BIOACTIVE METABOLITES AND BIOMARKERS FROM *RHIZOPHORACEAE* MANGROVES- A CHEMOTAXONOMIC APPROACH

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Ph.D. Thesis under the Faculty of Marine Sciences

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Certificate

This is to certify that the thesis entitled "**Bioactive Metabolites and Biomarkers from *Rhizophoraceae* mangroves- A Chemotaxonomic Approach**" is an authentic record of the research work carried out by Ms. Nebula Murukesh, under my supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Kochi-682016, in partial fulfilment of the requirements for Ph.D. degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any University.

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Declaration

I hereby declare that the thesis entitled **"Bioactive Metabolites and Biomarkers from *Rhizophoraceae* mangroves- A Chemotaxonomic Approach "** is an authentic record of the research work carried out by me under the guidance and supervision of Dr. N. Chandramohanakumar, Professor, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, and no part of this has previously formed the basis of the award of any degree, diploma, associateship, fellowship or any other similar title or recognition.

Nebula Murukesh

Kochi-16
December 2014

Dedicated to

My family, my strength…

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Preface

Mangroves are specialised ecosystems developed along estuarine sea coasts and river mouths in tropical and subtropical regions of the world, mainly in the intertidal zone. Hence, the ecosystem and its biological components is under the influence of both marine and freshwater conditions and has developed a set of physiological adaptations to overcome problems of anoxia, salinity and frequent tidal inundations. This has led to the assemblage of a wide variety of plant and animal species of special adaptations suited to the ecosystem.

The path of photosynthesis in mangroves is different from other glycophytes. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis. As they survive under extreme conditions of salinity, temperature, tides and anoxic soil conditions they may have chemical compounds, which protect them from these destructive elements. Mangroves are necessarily tolerant of high salt levels and have mechanisms to take up water despite strong osmotic potentials. Some also take up salts, but excrete them through specialised glands in the leaves. Others transfer salts into senescent leaves or store them in the bark or the wood. Still others simply become increasingly conservative in their water use as water salinity increases. A usual transportation or biosynthetic path as other plants cannot be expected in mangrove plants.

In India, the states like West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Andaman and Nicobar Islands, Kerala, Goa, Maharashtra, and Gujarat occupy vast area of mangroves. Kerala has only 6 km^2 total mangrove area with *Rhizophora apiculata*, *Rhizophora mucronata*, *Bruguiera gymnorrhiza*, *Bruguiera cylindrica*, *Avicennia officinalis, Sonneratia caseolaris, Sonneratia apetala* and *Kandelia candal*, as the important species present, most of which belong to the family *Rhizophoraceae*.

Rhizophoraceae mangroves are ranked as “major elements of mangroves” as they give the real shape of this unique and interesting ecosystem and these mangrove species most productive and typical characteristic ecosystem of World renowned. It was found that the *Rhizophoraceae* mangrove extracts exhibit several bioactive properties. Various parts of these mangroves are used in ethnomedicinal practices. Even though extracts from these mangroves possess therapeutic activity against humans, animal and plant pathogens, the specific metabolites responsible for these bioactivities remains to be elucidated. Various parts of these mangroves are used in ethnomedicinal practices. There is a gap of information towards the chemistry of *Rhizophoraceae* mangroves from Kerala.

Thorough phytochemical investigation can achieve the validity of ethnomedicines as well as apply the use of mangrove plants in the development of new drugs. Such studies can pave a firm base for their use in biomarker and chemotaxonomic studies as well as for the better management of the existing mangrove ecosystem. In this study, the various chemical parameters including minerals, biochemical components, bioactive and biomarker molecules were used to classify and assess the possible potentials of the mangrove plants of the true mangrove family *Rhizophoraceae* from Kochi.

The thesis is divided into six chapters. Chapter 1 is the introduction and reviews the chemical compositions as well as bioactivities of mangrove plants belonging to *Rhizophoraceae* family. It also deals with aim and scope of the present study. Chapter 2 provides the details of the plant materials and the analytical methodology adopted.

Chapter 3 is devoted to elemental, isotopic, mineral and biochemical compositions of the plants under consideration in order to evaluate the basic chemical nature of the plants for their classification. Chapter 4 gives the idea of occurrence of food flavonoids and their role in determining the nutritive, taxonomic as well as bioactive role in mangroves.

Chapter 5 deals with the variations in fatty acid profiles of common *Rhizophoraceae* mangrove species found in Kochi with the aim of determining if differences occur in the fatty acid profiles of mangrove tissues and examining the prospect of using individual or groups of fatty acids as taxonomic markers and biomarkers. Chapter 6 mainly deals with the distribution of alkanes and analyses their relevance as a chemotaxonomic tool.

The salient features of the present investigation are summarised at the end of the thesis.

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List of Abbreviations

TCHO	Total Carbohydrates
LMWC	Low Molecular Weight Carbohydrates
LPD	Total Lipid Content
PRT	Total proteins
PS	Polysaccharides
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
FA	Fatty acid
EFA	Essential fatty Acid
SAFA	Saturated fatty acid
MUFA	Mono unsaturated fatty acid
PUFA	Poly unsaturated fatty acid
BCL	Bruguiera cylindrica leaves
BCB	Bruguiera cylindrica bark
BGL	Bruguiera gymnorrizha leaves
BGB	Bruguiera gymnorrizha bark
KCL	Kandelia candel leaves
KCB	Kandelia candel bark
RAL	Rhizophora apiculata leaves
RAB	Rhizophora apiculata bark
RML	Rhizophora mucronata leaves
RMB	Rhizophora mucronata bark

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Chapter 1

INTRODUCTION

Contents

1.1 Mangrove ecosystem
1.2 Distribution of mangroves
1.3 Rhizophoraceae mangroves
1.4 Chemotaxonomy
1.5 Aim and scope of the present study

Plants have been important and indispensable sources of natural products for the health of human beings for a long period of time in the history and they possess great potential for producing new drugs (Nascimento *et al*., 2000; Kuete, 2010; Littleton *et al*., 2005). Medicinal plants have been used for centuries to cure human ailments and diseases because they contain components of curative value (Panda 2010). Plants are sources of compounds that have the ability to combat diseases, antimicrobial, antiviral and antifungal activities (Nascimento *et al*., 2000, Gazim *et al*., 2008, Premanathan *et al*., 1999). About 25% of the drugs prescribed worldwide come from plants. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). Tropical and subtropical areas of the world are conferred with abundance and diversity of flora and

herbs which have properties such as antimicrobial, antiviral and antifungal, remaining unexplored.

Mangrove vegetation is an important coastal ecosystem associated with tidal / mud flats and back water systems. They are also among the world's most productive ecosystems. Mangroves create unique ecological environments that host rich assemblages of species. They serve as custodians of their juvenile stock and form most valuable biomass (Odum, 1971). These ecosystems play a very dynamic and significant role in the estuarine mouths having a potentially high impact on the carbon budget of the global coastal zone.

1.1 Mangrove ecosystem

Mangroves form one of the world's most unique ecosystems because they thrive where no other trees can survive – in the transition zone between the ocean and land. These plants, and the associated microbes, fungi, plants, and animals, constitute the mangrove forest community or mangal. The mangal and its associated abiotic factors constitute the mangrove ecosystem. So, mangroves are wetland ecosystems, formed by special types of plants and animals of low lying tropical coasts, estuaries, deltas, backwaters and lagoons where they exist in conditions of high salinity, extreme tides, strong winds, high temperatures and muddy anaerobic soils (Kathiresan 2000 , Kathiresan and Bingham 2001).

Mangroves are a valuable ecological and economic resource. They protect and stabilise coastlines as well as enrich coastal waters. Throughout-welling of leaf litter and dissolved organic matter, mangroves act as detritus

source to the adjacent oligotrophic marine food webs, supporting valuable estuarine and coastal fisheries (Singh *et al*., 2005). Mangrove ecosystems are known to be potentially significant sources of organic matter to adjacent estuaries and coastal waters on a global scale (Jennerjahn and Ittekkot, 2002; Dittmar *et al*., 2006). They play a crucial role in the biogeochemical cycling of phosphorus, carbon, nitrogen and other nutrients (Ratheesh Kumar, 2011; Bunt, 1992).

Their unique features also make them ideal sites for experimental studies of biodiversity and ecosystem function. The factors like water level or tidal inundation, water and soil salinity, pH, sediment flux, oxygen potential, availability of anions and cations, hydrodynamics, stresses etc. are important in determining the habit and habitat selection, individual species distribution, succession pattern and interspecific competition among these mangroves. Mangroves provide food and a wide variety of traditional products for the residents. The mangrove leaves are useful contributors to the nutrient system of the mangrove environment. It is known that mangrove leaves contain sufficient amounts of minerals, vitamins and amino acids, which are essential for the growth, and nourishment of marine organisms and livestock. Mangrove foliage plays an important role in the formation of detritus, which is utilised by several estuarine and marine detritivorous organisms. Moreover, mangrove leaves make a superior fodder due to their high salt and iodine content (Bandaranayake, 2002). Extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens. Mangroves may be further developed as sources of high-value commercial products and fishery resources and as sites for a burgeoning ecotourism industry.

1.2 Distribution of mangroves

Mangroves occupy less than 1 % of the world's surface (Saenger, 2002) and are mainly found between the Tropic of Cancer and the Tropic of Capricorn on all continents covering an estimated 75 percent of the tropical coastline worldwide. There are more than 18 million ha of global mangroves inhabiting in 112 countries and territories in the tropical and subtropical region (Fig. 1.1 and 1.2) (Kathiresan and Bingham 2001; Spalding, 1997).

Mangroves are largely restricted to latitudes between 30° north and 30° south. Northern extensions of this limit occur in Japan (31°22'N) and Bermuda (32°20'N); southern extensions are in New Zealand (38°03'S), Australia (38°45'S) and on the east coast of South Africa (32°59'S) (Spalding, 1997; Yang *et al.*, 1997). Mangroves are not native to the Hawaiian Islands, but since the early 1900's, at least 6 species have been introduced there.

Around 34 major and 20 minor mangrove species belonging to about 20 genera in over 11 families have been recorded globally (Tomlinson, 1986). Mangroves of South and Southeast Asia form the world's most extensive and diverse mangrove systems comprising 41.4 percent of global mangroves. Indian mangroves make up 3.1 percent of the total global cover and are distributed along all the maritime states, except the union territory of Lakshadweep.

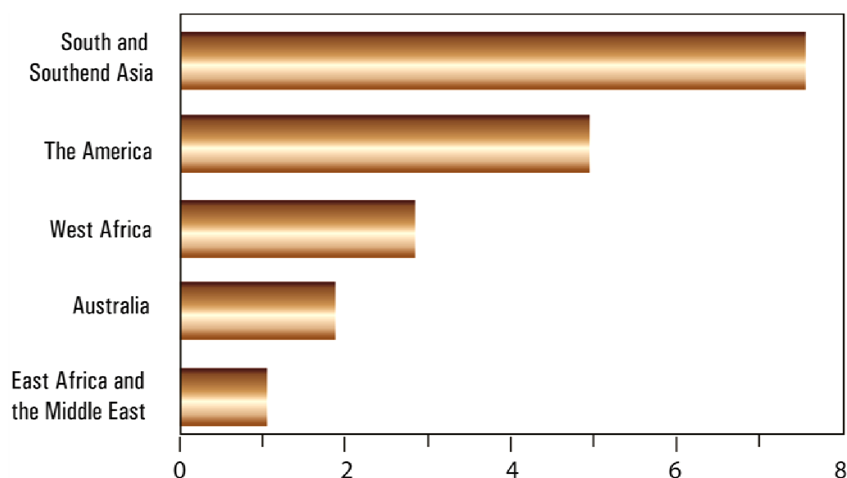


Fig. 1.1 Global coverage of mangrove forests (Kathiresan and Bingham, 2001).

1.2.1 Mangrove ecosystem of India

Mangroves in India account for about 3% of the global mangroves and 8% of Asian mangroves (SFR, 2009; FAO, 2007). Recent data available from State of Forest Report 2011 of the Forest Survey of India, Dehra Dun shows that mangrove cover in the country is 4,662.56 sq.km, which is 0.14 percent of the country's total geographical area. These mangrove habitats (69°E-89.5°E longitude and 7°N-23°N latitude) comprise three distinct zones: East coast habitats having a coast line of about 2700 km, facing Bay of Bengal, West coast habitats with a coast line of about 3000 km, facing Arabian sea, and Island Territories with about 1816.6 km coastline (Singh *et al.*, 2012). In India, the states like West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Andaman and Nicobar Islands, Kerala, Goa, Maharashtra, and Gujarat occupy vast area of Mangroves. The very dense mangrove comprises 1,403 sq.km (30.10 % of the mangrove cover), moderately dense mangrove is 1,658.12 sq.km (35.57%) while open

mangroves cover an area of 1,600.44 sq.km (34.33%). Distribution of mangroves along the Indian coast is presented in Table1.1.

