, K. K. 1997.Chemical composition of the volatile oil from the fruits of *Cinnamomum zeylanicum* Blume.*FlavFragr J.* 12: 331–333.

Jayaprakasha, G. K. and Rao, L. 2011. Chemistry, Biogenesis, and Biological Activities of Cinnamomum zeylanicum.*Crit. Rev. Food Sci. Nutr.* 51: 547-562

Joseph, J. 1981. Floral biology and variation in cinnamon.*Placrosym.* 4: 431 – 434.

84

Joshi, S. C., Padalia, R.C., Bisht, D.S. and Mathela, C.S. 2009. Terpenoid diversity in the leaf essential oils of Himalayan Lauraceae species. *Chem Biodiver.* 6: 1364-1373.

Joy, P. P., Thomas, J., Mathew, S., Jose, G. and Joseph, J. 1995.Aromatic Plants. Aromatic and Medicinal Plants Research Station. Odakkali, Asamannoor PO,Kerala.

Joy, P. P., Thomas, J., Mathew, S. and Jose, G. 1996. Elite cinnamon types for leaf oil and eugenol. Proc. Eighth Kerala Sci. Cong. 143-144.

Joy, P. P., Thomas J. and Samuel Mathew. 1998.Cinnamon (*Cinnamomumverum*Presl.) for flavour and fragrance. *FAFAI Journal*, 20(2):37-42.

Joy, P. P. and Maridass, M. 2008. Inter species relationship of *Cinnamomum*species using RAPD marker analysis.*Ethnobotanical Leaflets*. 12: 476-480.

Karthikeyan, S.2009. Flowering plants of India in 19th and 21st Centuries – A comparision.In: Krishnan, S. &Bhat, D.J. (Eds.), Plant and fungal biodiversity and bioprospecting. Goa University, Goa.19–30.

Kazemi, M., & Mokhtariniya, S. 2016.Essential oil composition of bark of *Cinnamomum zeylanicum.Journal of Essential Oil Bearing Plants*. 19(3): 786-789.

Khan, A., Safdar, M., Ali Khan, M. M., Khattak, K. N.and Anderson, R. A. 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care*.26: 3215–3218.

Khanuja, S. P., Shasany, A. K., Darokar, M. P., & Kumar, S. 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils.*Plant mol biol rep.* 17(1): 74-74.

Kit, Y. S. and Chandran, S. 2010. A Simple, rapid and efficient method of isolating DNA from Chokanan mango (*Mangifera indica* L) .*Afr J Biotechnol.* 9(36): 5805–5808.

Korner, C. 1999. Alpine Plant Life.Functional Plant Ecology of High Mountain Ecosystems.Springer, Berlin.

85

Krishnamoorthy, B.,Sasikumar, B., Rema, J., Gopalam, A.and J. Abraham. 1992. Variability and association studies in cinnamon (*Cinnamomum verum*).J.Presl Spices and Aromatic Crops. 1(2): 148-150.

Kuo, D. C., Lin, C.C., Ho, K.C., Cheng, Y.P., Hwang, S.Y. and Lin, T.P. 2010. Two genetic divergence centers revealed by chloroplastic DNS variation in populations of Kanehirae Hay.*Conser Gen.* 11:803-812.

Leela, N. K. (2008). Chemical Characterization of *Cinnamomum* Germplasm: Final Report 2005-08.

Lin, T.P., Cheng, Y.P. and Huang, S.G. 1997. Allozyme variation in four geographic areas of *Cinnamomumkaneirae.J Hered.* 88: 433 - 438.

Lungarini, S., Aureli, F. andConi, E. Coumarin and cinnamaldehyde in cinnamon marketed in Italy: a natural chemical hazard. *Food Additives and Contaminants A*. 2008.25(11):1297–1305.

M' Ribu, H. K. and Hilu, K. W. 1994.Detection of variation in Panicum millets through random amplified polymorphic DNA.*Theor. Appl. Genet.* 88: 412-416.

Mallavarapu, G. R., Ramesh, S., Chandrasekhara, R. S., Rajeswara, Rao B. R., Kaul, P. N. and Bhattacharya, A. K. 1995. Investigation of the essential oil of Cinnamon leaf grown at Bangalore and Hyderabad.*Flav Fragr J.* 10: 239–242.