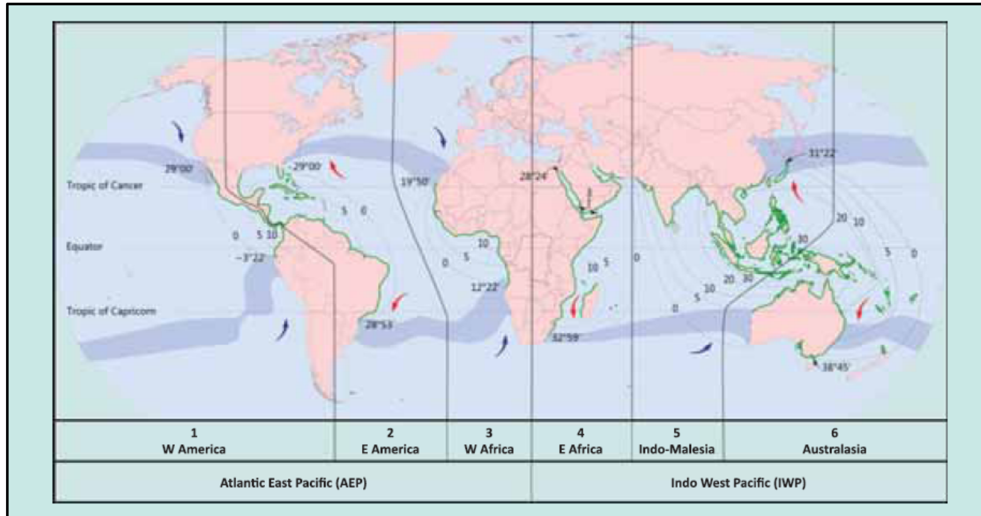


Fig. 1.2 Distribution of mangroves in different regions of the world showing their latitudinal extremes in relation to the 20ºC winter water temperature isotherm, and the decline in number of species with increasing latitude (Clough, 2013).

The mangrove ecosystem is basically of three types, the first being the deltaic mangroves located along the mouth of major estuaries on east coast and Gulf of Kachh and Khambhat Gulf on the west coast. These cover up to 53% of the total Indian mangroves out of which Sunderbans cover about 78%. Second types are the coastal mangroves which are found along the intertidal coastlines, minor river mouths, sheltered bays, and backwater areas of the west coast this constitute 12% of the mangrove area of India and lastly the island mangroves which are found along shallow protected intertidal zones of bay islands such as Lakshadweep and Andamans. They are approximately 16% of the total mangrove area (Ingole, 2005). The most

dominant mangrove species found along the east and west coast of India are *Rhizophora mucronata, Rhizophora apiculata, Bruguiera gymnorrhiza, Bruguiera parviflora,, Sonneratia alba, Sonneratia caseolaris, Cariops tagal, Heretiera littoralis , Xylocarpus granatum, Xylocarpus molluscensis, Avicennia officinalis, Avicennia marina, Excoecaria agallocha, Lumnitzera racemosa* (Kathiresan, 2003)

Table 1.1. Distribution of different types of Mangroves along the Indian Coast and their area (Source: Forest Survey India, 2011).

Sl No	State/UT	Very Dense Mangrove (Km^2)	Moderately Dense Mangrove (Km^2)	Open Mangrove (Km^2)	Total (Km^2)
1	Andhra Pradesh	0	126	226	352
2	Goa	0	20	2	22
3	Gujarat	0	182	876	1058
4	Karnataka	0	3	0	3
5	Kerala	0	3	3	6
6	Maharashtra	0	69	117	186
7	Orissa	82	97	43	222
8	Tamil Nadu	0	16	23	39
9	West Bengal	1038	881	236	2155
10	Andaman & Nicobar Islands	283	261	73	617
11	Daman & Diu	0	0.12	1.44	1.56
12	Puduchery	0	0	1	1
	Total	1403	1658.12	1601.44	4662.56

1.2.2 Mangroves of Kerala coast

Kerala lies towards the South - West coast of India, a segment barred by the Western Ghats. It extends between the latitudes 8" 18' and 12' 48' north and longitudes 71" *53'* and 77" 24' east with an area of about

38863km^2 (Ram and Shaji, 2013). Kerala with a coastline of about 580 km and 41 rivers emptying into the Lakshadweep Sea was once very rich in mangrove formations, perhaps next only to Sunderbans in the eastern part of the mainland of the country, extending to more than 70,000 ha of mangroves (Basha, 1992). Later this has been diminished to 4200 ha (Mohanan, 1997) of isolated patches consisting of few species.

According to a study by the Kerala Forest Research Institute, Peechi, the extent of undisturbed mangroves is reduced to just 150 hectares, mostly in Kannur, Kozhikode, and Ernakulam. The potential mangrove area, however, is estimated to be 1,670 hectares which include 755 hectares in Kannur and 293 hectares in Kozhikode. As per the report by Forest survey of India (SFR 2011), Kerala has 6km^2 of mangrove land (Table1). The important mangrove patches existing now in Kerala are mangroves of Veli, Quilon, Kumarakom, Kannamali, Mangalavanam, Chetwai, Nadakkavu, Edakkad, Pappinisseri and Kunjimangalam which have been singled out for conservation and rehabilitation (Suma, 1995). Study conducted by the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology identified 14 species of mangroves along Kerala Coast. Important mangrove species are *Rhizophora apiculata, Rhizophora mucronata*, *Bruguiera gymnorrhiza*, *Bruguiera cylindrica*, *Avicennia officinalis*, *Sonneratia caseolaris*, *Sonneratia apetala*, *Kandelia candal.*

The higher population density in Kerala coast has resulted in tremendous pressure on the natural ecosystem, particularly the mangroves. The wetlands of Kerala included a large mangrove swamp centuries ago and anthropogenic activities modified the system into settlements, agricultural

fields, filtration ponds and prawn culture fields. Vast areas of mangal lands were cleared and reclaimed for urbanisation, construction of harbours, ports, prawn farming, coconut plantation and rice fish culture (Gopalan *et al.*, 1983). These reclaimed lands of the coastal areas are underdeveloped and rice culture or brackish water fish and prawn culture are commonly practiced there.

1.3 *Rhizophoraceae* mangroves

The *Rhizophoraceae* is a pantropical family of about 16 genera and 120 species of trees and shrubs (Naskar and Mandal, 1999). A detailed taxonomic history was described by Juncosa and Tomilson (1988). The family name originates with the genus Rhizophora of Linnaeus in 1753, preceded by Rumphiu's Mangium. Both Linnaeus and Rumphiu's used their generic names respectively, *Rhizophora* and *mangium*, to include all the plants of mangroves in that period. Rhizophoraceae was formally designated as the "order *Rhizophoreae*", comprising *Bruguiera* Lamk., *Rhizophora* Linnaeus and *Carallia* Roxburgh. Later the family was subdivided into *Legnotidae* and *Rhizophoreae* sensu stricto, which still included *Carallia.* Subsequently, these two sections were raised to family status but *Carallia* was transferred to the *Legnotidae*. Therefore, this is the first inception of the first recognition of the *Rhizophoreae* as 'Mangrove *Rhizophoraceae*' (Juncosa and Tomilson, 1988).

The *Rhizophoraceae* family of true mangroves have twenty four species in four genera which include *Bruguiera* containing seven species, *Ceriops* containing five species, *Kandelia* containing two species and

Rhizophora having ten species (Nebula et. al, 2013). The distribution of species in *Rhizophoraceae* family is given in Table 1.2.

Table 1.2 Mangroves of *Rhizophoraceae* family of true mangroves

Bruguiera	***Ceriops***	***Kandelia***	***Rhizophora***
B. cylindrica	*C. decandra*	*K. candel*	*R. apiculata*
B. exarista	*C. tagal*	*K. obovata*	*R. harrisoni*
B. gymnorrizha	*C. tagal var. australasica*		*R. lamarckii*
B. hainessi	*C. tagal var. typical*		*R. mangle*
B. parviflora	*C. Zeppeliana Blume*		*R.mucronata*
B. sexangula			*R.racemosa*
B.sexangula var. rhynchopetala			*R. samoesis*
			R. selala
			R.stylosa
			R. annamalayana

1.3.1 Morphological features

Mangroves are highly adapted to the coastal environment, with exposed breathing roots, extensive support roots and buttresses, salt-excreting leaves, and viviparous water-dispersed propagules. These adaptations vary among taxa and with the physico-chemical nature of the habitat (Duke, 1992). All the true mangroves of the four genera of the tribe *Rhizophoraceae* mostly grow in the intertidal silty clay and loam soil, frequently inundated with high saline tidal seawater or brackish water; their

salt resistance efficiencies are very high for having several morphological and anatomical features.

Medium to tall trees, occasionally shrubs or small trees; much spreading diffuse woody branches, bark grayish to whitish gray and mostly contain high percentage of red tannin. Aerial roots, stilt roots, bow roots, prop roots, knee roots and plank roots or flanges, these roots mostly support the trunks in the clayey silted up sediments within the intertidal zones of the mangrove habitats. Their shoots have differentiated into nodes and congested internodes; internodes 0.1 cm to 1.0 cm long, initially covered by brown thin papery covers; apical shoot green , in the shoot apex the green leaves are clustered; rough brown layers encircling the nodes and continued below it two leaf scars present on both sides.

Phyllotaxis bijugate and leaves are mostly arranged densely on the apical part of the shoots and distinct leaf scars are present on the basal part of the branches. Leaves are mostly broad, thick, leathery, highly cuticularised, waxy, dark green, long petioled. Leaves are opposite decussate, simple, exstipulate, petiolate, petiole 2cm- 5cm long, slightly flattened at the base; 0.3 cm- 0.45 cm broad, glabrous, green, pulvinous, flexible; lamina broadly ovate, elliptic, entire, acute or mucronate; tapering both apically and basally 12.0 cm- 20.5 cm long X 5.0 cm – 8.0 cm broad; dorsiventral, dorsal side waxy, shiny, coriaceous, dark green, mid vein distinct in ventral side, unicosted reticulate venation inconspicuous.

Flower solitary cyme, aggregrate cyme, mostly 0.5 cm- 4.0cm long with peristant calyx teeth; fruit capsule. Germination viviparous and hypocotyles ranges 5.0cm- 75.0 cm long, pendulous from the mother plant.

pollination by wind, insects and birds. Infloresence cymes, opposite decussate, ebracteate, penduncle 1.8 cm long 5.5 cm long, 2 flowers or 7 flowers on each penduncle; penduncle erect, slightly bent. Flowers ebracteate, pedicellate or sessile, pedicel upto 2.0 cm long, complete, bisexual, regular, erect, odourless, upto 4 cm long, globose; sepals- 4, polysepalous, superior, ovate, entire, acute, fleshy, valvate, persistent in the fruit, upto 1.4 cm long X 0.7 cm broad; petals- 4, polypetalous, superior, lanceolate, entire, acute, deciduous, alternate to sepal, 1.0 cm long X 0.25 braod, shorter than sepals; stamens 8-32, free, filament, short or sessile, anther bilobed, inserted, introrse, sagittate, basifixed, upper surface same levelled, yellowish, superior, longitudinally dehiscent; carpels- 2-3, syncarpy; ovary globose, inferior, 2-4 chambers, 2-6 ovules in each chamber, axile palcentation; style-1, terminal, short, stigma-2, bifid, short.

Fruits capsule, 2.0 cm- 7.0 cm long, 7.0 cm – 9.5 cm diameter, oval, broad in middle and tapering towards both ends, brownish in dry condition, with four persistent sepals; seed one, covered by thick indehiscent pericarp; after maturation the seed bifurcate transversely within the fruit; upper potion with tapering appendages, bagged into furrow bascate like cavity. Seed- 1.8 cm long x 2.2 cm diameter; hypocotyle cylindrical; germination viviparous, epigeal.

1.3.2 Chemical constituents

As per the reports, the major chemical constituents of plants of the family R*hizophoraceae* comprise diterpenoids, triterpenoids, phenolic compounds and steroids. Terpenoids are the predominant constituents of this family. Out of 24 species, reports on chemical constituents of only 16

species are available. Not many studies have been reported on the chemical composition of plants of this family.

The metabolic pattern of the genus *Bruguiera* has been extensively characterised by a suite of di and triterpenes. In addition, they also produce flavonoids, tropane derivatives and cyclic polysulphides. 22 metabolites from B. *cylindrica*, 54 metabolites from *B. gymnorrhiza*, 9 metabolites from *B. exaristata*, 6 from *B. parviflora*, 2 metabolites from from *B. sexangula* and 40 metabolites from *B. sexangula var rhynchopetala* were identified so far. A detailed list of chemical compounds identified from *Bruguiera* is given in Table 1.3 in which the number in bracket designates the structure number.

The plants of the genus *Ceriops* valued for their rich tannin content and are a rich source of pentacyclic triterpenoids (Ghosh *et al*. 1985). The 30 metabolities from *C.decandra* and 72 metabolites from *C.tagal* are known till date. Thus a total of 93 metabolites including 39 diterpenoids and 52 tritepenoids along with 2 steroids have been reported so far from this genus. Dolabranes (diterpenoids) are the marked metabolites of *C. tagal*. These compounds can be used as chemotaxonomic markers of this plant. The chemical constituents identified from *Ceriops* are listed in Table 1.4.

Plant	Compound Class and Name	Plant Part	References
B. cylindrica	***Alkaloids*** Brugine **(1)**, Tropine **(2)**, Tropine acetate **(3)**, Tropine benzoate **(4)**, Tropine isobutyrate **(5)**, Tropine isovalerate **(6)**, Tropine n-butyrate **(7)**, Tropine propionate **(8)**	Stem&bark, stem bark	Katu and Takahashi, 1975; Loder and Russel, 1969
	D-Friedooleananes (Triterpenoids) 3α - taraxerol **(9)**, 3α-E-caffeoyltaraxerol **(10)** , 3α-E-coumaroyltaraxerol **(11)**, 3α-E-feruloyltaraxerol **(12)** , 3α-Z-coumaroyltaraxerol **(13)** , 3α-Z-feruloyltaraxerol**(14)** , 3β – taraxerol **(26)** , 3β-E-feruloyltaraxerol **(17)** , 3β-Z-feruloyltaraxerol **(23) ,**	Fruits, hypocotyls	Laphookhieo et al., 2004a; Karalai and Laphookhieo, 2005
	Lupanes (Triterpenoids) 3α - lupenol **(32)**, 3α-E-coumaroyllupeol **(34)**, 3α-Z-coumaroyllupeol**(37)** , 3β-E-caffeoyllupeol B**(44)** , 3β-E-coumaroyllupeol **(45)**, 3β-Z-coumaroyllupeol**(55)** Lupenone **(64),** Lupeol **(65)**	Fruits, hypocotyls	Karalai and Laphookhieo, 2005
	Kauranes(Diterpenoids) ent-kaur-16-ene-13, 19 diol **(115)**	Roots	Salae et al., 2007
	Sulphur compounds 4 Hydroxy-1,2-dithiolane **(144)** Brugierol **(145)**, Isobrugierol **(149)**	Stem and bark	Katu and Takahashi,1975
B. exaristata	***Alkaloids*** Tropine **(2)**, Tropine acetate **(3)**, Tropine benzoate **(4),** Tropine isobutyrate **(5)**, Tropine isovalerate **(6),** Tropine n-butyrate **(7)**, Tropine propionate **(8)**	Stem bark	Loder and Russel, 1969

Plant	Compound Class and Name	Plant Part	References
B. cylindrica	***Alkaloids*** Brugine **(1)**, Tropine **(2)**, Tropine acetate **(3)**, Tropine benzoate **(4),** Tropine isobutyrate **(5)**, Tropine isovalerate **(6),** Tropine n-butyrate **(7)**, Tropine propionate **(8)**	Stem&bark, stem bark	Katu and Takahashi, 1975; Loder and Russel, 1969
	D-Friedooleananes (Triterpenoids) 3α - taraxerol **(9)**, 3α-E-caffeoyltaraxerol **(10)** , 3α-E-coumaroyltaraxerol **(11)**, 3α-E-feruloyltaraxerol **(12)** , 3α-Z-coumaroyltaraxerol **(13)** , 3α-Z-feruloyltaraxerol**(14)** , 3β – taraxerol **(26)** , 3β-E-feruloyltaraxerol **(17)** , 3β-Z-feruloyltaraxerol **(23) ,**	Fruits, hypocotyls	Laphookhieo et al., 2004a; Karalai and Laphookhieo, 2005
	Lupanes (Triterpenoids) 3α - lupenol **(32)**, 3α-E-coumaroyllupeol **(34)**, 3α-Z-coumaroyllupeol**(37)** , 3β-E-caffeoyllupeol B**(44)** , 3β-E-coumaroyllupeol **(45)**, 3β-Z-coumaroyllupeol**(55)** Lupenone **(64),** Lupeol **(65)**	Fruits, hypocotyls	Karalai and Laphookhieo, 2005
	Kauranes(Diterpenoids) ent-kaur-16-ene-13, 19 diol **(115)**	Roots	Salae et al., 2007
	Sulphur compounds 4 Hydroxy-1,2-dithiolane **(144)** Brugierol **(145)**, Isobrugierol **(149)**	Stem and bark	Katu and Takahashi,1975
B. exaristata	***Alkaloids*** Tropine **(2)**, Tropine acetate **(3)**, Tropine benzoate **(4),** Tropine isobutyrate **(5)**, Tropine isovalerate **(6),** Tropine n-butyrate **(7)**, Tropine propionate **(8)**	Stem bark	Loder and Russel, 1969

	Carbohydrates 1-D-1-O-methyl muco inositiol **(161)**	Leaves	Richter et al.,1990
B. gymnorrhizha	***Lupanes (Triterpenoids)*** Betulin **(57)** Lupeol **(66)**	Leaves	Ghosh et al., 1985
	Oleanane (Triterpenoids) Oleanolic acid **(70)** β-amyrin **(71)**	Leaves	Ghosh et al., 1985
	Ursanes (Triterpenoids) Ursolic acid **(73)** α-amyrin **(74)**	Leaves	Ghosh et al., 1985
	Dammaranes (Triterpenoids) Bruguierin **A (75)**, Bruguierin B **(76)**, Bruguierin C **(77)**	Flowers	Homhual et al., 2006a
	Triterpene alcohol Gymnorhizol (3-epi-δ-amyrin) (**85**)	Leaves	Ganguly and Sarkar, 1978
	Fatty acids linoleic acid **(88)**, linolenic acid **(89)** , palmitic acid **(90)**	Leaves	Hogg and Gillan, 1984
	Steroids -O-α-L-Rhamnopyranosyl- (+)-Catechin-(4α->2)phloroglucinol **(91),** Campesterol **(92),** Cholesterol **(93).** Sitosterol **(96),** Stigmaste-7-en-3β-ol **(98),** Stigmasterol **(99)**	Bark, leaves	Achamadi et al., 1994; Ghosh et al., 1985
	Kauranes(Diterpenoids) 13-hydroxy-16-ent-kauren-19-al **(103),** 16 □,17-dihy- droxy-ent-9(11)-kaurene-19-al **(107),** 16 □,17-dihydroxy-ent-9(11)-kauren-19-oic acid **(108),** 16-ent- kaurene-13,19-diol **(115),** 16-ent-kauren-19-ol **(110),** 16□□ H-17,19-ent-kauranediol **(104),** 16□□ H-17-hydroxy- ent-kauran-	Stem, bark	Han et al., 2004; Subrahmanyam et al., 1999

	19-oic acid **(105),** 16□□,- 17-dihydroxy-ent-kauran-19-al **(106),** 17-chloro-13,16□-dihydroxy-ent-kauran-19-al **(111),** Steviol **(120),** methyl (16R)-13,17-epoxy-16-hydroxy-ent- kaur-9(11)- en-19-oate **(119),** methyl 16α,17-dihydroxy-ent-kaur-9(11)-en-19-oate **(116),** methyl-16 □,17-dihydroxy-ent-kauran-19-oate **(117)**		
	Pimaranes (Diterpenoids) 15(S)-isopimar-7-en-15,16-diol **(123),** ent-8(14)-pimarene-15R,16-diol **(128),** ent-8(14)-pimarene-1α,15R,16-triol **(129),** isopimar-7-ene-1β,15R,16-triol **(130),** (5R,9S,10R,13S,15S)ent-8(14)-pimarene-1-oxo-15R,16-diol **(122)**	Stem, root bark	Han et al., 2005a; Subrahmanyam et al., 1999
	Beyeranes (Diterpenoids) (4R,5S,8R,9R,10S,13S)-ent-17-hydroxy-16-oxobeyeran-19-al **(135)**	Stem	Han et al., 2004
	Sulphur compounds Brugierol **(145),** Bruguiesulfurol **(146),** cis- 3,30-dihydroxy-1,5,10,50-tetrathiacyclodecane **(147),** Gymnorrhizol **(148),** Isobrugierol **(149),** Neogymnorrhizol **(150),** Trans-3,30-dihydroxy-1,5,10,50-tetrathiacyclodecane **(151)**	Flowers, leaves and stem	Homhual et al., 2006b; Huang et al, 2009
	Aromatic compounds 1-(3-hydroxyphenyl)-hexane-2,5-diol **(153),** 2,3-dimethoxy-5-propylphenol **(154),** 3-(3-hydroxybutyl)-1,1-dimethylisochroman-6,8-diol **(155),** Bruguierol A **(156),** Bruguierol B **(157),** Bruguierol C **(158),** Bruguierol D **(159)**	Stem, branch	Han et al., 2005b; Han et al., 2007
	Lupanes (Triterpenoids) 3β-Z-caffeoyllupeol **(38),** 3β-Z-coumaroyllupeol **(55),** lupenone **(64),** lupeol **(65),** Dioslupecin A **(57)**	Fruits	Chumkaew et al., 2005

	Lanostanes(Triterpenoids) sexangulic acid **(86)**	stem	Li et al., 2010
B. parviflora	***D-Friedooleananes (Triterpenoids)*** Taraxerone **(25)**	Stem	Bao and Lin, 2006
B.sexangula	***Lupanes (Triterpenoids)*** Lupenone **(64),** Lupeol **(65),** trans-hydroxy-cinnamoyl lupeol **(66)**	Stem	Bao and Lin, 2006
B. sexangula var. rhynchopetala	***Oleanane (Triterpenoids)*** β-amyril palmitate **(72)**	Stem	Bao and Lin, 2006
	Squalene*(Triterpenoid)* Squalene **(87)**	Stem	Bao and Lin, 2006
	Steroids Daucosterol **(94)** β -Sitosterol (**96)** α-Hydroxy-sitosterol **(100)**	Stem	Bao and Lin, 2006
	Kauranes(Diterpenoids) (16R)-13,17-epoxy-16-hydroxy- ent-kaur-9(11)-en-19-al **(101)** , 16 □,17-dihy- droxy-ent-9(11)-kaurene-19-al **(107),** 16,17-dihydroxy-19-nor- ent-kaur-9(11)-en-3- one **(109) ,** Ceriopsin F **(113) ,** methyl (16R)-13,17-epoxy-16- hydroxy-ent- kaur-9(11)- en-19-oate **(119) ,** methyl 16α,17-dihydroxy-ent-kaur-9(11)-en-19-oate **(116)**	Stem	Bao et al., 2005
	Pimaranes (Diterpenoids) (1αH,15R)-ent-pimar-8(14)-ene-1,15,16-triol **(121)**	Stem	Bao et al., 2005

	Beyeranes (Diterpenoids) ent-17-hydroxy-16-oxobeyer-9(11)-en-19-al **(136)**	Stem	Bao et al., 2005
	Sulphur compounds brugierol **(145),** isobrugierol **(149)**, (–)-3,4-dihydro-3-hydroxy-7-methoxy-2H-1,5- benzodithiepine-6,9-dione **(152)**	Stem	Bao et al., 2005
	Benzoquinone 2,6-dimethoxy-1,4-benzoquinone **(162)**	Stem	Bao et al., 2005
	Phenolic glycosides 1-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-3,4,5-trimethoxybenzene **(163),** 3,4,5-trimethoxyphenyl-β-D-glucopyranoside **(164),** Rhyncoside A **(168)** Rhyncoside B **(169)** Rhyncoside C **(170)** Rhyncoside D **(171)**	Stem	Bao et al., 2007
	Flavonoids Myricetin-3-O-rutinoside **(199)** Nicotiflorin **(200),** Rutin **(205)** Tricin **(206)**	Stem	Bao et al., 2007
	Lignans (+)-5′-methoxyisolariciresinol-9′-β-D- xylopyranoside **(207)** (+)-lyoniresinol-3α-O-α-L-rhamnopyranoside **(208)** Brugunin A **(209)** Hedyotisols A **(210)** Hedyotisols B **(211)** Hedyotisols C **(212),** Lyoniside **(213),** Rhynocoside E **(214),** Rhynocoside F **(215)**	Stem	Bao et al., 2007

Table 1.4. Chemical constituents of *Ceriops*

Plant	Compound Class and Name	Plant Part	References
Ceriops decandra	***Beyeranes (Diterpenoids)*** Ceriopsin A **(137),** Ceriopsin B **(138),** Ceriopsin G **(139),** Isosteviol **(140),**	Roots	Anjaneyulu and Rao; 2002, Anjaneyulu and Rao, 2003
	Kauranes (Diterpenoids) Cerioprin E **(112),** Ceriopsin F **(113),** Steviol **(120),** Methyl-ent-16β,17-dihydroxy-9(11)-kauren-19-oate **(118),** ent-16β,17-dihydroxy-9(11)-kauren-19-oic acid **(114)**	Roots	Anjaneyulu et al., 2002a; Anjaneyulu and Rao, 2003
	Ursane (Triterpenoid) Ursolic acid **(73)**	Leaves	Ponglimanont and Thongdeeying, 2005
	Lupanes (Triterpenoids) 30-nor-lup-3β-ol-2-one **(29),** 3αbetulinic acid **(33),** 3β-E-caffeoyllupeol **(44),** 3β,20-dihydroxylupane **(40),** 3β-E-coumaroyllupeol **(45),** 3β-E-feruloylbetulin **(46),** 3β-E-feruloyllupeol **(48),** 3β-hydroxylupan-29-oic acid **(49),** 3β-Z-coumaroyllupeol **(55),** 3β-Z-feruloyllupeol **(56),** Betulin **(57),** Betulinaldehyde **(58),** Betulinic acid **(59),** Lup-20(29)-en-3β,30-diol **(62),** Lupenone **(64),** Lupeol **(65)**	Leaves	Ponglimanont and Thongdeeying, 2005
	Pimaranes (Diterpenoids) 8,15Repoxypimaran- 16-ol **(125),** Ceriopsin C **(126),** Ceriopsin D **(127)**	Roots	Anjaneyulu and Rao, 2003; Anjaneyulu and Rao, 2002
	Dolabranes (Diterpenoids) Tagalsin A **(223),** Tagalsin B **(224),** Tagalsin C **(225),** Tagalsin D **(226),** Tagalsin E **(227),** Tagalsin F **(228),** Tagalsin G **(229),** Tagalsin H **(230),** Tagalsin O **(231),** Tagalsin P **(232),** Tagalsin Q **(233),** Tagalsin R **(234),** Tagalsin S **(235),** Tagalsin T **(236),**	Stem and twigs, aerial parts, roots	Zhang et al., 2005a; Ouyang et al., 2010; Chen et al., 2011; Hu et al., 2010; Fun et