Mani, M.S. 1965. Key to plant galls from India. *Bull. Bot. Surv. India*. 7 (1- 4): 89–127.

Manosi, D., Suvra, M., Budhimanta, M., and Jayram, H. 2013.Ethnobotany, phytochemical and pharmacological aspects of *Cinnamomum zeylanicum* blume.*Int. Res. J. pharm.* 4: 58-63.

Maridass, M .and Victor, B. 2008. Ethnobotanical uses of *Cinnamomum* species, Tamil Nadu, India.Ethnobotanical Leaflets. 12: 150-155.

Mathew, S. and Emilia A. T. 2006. Studies on the antioxidant activities of Cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. *Food Chem.* 94.520-528.

Morrison, D. A., and Weston, P.H., 1985. Analysis of morphological variation in a field sample of *Caladeniacatenata*(smith) *Druce* (Orchidaceae).*Aust J Bot.* 33:185-196.

Maridass, M. and Victor, B. 2008. Ethnobotanical uses of *Cinnamomum* species, Tamil Nadu, India. *Ethnobotanical Leaflets*. 12:150-155.

Mazimba,O., Wale, K., Kwape,T. E. Shetonde,O., Mihigo. and Kokengo, B. M. 2015. *Cinnamomumverum*: Ethylacetate and methanol extracts antioxidant and antimicrobial activity. *J. Med. Plants. Stud.* 3(3): 28-32.

Muthuramkumar, S. 2002. Diversity and ecology of lianas in a tropical evergreen forest in the Anamalais, Western Ghats, India. Ph.D. Thesis, Pondicherry University, Pondicherry.

Mohammad, R. M., Mohammad, R. F. M., Ali. E. and Darab, Y. 2015. Genetic relationships of Iranian *Hypericumperforatum*L.wild populations as evaluated by ISSR markers.*Plant SystEvol.* 301(2):657–665.

Nath, S. C., Pathak, M. G. and Baruah. 1996. Benzyl Benzoate, the major component of the leaf and stem bark oil of *Cinnamomumzeylanicum*Blume. *J. Essential Oil Res.* 8:327-328.

Nayar, M.P. 1996. Hot spots of endemic plants of India, Nepal and Bhutan. TBGRI, Thiruvananthapuram.

Nayar, T.S., Beegam, R., Mohanan, N. and Rajkumar, G. 2006. Flowering Plants of Kerala.Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram.

Okada, H. and Tanaka, R. 1975. Karyological studies in some species of Lauraceae. Taxon 24: 271–280.

Pandey, S. and Singh, R. 2014. Phytochemical screening of selected medicinal plant *Cinnamomumzeylanicum*bark extract. *Int. J. Res. Sci. Publ.* 4(6): 2250-3153.

Patel, Kirti., Ali, Sadaquat., Sotheeswaran., Subramanium., Dufour. and Jean-Pierre. 2007. Composition of the leaf essential oil of *Cinnamomumverum* (Lauraceae) from Fiji Islands. *J. Essential Oil Bearing Plants*. 10: 374-377.

87

Paranagama, P.A., Wimalasena, S., Jayatilake, G.S., Jayawardena, A.L., Senanayake, U.M. and Mubarak, A.M. 2001. A comparison of essential oil constituents of bark, leaf, root and fruit of cinnamon (*Cinnamomumzeylanicum*Blume.) grown in Sri Lanka. *J. National. Sci. Foundation Sri Lanka*. 29(3&4):147-153.

Peterson, D. W., George, R. C., Scaramozzino, F., LaPointe, N. E., Anderson, R. A., Graves, D. J. and Lew, J. 2009. Cinnamon extract inhibits tau aggregation associated with Alzheimer's disease in vitro. J Alzheimers Dis. 17(3): 585-97.

Pooja, A., Arun, N., and Maninde, K. 2013.GC-MS profile of volatile oils of *Cinnamomum zeylanicum* Blume and *Ocimumkilim* and *scharicum* Baker ex Gurke.*Int. J. Pharm. Sci. Rev. Res.* 19(2): 124-126.

Prakashkumar, R., Anoop, K. P., Ansari, R., Sivu, A. R., Pradeep, N. S. and Madhusoodanan, P. V. 2015. Analysis of genetic diversity of Lagenandraspp.(Araceae) of Kerala (South India) using ISSR Markers.*Int J Sci Res.* 4(6):775–777.