Ceriops tagal	Tagalsin U **(237),** (5S*,8S*,9S*,10R*,13S*)-3-hydroxy-16- nor-2-oxodolabr-3-en-15-oic acid **(219),** (5S*,8S*,9S*,10R*,13S*)-3,16- dihydroxydolabar-3-ene-2,15–dione **(218),** (5S*,8S*,9S*,10R*,13S*)-2-hydroxy-16 -nor-3-oxodolabr-1,4(18)-dien-15-oic acid **(217),** (5S*,8S*,9S*,10R*,13S*)-dolabr-3-ene-15-,16-diol **(220),** (5S*,8S*,9S*,10R*,13S*)- dolabr-4(18)-ene-15,16 diol **(216),** erythroxydiol Y **(222),**		al., 2006; Chantrapromma et al., 2007
	Dolabr-4(17),15(16)-dien-3-one **(238),** 7-glycoloyl-2-hydroxy-1,4b,7, 10a-tetramethyl-4a,4b,5,6,7,8,8a,9,10,10a-decahydrophenanthren -3(4H)-one **(239)**	Stems and twigs, roots	Zhang et al., 2005b; Chen et al., 2008; Chen et al., 2011
	Dimeric diterpenoids		
	Tagalsins I **(244),** Tagalsins J **(245),** Tagalsin L **(241),** Tagalsin M **(242),** Tagalsin N **(243),** 8(14)-enyl-pimar-2'(3')-en-4'(18')-en-15'(16')-endolabr-16,15,2',3'-oxoan-16-one **(240)**	Hypocotyls and fruits	Pakhathirathien et al., 2005
	Dammarane (Triterpenoids)		
	Cereotagalol A **(78),** Cereotagalol B **(79),** Cereotagaloperoxide **(80),** Dammarenediol II **(81),** Fouquierol **(82),** Isofouquierol **(83),** ocotillol II **(84)**	Hypocotyls and fruits	Pakhathirathien et al., 2005
	Oleananes (Triterpenoids)		
	Oleanolic acid **(70)**	Hypocotyls and fruits, aerial parts, roots	Pakhathirathien et al., 2005; Chacha, 2011; Wang et al., 2010; Chen et al., 2011
	Lupanes (Triterpenoids)		
	28-hydroxylup- 20(29)-en-3-one **(27)** 3-oxolup-20(29)-en-28-oic acid **(31)** 3αbetulinic acid **(33)** 3α-O-transcoumaroylbetulinic acid **(35)** 3α-O-trans-feruloylbetulinic acid **(36)** 3β- O-cis-coumaroylbetulin **(39)** 3β-acetylbetulinic acid **(41)** 3β-E-caffeoylbetulin **(42)** 3β-E-caffeoylbetulinic acid **(43)**	Roots, stems and twigs	Chen et al., 2008; Hu et al., 2010; Chacha, 2011

	3β-E-coumaroyllupeol **(45)** 3β-E-feruloylbetulin **(46)** 3β-E-feruloylbetulinic acid **(47)** 3β-E-feruloyllupeol **(48)** 3β-O- cis-coumaroylbetulinic acid **(50)** 3β-O- trans-feruloylbetulin **(51)** 3β-O-cis-feruloylbetulin **(52)** 3β-O-trans- coumaroylbetulinic acid **(53)** 3β-O-trans-coumaroylbetulin **(54)** 3β-Z-feruloyllupeol **(56)** Betulin **(57)** Betulinic acid **(59)** betulonic acid **(60)** lup-20(29)-en-3β,28-diol **(61)** lup-20(29)-en-3β-hydroxy-28-oic **(63)** Lupeol **(65)**		
	<u>Pimaranes (Diterpenoids)</u> ent- 8(14)-pimarene-15R,16-diol **(128)** isopimar-8(14)-en-15,16-diol **(131)** isopimar-8(14)-en-16-hydroxy-15-one **(132)** methoxy-ent-8(14)-pimarenely-15-one **(133)**	Stems and twigs	Hu et al., 2010
	<u>Abietane (Triterpenoid)</u> abieta-8,11,13—trien-18-oic acid **(246)** ***<u>Steroids</u>*** Stigmasterol **(99)**, β-sitosterol **(96)**	Roots	Chen et al., 2008

There are two species in the mangrove genus *Kandelia; Kandelia candel* and *Kandelia obovata*. Only one report is available regarding the chemical constituents of plants of this genus. A few tannin compounds have been reported from *K. candel*. *K. obovata* still remains uninvestigated for its chemical constituents. 24 phenolic compounds including three propelargonidin dimmers, three procyanidin trimers, fourteen proanthocyanidins and four flavan-3-ols have been isolated from the bark of *Kandelia candel Druce* (Hsu *et al*., 1985) (Table 1.5).

Mangrove genus *Rhizophora* has ten species; *R. apiculata, R. harrisonii, R. lamarckii, R. mangle, R. mucronata, R. racemosa, R. samoesis, R. selala, R. stylosa and R.annamalayana.* Among these, only reports on chemical constituents of *R. apiculata, R. mangle, R. mucronata,* and *R. stylosa* are available. These reports reveal a total of 34 metabolites from R. apiculata, 2 metabolites from R. mangle, 25 metabolites from R. mucronata and 35 metabolites from R. stylosa (Table 1.6).

Table 1.5. Chemical constituents of the bark of *Kandelia candel*

Compound Class	Compound Name
Propelargonidin dimers	Afzelechin-(4α→8)- afzelechin **(181)**
	Afzelechin-(4α→8)- catechin **(182)**
	Afzelechin-(4α→8)- epicatechin **(183)**
Procyanidin trimers	Epicatechin-(4β→6)-epicatechin-(4β→6)-epicatechin **(189)**
	Epicatechin-(4β→6)-epicatechin-(4β→8)- catechin **(190)**
	Epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin **(191)**
Proanthocynadins	Cinchonain Ia **(185)**
	Cinchonain Ib **(186)**
	Cinchonain IIa **(187)**
	Cinchonain IIb **(188)**
	Kandelins A-1, A-2, B-1, B-2, B-3, B-4 **(194-199)**
	proanthicyanidin B-1, B-2, C-1**(202-204)**
	proanthicyanidin trimer **(205)**
Flavan-3-ols	(-)-epicatechin **(173)**
	(+)-afzeleczhin **(174)**
	(+)-catechin **(175)**
	(+)-gallocatechin **(177)**

Table 1.6. Chemical constituents of the genus *Rhizophora*

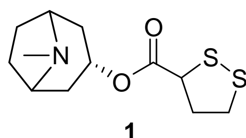
Plant	Compound Class and Name	Plant Part	References
R. apiculata	***D- Friedooleananes (Triterpenoids)***	Leaves	Kokpol et al., 1990
	Careaborin (3β-E-p-coumaroyltaraxerol) (**18**), Taraxerol **(28),** Taraxeryl cis-p –hydroxycinnamate **(24)**		
	Lupanes (Triterpenoids)	Stem	Gao et al., 2011
	Lupeol **(65)**		
	Aliphatic alcohols	Heartwood	Kokpol et al. 1993
	Dotriacontanol **(247),** Hentriacontanol **(248),** Nonacosanol **(249),** Octacosanol **(250)**, Triacontanol **(251)**		
	Aliphatic saturated carboxylic acids	Heartwood	Kokpol et al., 1993
	Doicosanoic **(252),** Henicosanoic **(253),** Hentriacontanoic **(254),** Heptacosanoic **(255),** Hexatriacontanoic **(256),** Octacosanoic **(257),** Pentacosanoic **(258),** Tetracosanoic **(259),** Tetratriacontanoic **(260),** Triacontanoic **(261),** Tritriacontanoic **(262)**		
	Steroids	Heartwood, stem	Kokpol et al., 1993; Gao et al., 2011
	Campesterol **(92),** Ergosta-7,22-dien-3b-ol **(95),** Ergosta-7,22-dien-3b-ol **(95),** Sitosterol **(96),** Sitosteryl 3-glucoside **(97),** Stigmasterol **(99)**		
	Aromatic compound	Heartwood	Kokpol et al., 1993
	Syringaldehyde **(160)**		
	Benzoquinone	Heartwood	Kokpol et al., 1993
	2,6- dimethoxy-p-benzoquinone **(162)**		
	Labdanes(Diterpenoids)	Roots	Saxena and Garg, 1994
	Apiculol **(263)**		

	Pimaranes (Diterpenoids)	Stem	Gao et al., 2011
	15(S)-isopimar-7-en-1-oxo-15,16-diol **(124)**	Stem	Gao et al., 2011
	Kauranes (Diterpenoids)		
	13,16α,17-trihydroxy-ent- 9(11)-kauren-19-oic acid **(102)** 16(R)-13,17-epoxy-16-hydroxy-ent-kaur-9(11)-en-19- al **(101)** ent-12,17-epoxy- 16β-hydroxy-9(11)-kauren-19-oate **(112)** Methyl-ent-16β,17-dihydroxy-9(11)- kauren-19-oate **(118)** Methyl-ent-kaur-9(11)-ent-13,17-expoxy- 16-hydroxy-19-oate **(119)**		
	D- Friedooleananes (Triterpenoids)		
	Taraxerol **(28)**	Leaves and stems	Williams, 1999
	Lupanes (Triterpenoids)		
R. mangle	Trans- hydroxycinnamoyllupeol **(66)**	Leaves and stems	Williams, 1999
	D- Friedooleananes (Triterpenoids)		
	3β-E-caffeoyltaraxerol **(16),** 3β-Z-caffeoyltaraxerol **(22),** 3β-Z-p-coumaroyltaraxerol **(24),** 3β-E-p-coumaroyltaraxerol **(18),** Taraxerol **(28)**	Fruits	Laphookhieo et al., 2004
R. mucronata	***Lupanes (Triterpenoids)***		
	Lupeol **(65)**	Leaves, stem bark Stem bark	Ghosh et al.,1985; Rohini and Das 2010
	Oleananes (Triterpenoids)		
	3β-O-(E)-(4-methoxy) cinnamoyl- 15 α-hydroxyl β-amyrin **(68)** Oleanolic acid **(70)** β-amyrin **(71)**	Leaves, root bark	Rohini and Das 2010; Ghosh et al., 1985; Rao et al., 2005
	Ursanes (Triterpenoids)		
	Ursolic acid **(73),** α-amyrin **(74)**	Leaves, root bark Root bark	Ghosh et al., 1985; Rao et al., 2005; Rao et al., 2005
	Steroids		
	Daucosterol **(94),** Sitosterol **(96)**	Roots	Anjaneyulu and

	Labdanes(Diterpenoids) Rhizophorin A **(264)**	Roots	Rao, 2001; Anjaneyulu et al., 2002b
	Beyeranes (Diterpenoids) Rhizophorin B **(141),** Rhizophorin C **(142),** Rhizophorin D **(143)**	Roots	Anjaneyulu et al., 2002b
	Pimaranes (Diterpenoids) Rhizophorin E **(134)**	Fruits	Anjaneyulu et al., 2002b
	Sesquiterpene Mucronatone **(265)**	Roots	Laphookhieo et al., 2004b
	Carbohydrate 1-D-O-methyl-muco-inositol **(161)**	Stem bark	Richter et al., 1990
	Hopanoid Adian-5-en 3-ol **(266)**	Root bark	Rohini and Das, 2010
	Phenolic compounds Atranorin **(165)**	Root bark	Rao et al., 2005
	Xanthone(aromatic ketone) Lichixanthone **(267)**	Root bark	Rao et al., 2005
R. stylosa	***Aliphatic ketone*** Palmitone **(268)**	Stems and twigs, leaves	Rao et al., 2005
	D- Friedooleananes (Triterpenoids) 3β -O-(E)-coumaroyl-taraxerol **(15),** 3β-O-(Z)-coumaroyl-taraxerol **(19),** 3β-taraxerol acetate **(20),** 3β-taraxerol formate **(21),** Careaborin (3β-E-p-coumaroyltaraxerol) (**18**), Taraxerol **(28),** Taraxerone **(26)**	Stems and twigs	Li et al., 2008; Yang et al., 2008

	Oleananes (Triterpenoids)	Leaves	
	3β-O-(E)-coumaroyl-15α-hydroxy-β-amyrin **(69)**	Leaves	Li et al., 2008
	Steroids		
	Daucosterol **(94),** Sitosterol **(96)**	Stems, stems and twigs	Yang et al., 2008
	Phenolic compounds		
	Protocatechuic acid **(167),** Isovanillic acid **(166)**		Yang et al., 2008
	Flavonoids		
	Rutin **(205),** Astilbin **(183),** 3,7-O-diacetyl (–)-epicatechin **(178),** (–)-epicatechin **(172).** 3-O-acetyl (–)-epicatechin **(179),** 3,3′,4′,5,7-O- pentaacetyl (–)-epicatechin **(177),** (+)-afzelechin **(173),** (+)-catechin **(174),** Proanthocyanidin B2 **(202),** Glabraoside A **(191),** Glabraoside B **(192),** Cinchonain IIa **(186),** Cinchonain IIb **(187),** (+)-catechin 3-O-α-L-rhamnoside **(175),** Cinchonain Ia **(186),** Cinchonain Ib **(185)**		Takara et al., 2008, Li et al., 2007

Alkaloids



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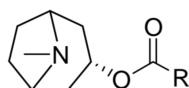


2 R= H

3 R= Me

4 R= Ph

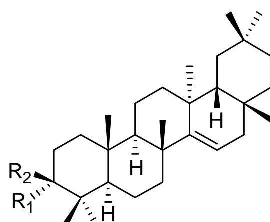
5 R= $CH(CH_3)_2$

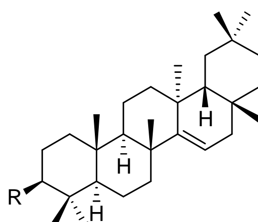
6 R= $CH_2CH(CH_3)_2$

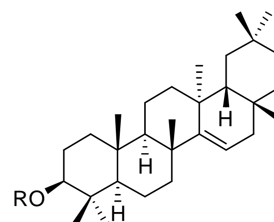
7 R= $(CH_2)_2CH_3$

8 R= CH_2CH_3

D-Friedooleananes





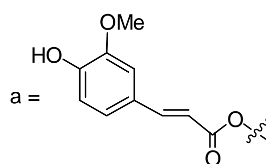


9 R_1= OH, R_2= H
10 R_1= c, R_2= H
11 R_1= b, R_2=H
12 R_1= a, R_2= H
13 R_1= d, R_2= H
14 R_1= e, R_2= H
17 R_1= H, R_2= a
23 R_1= H, R_2= b
26 R_1= H, R_2= OH
25 R_1+R_2=O

16 R = E- coumaroyl
18 R = E- coumaroyl
22 R = Z-caffeoyl
24 R = Z- coumaroyl

15 R= E- Coumaroyl
19 R= Z- Coumaroyl
20 R= Acetyl
21 R= Formyl

a =



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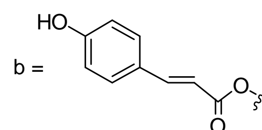
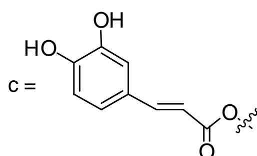


c =



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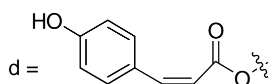

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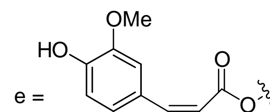
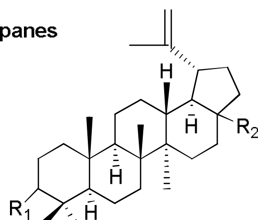
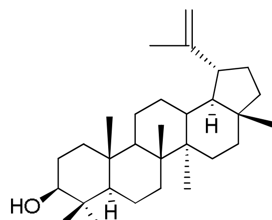


Lupanes





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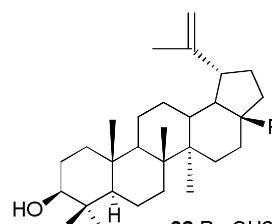
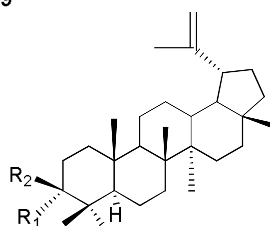


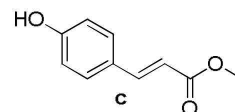
32 R =CH3

33 R= COOH

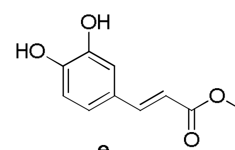
27 R1 =Ketone, R2= CH2OH
28 R1 = α-OH, R2=CH2OH
30 R1 =α-OH, R2= COOH
31 R1= Ketone, R2=COOH
35 R1=α-O-trans coumaroyl, R2= COOH
36 R1=α-O- trans feruloyl, R2=COOH
39 R1=β–O- cis coumaroyl, R2= CH2OH
50 R1= β-O- cis coumaroyl, R2= COOH
51 R1=β-O- trans feruloyl, R2=CH2OH
52R1=β-O- cis feruloyl, R2= CH2OH
53 R1=β-O- trans coumaroyl, R2= COOH
54 R1=β-O- trans coumaroyl, R2= CH2OH



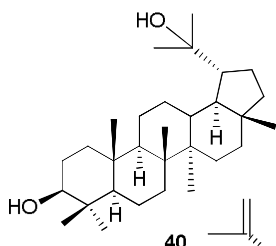
34 R1= E-c, R2=H
37 R1= Z-c, R2=H
38 R1=H, R2= Z-e
44 R1= H, R2= E-e
45 R1=H, R2= E-c
55 R1= H, R2= Z-c
65 R1+R2= O



c



e



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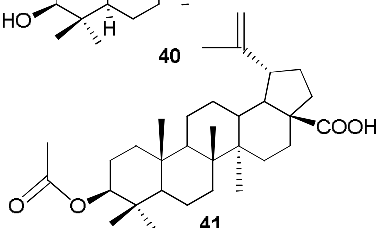

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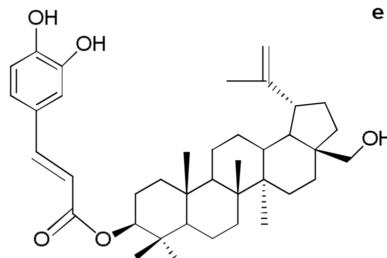

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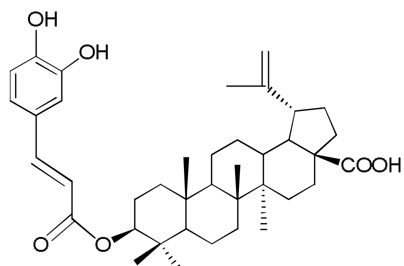

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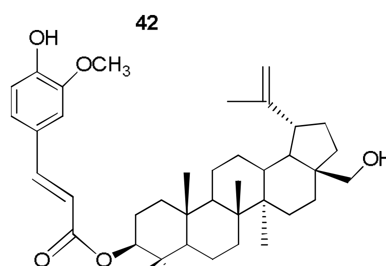

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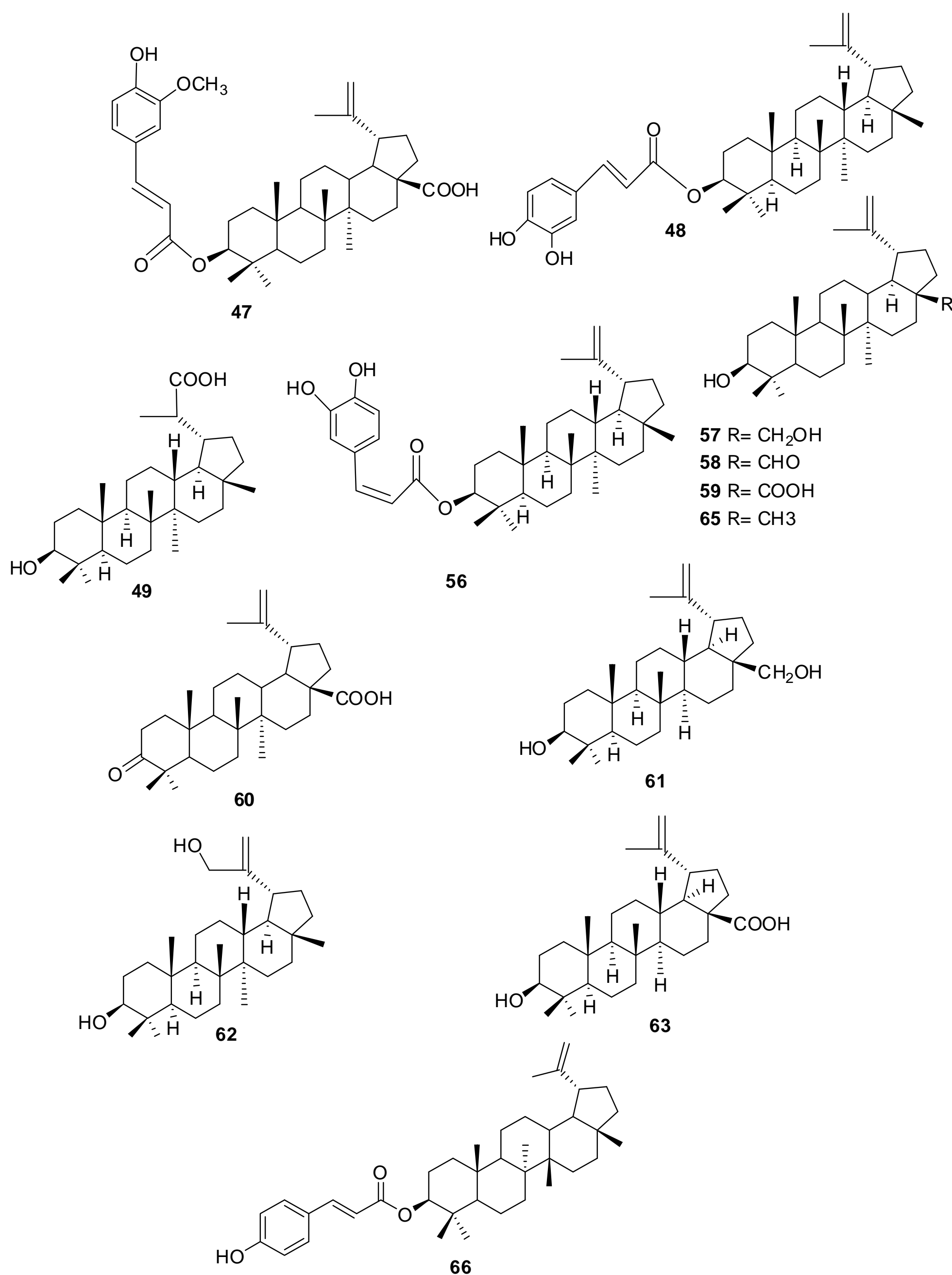
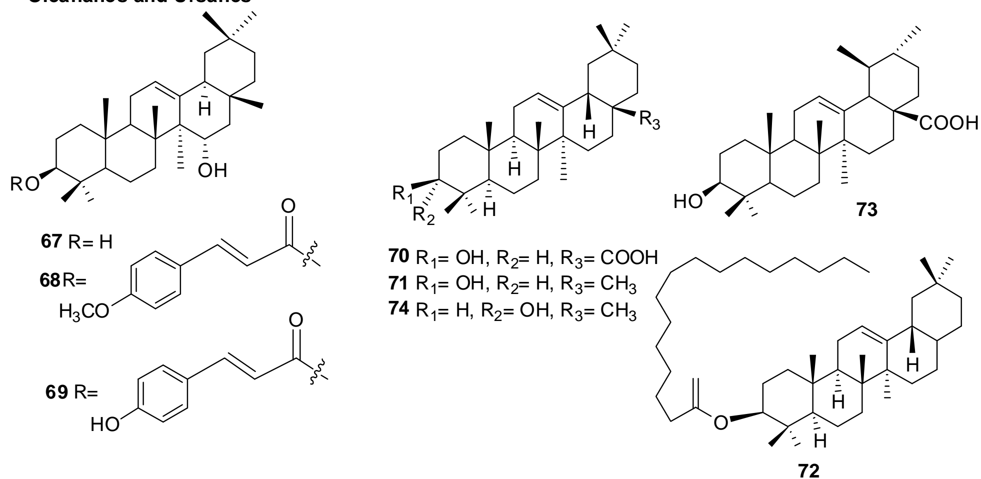
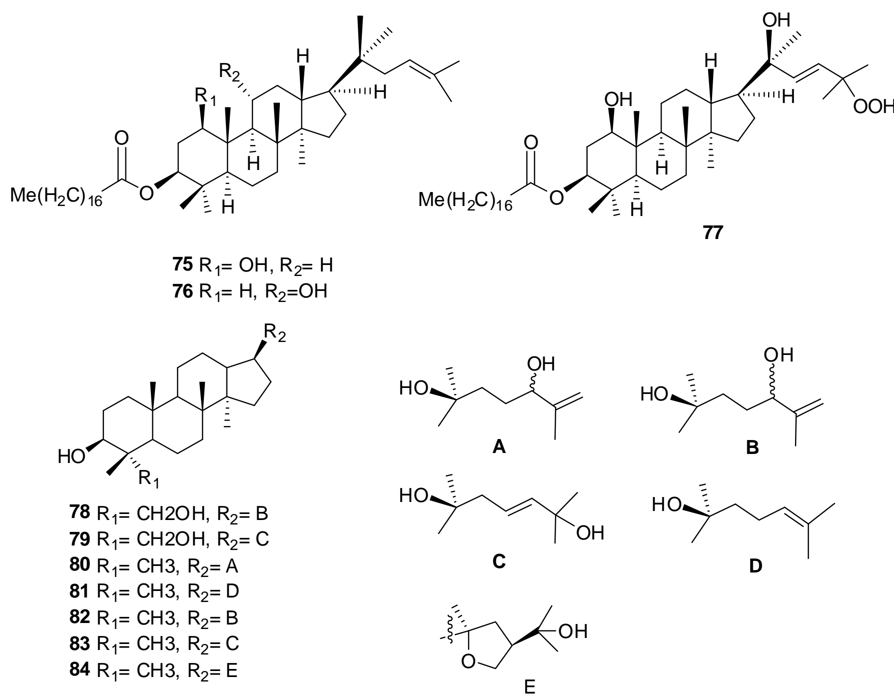