Qin, B., Nagasaki, M., Ren, M., Bajotto, G., Oshida, Y., and Sato, Y. 2003. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. *Diabetes Res Clin Pract.*62: 139–148.

Qui, L.J., Xing, L.L., Guo, Y., Wang, J. and Chang, R.Z. 2013. A platform for soybean molecular breeding: the utilization of core collections for food security. *Plant Mol. Biol.* 83: 41-50.

Rajapakse, R.H.S. and Kulasekera, V. L. 1982.Some observations on insect pests of cinnamon in Sri Lanka.*Entomon*. 7(2): 221- 223.

Rajeev, P. and Dinesh, R. 2005. Cinnamon (Extension Pamphlet), Director, Indian Institute of Spices Research. 1–7.

Ranasinghe, P., Jayawardana, R., Galappaththy, P., Constantine, G. R., Vas G. N. and Katulanda, P. 2012. Efficacy and safety of 'true' Cinnamon (*Cinnamomum zeylanicum*) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. *Diab Med.* 29: 1480–1492.

Ranasinghe, P., Pigera, S., Premakumara, G. S., Galappaththy, P., Constantine, G. R., and Katulanda, P. 2013. Medicinal properties of "true" cinnamon (*Cinnamomum zeylanicum*): a systematic review. *BMC Complementary and Alternative Medicine*.13:275.

Raghu, A.V., Unnikrishnan K. P., Hashim, K.M., Balachandran, I. and Mohanan K.V. 2007.Studies on morphological and phytochemical variability of different populations of Tribulusterrestris.*Int J Plant Breeding and Genetics*. 1(2): 95-100.

Ramasubramanian B., 2005. Studies on variability, genetic divergence and crop improvement in Tea (Camellia assamica (Masters) Wight).Ph. D Thesis, University of Calicut, Kerala, India.

Ravindran, P.N., Babu, K.N. and Shylaja, M. 2004. Cinnamon and cassia, The genus *Cinnamomum*. *Medicinal And Aromatic Plants – Industrial Profile*, 361 pp.

Ravindran, S., Krishnaswamy, N. R., Manilal, K.S. and Ravindran, P.N. 1991. A cluster analysis study of *Cinnamomum* from Kerala, *India.Feddes Report*. 102: 13-21.

Ravindran P.N. and Nirmal Babu, K., 1994. Chemotaxonomy of south Indian Piper.*J Med and Aromatic Plant Sci*. 3(1): 6-13.

Ravindran S., Krishnaswamy N.R., Manilal. K.S. and Ravindran, P.N., 1992. Chemotaxonomy of *Cinnamomum*Schaef.occurring in Western Ghats.*J Indian Bot Society*. 71: 37-41.

Salimath, S. S., De Oliveira, A. C. and Godwin, I. D. 1995. Assessment of genomic origin and genetic diversity in the genus Eleusine with DNA markers. *Genomic*. 38: 757-763.

Sang, B. L. and Soren, K. R. 2000. Molecular markers in some medicinal plants of the Apiaceae family.*Euphytica*. 114: 87-91.

Sandigawad, A.M. and Patil, C.G. 2011.Genetic diversity in *Cinnamomumzeylanicum*Blume.(Lauraceae) using random amplified polymorphic DNA (RAPD) markers.*African J. Biotech*. 10: 3682-3688.

Singh, Gurdip, Maurya, S. and Catalan, C A. N. 2007. "A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents." Food and chemical toxicology 45(9) : 1650-1661.

Sultana, N. and Ata, A. 2008. Oleanolic acid and related derivatives as medicinally important compounds. *J Enzyme Inhib Med Chem*. 23(6): 739-756.

Sproll, C., Ruge, W., Andlauer, C., Godelmann, R., and Lachenmeier, D. W. 2008. HPLC analysis and safety assessment of coumarin in foods.*Food Chem.* 109(2):462–469.

Takasao, N., Tsuji-Naito, K., Ishikura, S., Tamura, A. and Akagawa, M. 2012. Cinnamon extract promotes type I collagen biosynthesis *via* activation of IGF-I signaling in human dermal fibroblasts. *J Agric Food Chem.* 60: 1193-200.