OH
OCH_3
COOH
O
47
48
HO
OH
R
COOH
HO
H
49
56
57 R= CH_2OH
58 R= CHO
59 R= COOH
65 R= CH3
60
CH_2OH
61
62
63
66

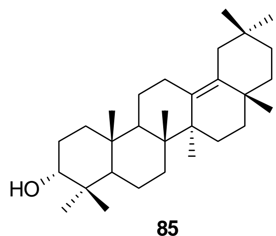
Oleananes and Ursanes



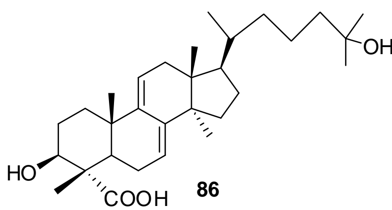
Dammaranes



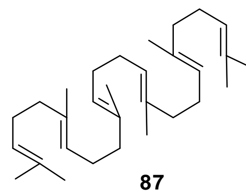
Triterpene alcohol



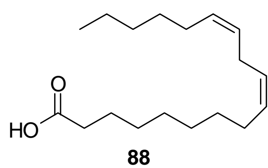
Lanostane

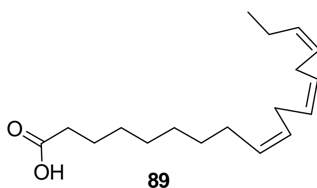


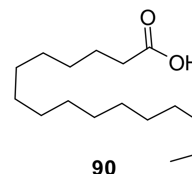
Squalene



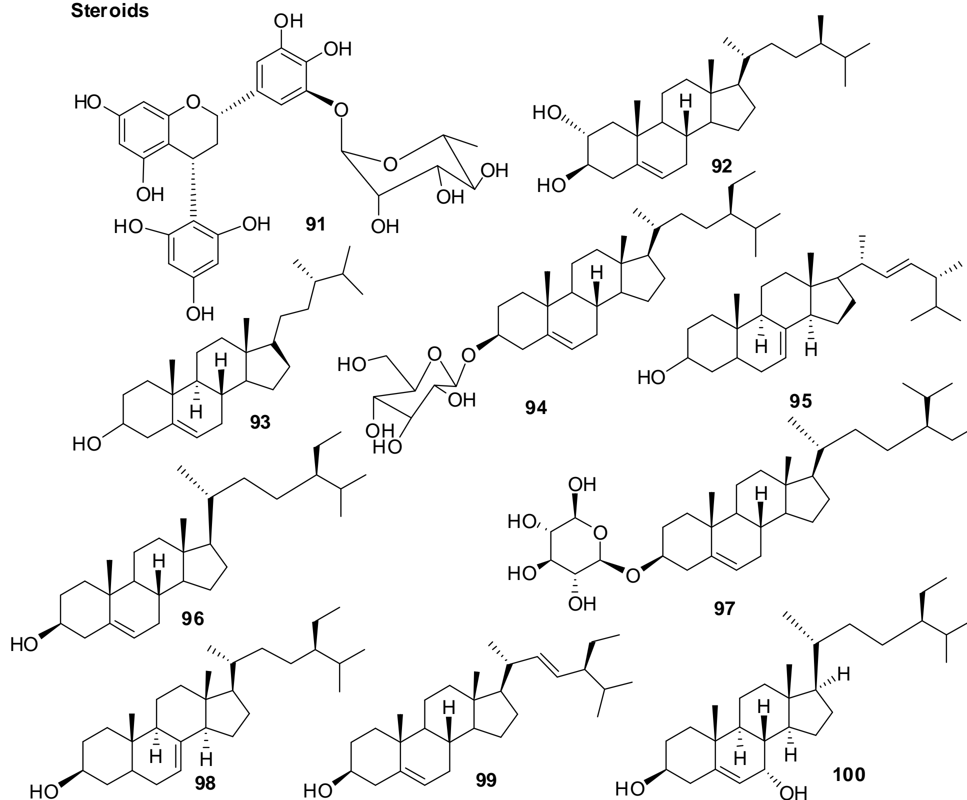
Fatty acids

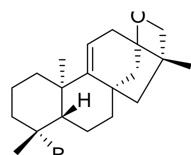


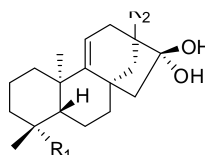


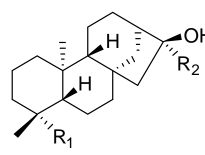


Steroids





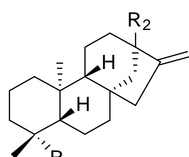


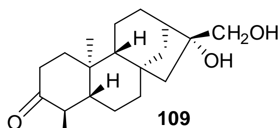


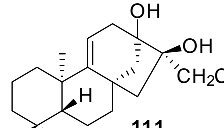
101 R= CHO
119 R= CO_2Me

102 R_1= COOH, R_2= OH
107 R_1= CHO, R_2=H
108 R_1= COOH, R_2= H
116 R_1=COOMe, R_2=H

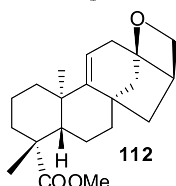
104 R_1=CH_2OH, R_2= H
105 R_1= COOH, R_2= H
106 R_1= CHO, R_2= OH
117 R_1= COOMe, R_2=OH

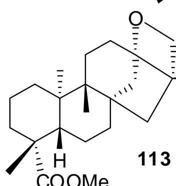


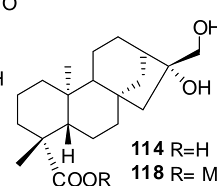




110 R_1= CH_2OH, R_2= H
103 R_1= CHO, R_2=OH
115 R_1= CH_2OH, R_2= OH
120 R_1=COOH, R_2=OH

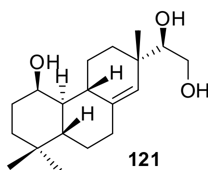


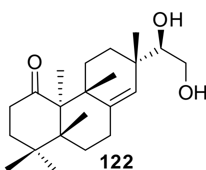


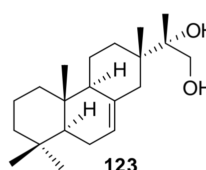


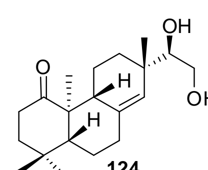
114 R=H
118 R= Me

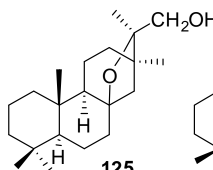
Pimaranes

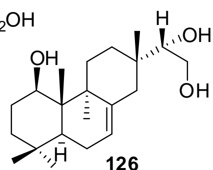


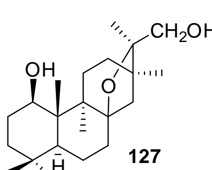


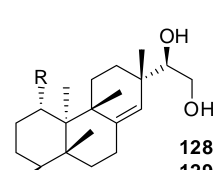




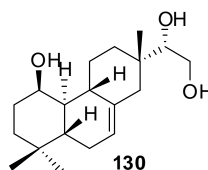


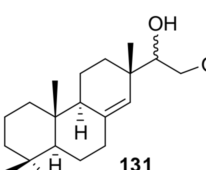


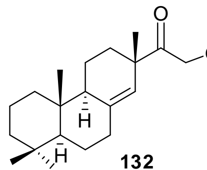


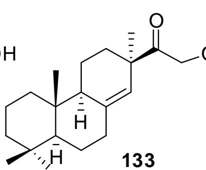


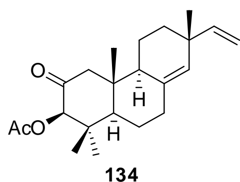
128 R= H
129 R= OH





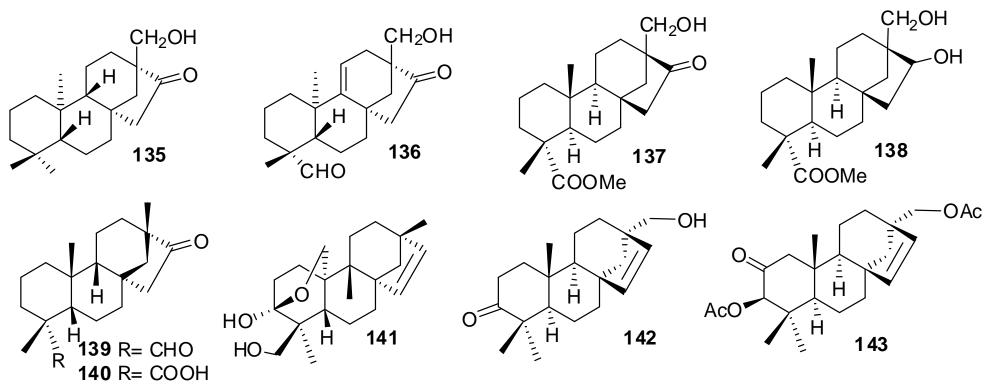




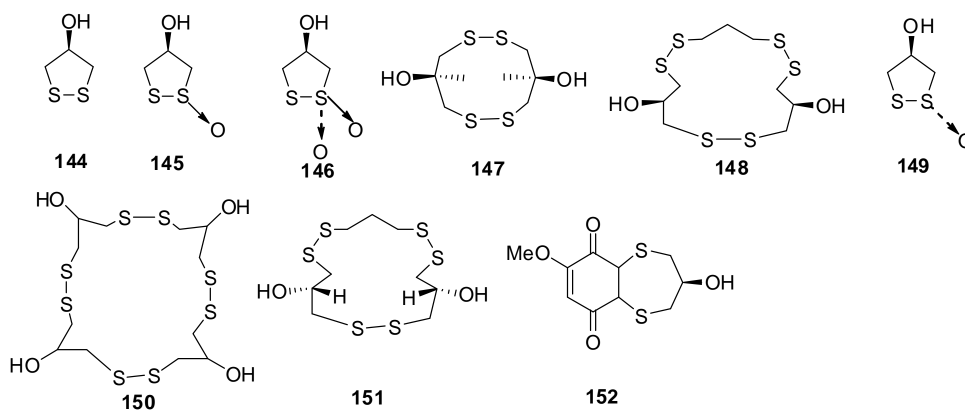


134

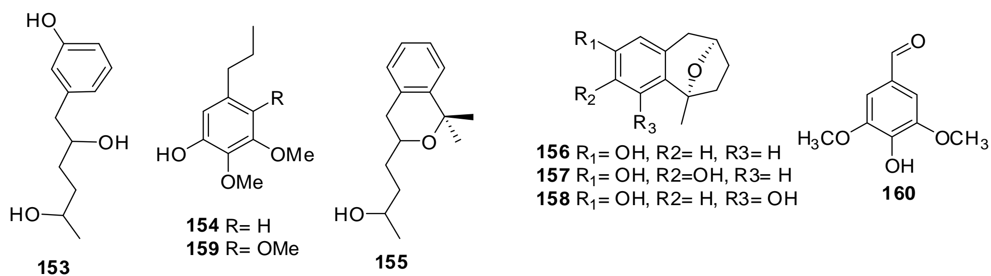
Beyeranes



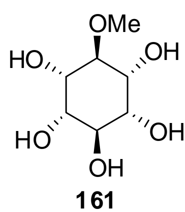
Sulphur compounds



Aromatic compounds



Carbohydrate **Benzoquinone**



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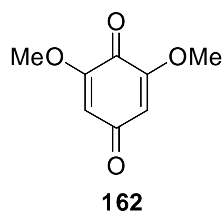

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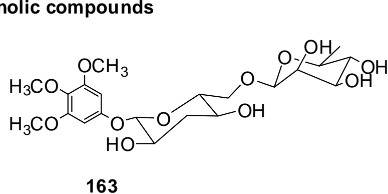
Phenolic compounds



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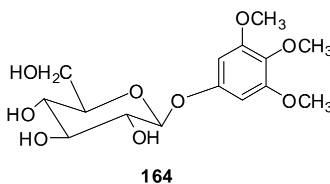

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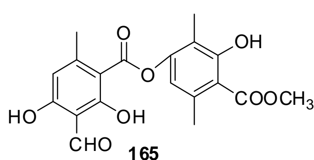

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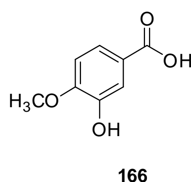

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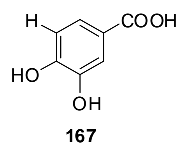

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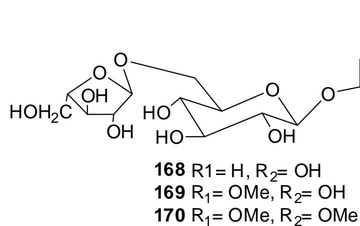
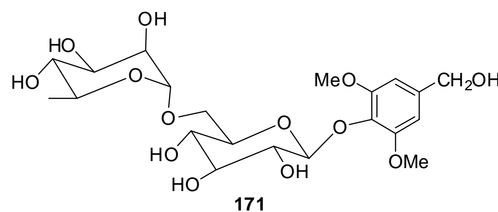


168 R1= H, R_2= OH
169 R_1= OMe, R_2= OH
170 R_1= OMe, R_2= OMe



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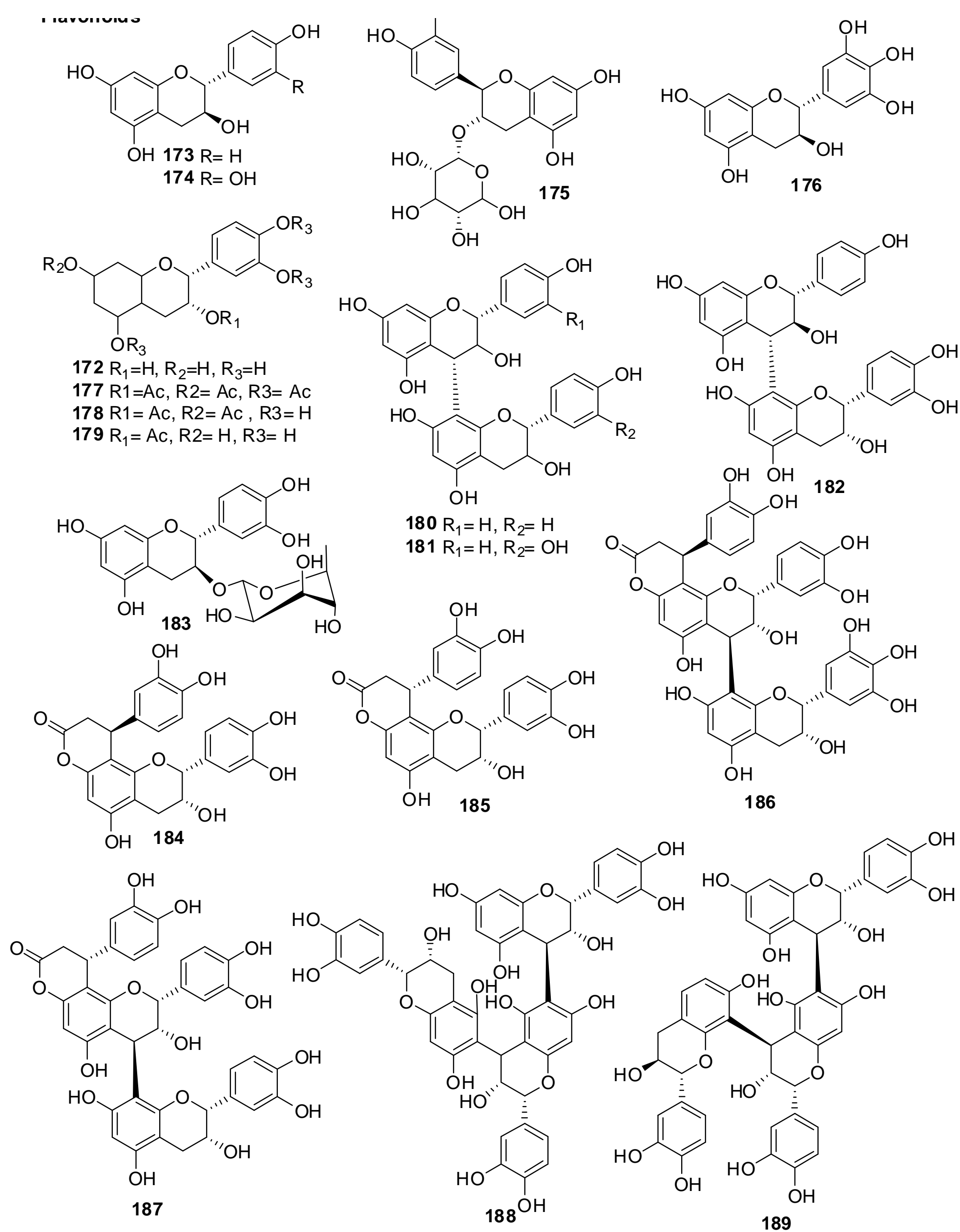
173 R= H
174 R= OH
175
176
172 R1=H, R2=H, R3=H
177 R1=Ac, R2= Ac, R3= Ac
178 R1= Ac, R2= Ac , R3= H
179 R1= Ac, R2= H, R3= H
180 R1= H, R2= H
181 R1= H, R2= OH
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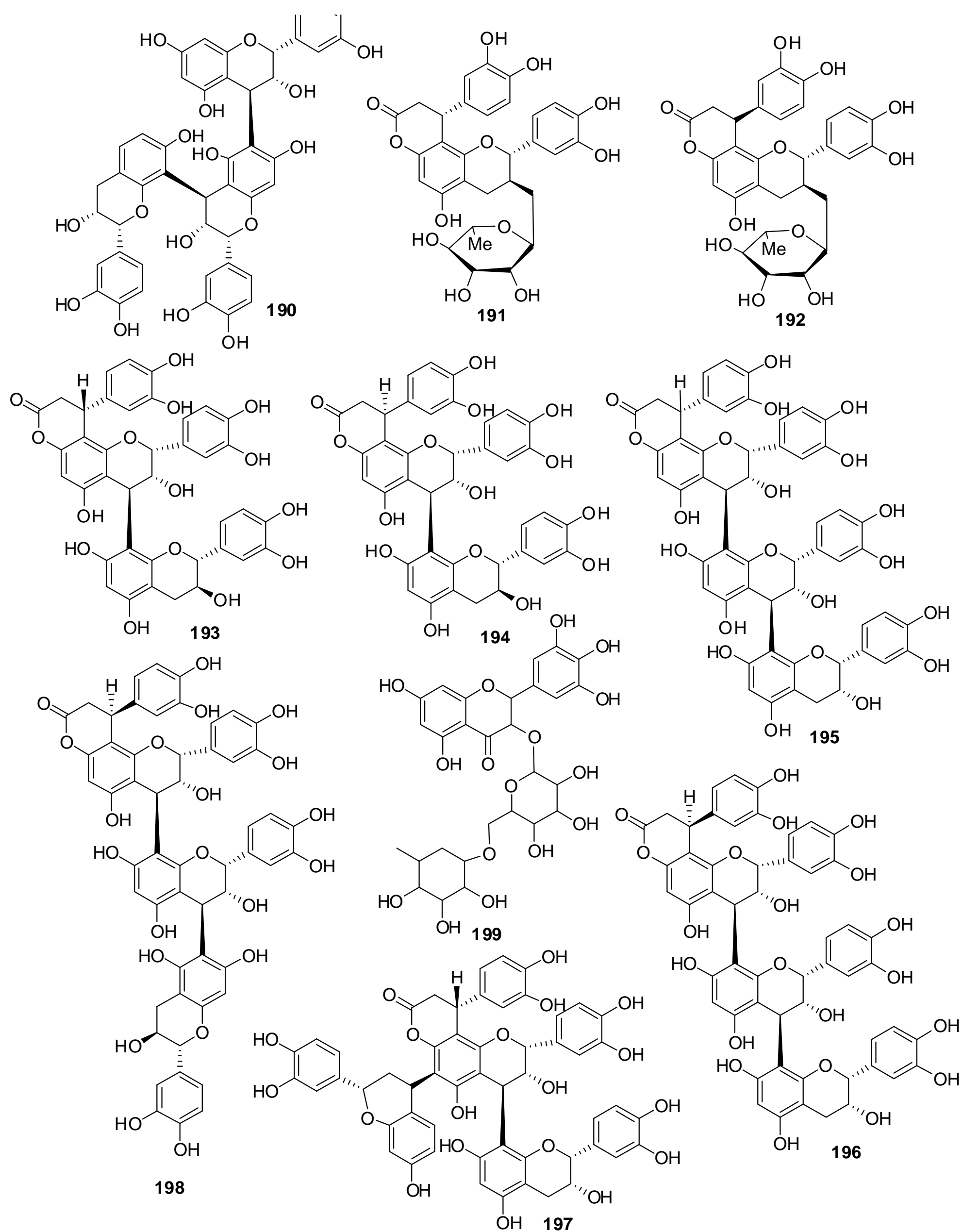
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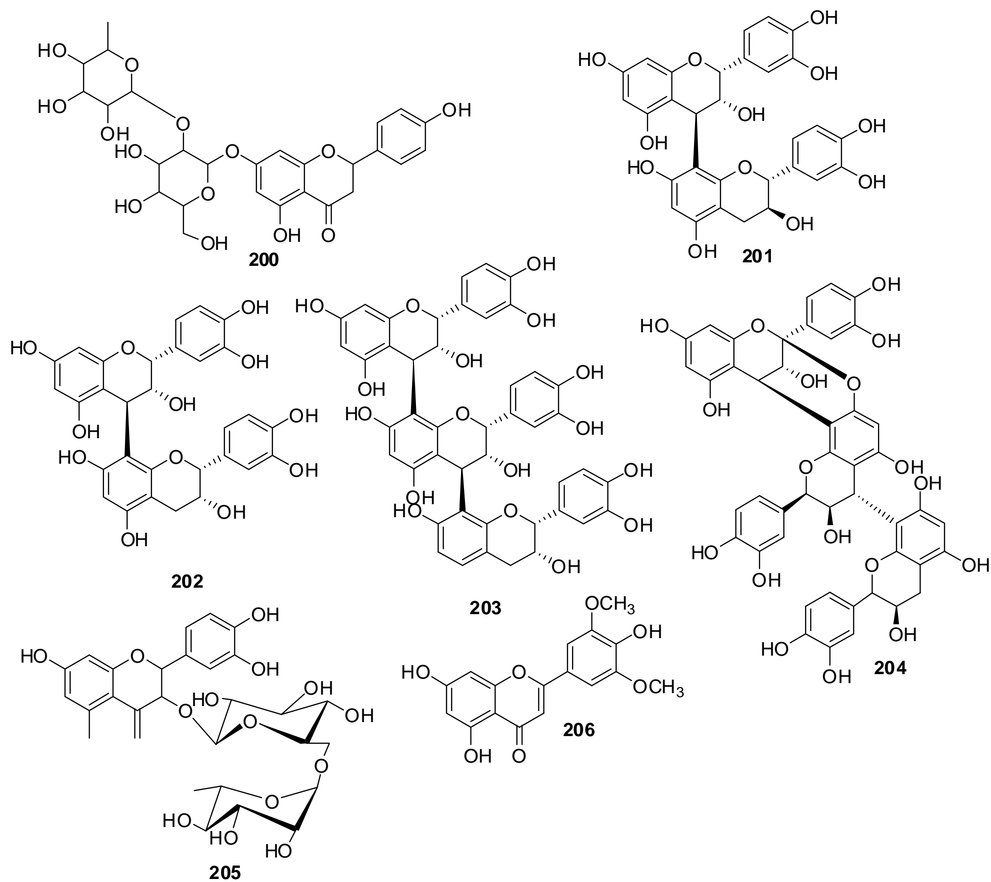
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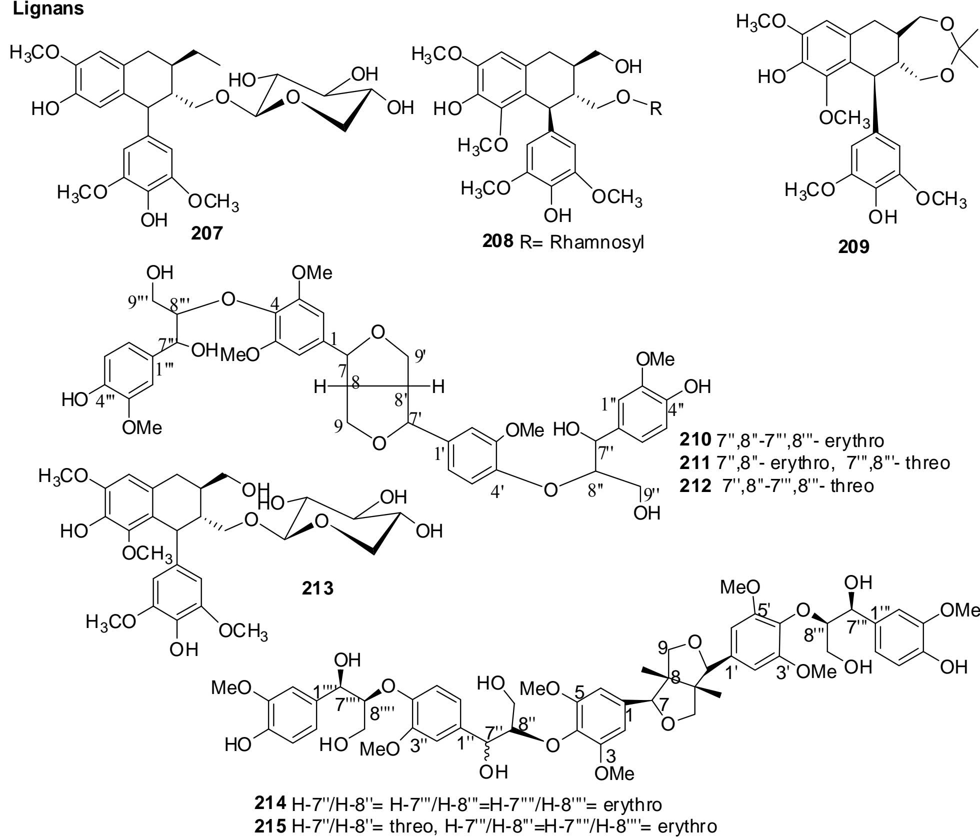
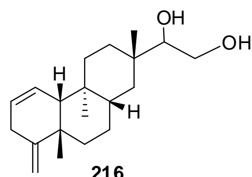
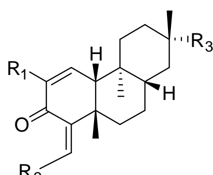
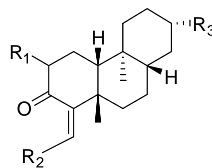
Lignans