Theis, N. and Lerdau, M. 2003. The evolution of function in plant secondary metabolites.*Int J Plant Sci.* 164:93-103.

Toni, M. Kutchan. 2005. A role for intra and intercellular translocation in natural product biosynthesis.*CurrOpin Plant Biol.* 8:292–300.

Tsuji-Naito, K. 2008. Aldehydic components of Cinnamon bark extract suppresses RANKL-induced osteoclastogenesis through NFATc1 downregulation. *Bioorganic and Med Chem.* 16: 9176-83.

Unlu, M., Ergene, E., Unlu, G. V., Zeytinoglu, H. S. and Vural, N. 2010. Composition, antimicrobial activity and *in vitro* cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae).Food Chem Toxicol. 48(11): 3274-80.

UN comtrade 2011.United Nations Commodity Trade Statistics Database.*http://comtrade.un.org/db/* (accessed April 8, 2013).

Wang, Y.H., Avula, B., Nanayakkara, N.P., Zao, J. and Ikhlas, A. 2013. Cassia cinnamon as a source of coumarin in cinnamon flavoured food and food supplements in the United states. *J. Agric. Food chem.* 61: 4470-4476.

Wijesekera., R. O. 1978. Historical overview of the cinnamon industry.*Crit. Rev. Food. Sci. Nutr.* 10: 1–30.

Wijesinghe, K.G.G. and Gunarathna, W. D. L. 2001. Characterization of true Cinnamon (*Cinnamomumverum*Persl) based on leaf morphology and their relationship with yield and quality. Sri Lanka Association for the Advancement of Science.57th Annual Session. Proc. Part I: 42 pp.

Yang, C.H. Li, R.X., and Chuang, L.Y., 2012. Antioxidant Activity of various parts of *Cinnamomum* cassia extracted with different extraction methods. *Molecules.* 17 (6): 7294-7304.

Zidorn, C.,Schubert, B. and Stuppner, H. 2005. Altitudinal differences in the contents of phenolics in flowering heads of three members of the tribe Lactuceae (Asteraceae) occurring as introduced species in New Zealand. *J. Bot.* 39: 141-146.

EVALUATION OF PHYTOCHEMICAL DIVERSITY OF *CINNAMOMUM VERUM* J. PRESL IN SOUTH INDIA

ATHIRA S. NAIR

(2012-09-109)

Abstract of the thesis
submitted in partial fulfillment of the
requirement for the degree of

B. Sc. – M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture
Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522
KERALA, INDIA
2017

ABSTRACT

Cinnamon, obtained from the inner bark of *Cinnamomum verum* J. S. Presl (Syn. *C. zeylanicum* Blume), is the second largest spice used in the world. There are several ambiguities regarding the origin, distribution, diversity and authenticity of Cinnamonand the present study evaluates the intra species diversity of *C. verum* distributed in south India through morphological, phytochemical and genetic analyses. Field study was conducted across south India and revealed that cinnamon (*C. verum*) is naturalized especially in homesteads of Kerala. Large scale plantations were rare and it is suggested to take initiatives to protect existing plantations as a genetic pool and also to promote large scale cultivation of cinnamon in Kerala to get good quality cinnamon. Morphological characters of leaves including qualitative and quantitative characteristics were evaluated and variations were observed among different accessions. The interspecies chemical diversity was studied based on volatile as well as non volatile chemical profiling. HPTLC analysis was carried out to determine presence of marker compounds in solvent extracts, while GC-MS analysis of essential oils were carried out for volatile chemical profiling. The chemical profiles showed significant variation between the accessions and were grouped in to different clads based on their chemical compositions. Content oftoxiccoumarin, which is a benchmark in the authenticity of cinnamon, was evaluated and results showed that authentic sample contain negligible or below detection limit of coumarin compared to market samples and cassia cinnamon. The genetic diversity was also analyzed among the different accessions of cinnamon collected. Highly polymorphic, four ISSR primers were used for genetical study and the genetic diversity was assessed using dendrogram that grouped 12 accessions in to 4 groups. The results revealed the existence of variationwithin the studied cinnamon accessions which may be caused by cross pollination, and due to the influence of other biogeographic conditions. The accession collected from the remaining of Cinnamon plantation at Ancharakkandy, Kannur emerged as a promising accession with favorable flavor qualities.