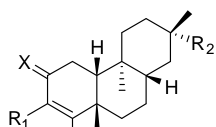


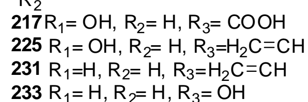
Dolabranes

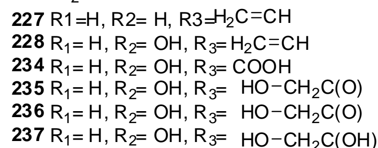


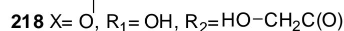


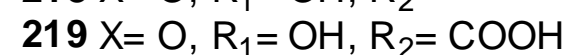


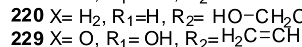


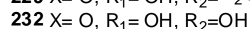


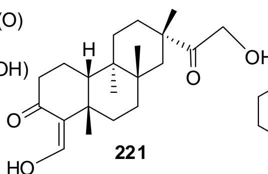


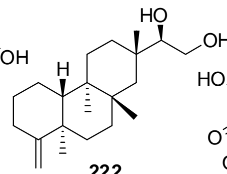


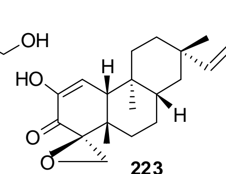


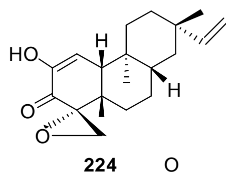


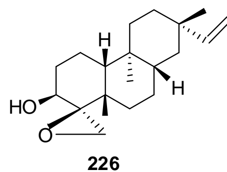


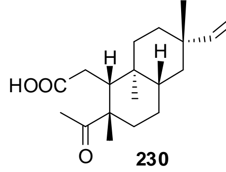


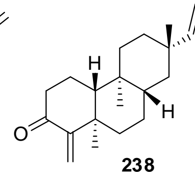


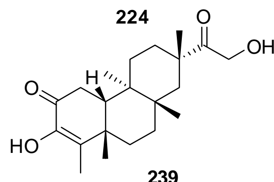












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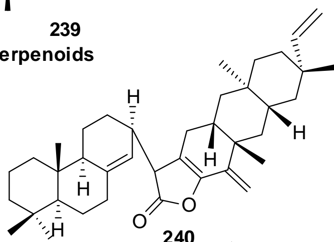
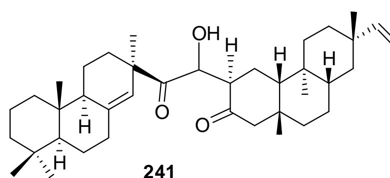
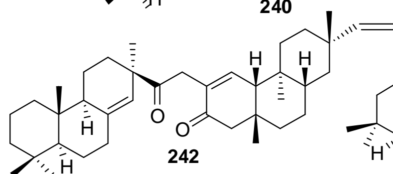
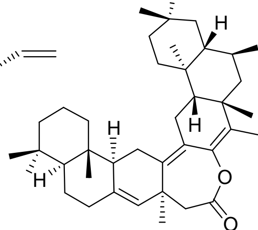
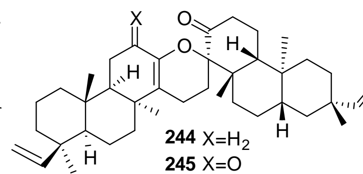


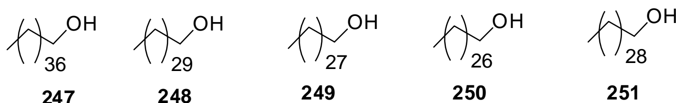




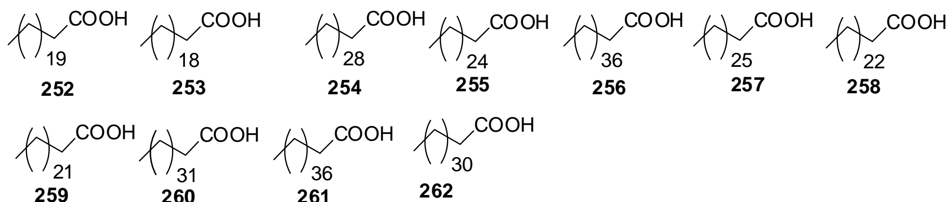


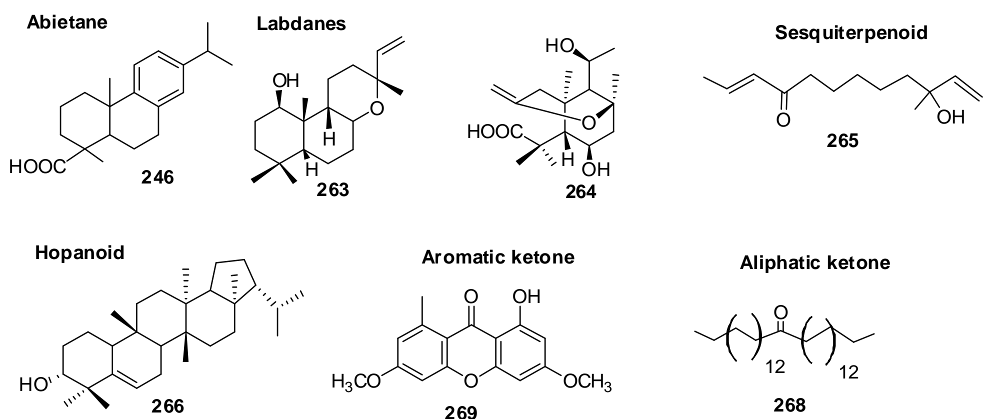


Aliphatic alcohols



Aliphatic acids





1.3.3 Bioactivities

Rhizophoraceae mangrove plants are potential sources of biologically active chemicals that are discernible from their wide spread application in ethnopharmaceutical practices. Various reports are available regarding the biological activities of these mangrove extracts. The antibacterial (Ravikumar *et al.*, 2010; Baskaran and Mohan, 2012), antifungal (Afzal *et al.*, 2005), antiviral (Premanathan *et al.*, 1999), insecticidal (Kabaru and Gichia, 2009), antidiarrhea (Das *et al.*, 2009), antidiabetic (Alarcon-Aguilara *et al.*, 1998), antiseptic (Fernandez *et al.*, 2002) and antioxidant (Banerjee *et al.*,

2008; Rahim *et al*., 2008) activities of *R. mucronata* have been reported. The components of crude alkaloid mixtures from *B. sexangula* and *B. exarista* were identified as tumor inhibitors (Loder and Russell, 1969). A polysaccharide extracted from the leaves of *B. cylindrica*, *R. apiculata* and *R. mucronata* of *Rhizophoraceae* along with some other mangrove plants exhibit positive activity against human immunodefiency viruses (Premanathan *et al*., 1999). All parts of *C. decandra* has proven antiviral activity (Premanathan *et al*., 1999). It also possess promising antibacterial (Chandrasekaran *et al*., 2009), anti-inflammatory (Hossain *et al*., 2011), and antidiabetic activity (Nabeel *et al*., 2010). The leaves and bark extract of *Ceriops tagal* shows antibacterial activity (Arivuselvan *et al*., 2011). Phenolics are important components in the leaf extract and hypocotyls of *K. candel* and show excellent antioxidant activities (Zhang *et al*., 2010; Wei *et al*., 2010). Therefore, the *K. candel* can be a good source for further development as an antioxidant medicine. During the study on the antibacterial activities of mangrove extracts against two antibiotic resistant pathogenic bacteria *Staphylococcus aureus* and *Proteus Sp* it was found that the ethyl acetate extract of *B. Sexangula* and *R. apiculata* also possess promising antibacterial activity (Abeysinghe, 2010). In a study it is reported that the gallic acid from hydrolysable tannin extracted from the barks of *R. apiculata* possessed a significant antiyeast (anticandidal) activity towards some yeast species of medically importance. It is anticipated that gallic acid from *R. apiculata* can find its potential as a novel of antiyeast agent might be useful to cure particularly candidiasis (Hong *et al*., 2011). Alcoholic extract of the leaves of *Rhizophora apiculata* from the mangrove forest of Sunderbans, West Bengal, India was prepared and manglycemic/anti-

hyperglycemic activity was studied in fed rats, glucose loaded rats and streptozotocin induced diabetic rats. The results of this study reveal that this plant extract has potential hypoglycemic action (Sur *et al*., 2004). The cholinesterase inhibition activity of *R. lamarckii* was established by Suganthy *et al*., 2009. The antihyperglycaemic effect of *R. mangle* was studied (Alarcon-Aguilara *et al*., 1998). The leaf extracts of three mangrove plants; *R. mucronata*, *R. apiculata* and *R.annamalayana,* were found to have potential anti-diabetic capacity due to the presence of an insulin-like protein (Alikunhi *et al*., 2012). Their work provides scientific support for the use of the mangroves in folklore medicine for the treatment of diabetes.

Hydroxydithiolane 1-oxides, brugierol and isobrugierol isolated from the flowers of *B. gymnorrhiza*, activated antioxidant response element (ARE luciferase activation). Bruguierin A, brugierol and isobrugierol also inhibited phorbol ester-induced NFκB (nuclear factor-κB) luciferase activation while Bruguierin A and brugierol selectively inhibited cyclooxygenase-2 (COX- 2) activity (Homhual *et al*., 2006a, 2006b). The compounds 16αH-17,19-ent-kauranediol; 13-hydroxy-16-ent-kaurene-19-ol and 16-ent-kaurene-19-ol isolated from *B. gymnorrhiza* showed promising activity against K-562 (human chronic myeloid leukemia) and L-929 (mouse fibroblasts) of which 16-ent-kaurene-19-ol showed the greatest selectivity for K-562 (IC_{50} 6.8 µg/ml) (Han *et al*., 2004). (5R,9S,10R,13S,15S)ent-8(14)-pimarene-1-oxo-15R,16-diol from the stem of *B. gymnorrhiza* showed moderate cytotoxic activities against L-929 (Han *et al*., 2005b).

The 15 membered macrocyclic polydusulfide, gymnorrhizol, possessing an unprecedented novel carbon skeleton isolated from *B. gymnorrhiza* exhibits potent inhibitory acitivity against protein tryrosine phosphatase 1B (PTP1B), an enzyme involved in regulation of insulin signaling and is regarded as a key for treatment of type III diabetes and obesity (Huang *et al*., 2009). One of the aromatic compounds extracted from the stem of *B. gymnorrhiza* , Bruguierol C showed moderate activity against Gram-positive and Gram-negative bacteria including mycobacteria and resistant strains. (Han *et al*., 2005b).

The lupane caffeoyl ester, 3-(Z)-caffeoyllupeol from *B. parviflora* exhibited antimalarial activity. (Chumkaew *et al*., 2005) Sexangulic acid obtained from *B. sexangula* showed moderate in vitro cytotoxicity against human lung cancer (A-549) and human luekaemic (H-L60) cell lines at a concentration of 5µg/ml (Li *et al*., 2010). Tagalsin C found in *C. tagal* was found to exhibit moderate cytotoxicity against HeLa human cervical carcinoma cell lines (Ouyang *et al*., 2010). The dimeric diterpenoid, 8(14)-enyl-pimar-2'(3')-en-4'(18')-en-15'(16')-endolabr-16,15,2',3'-oxoan-16-one and the other terpenoids; Tagalsin C, Tagalsin I, lup-20(29)-ene-3β,28-diol, 3-oxolup-20(29)-en-28-oic acid and 28-hydroxylup-20(29)-en-3-one isolated from the roots of the mangrove plant *C. tagal* exhibited antifouling activity against cyprid larvae of the barnacle without significant toxicity(Chen *et al*., 2011). The other non toxic antifouling compounds identified were ethoxy-ent-8(14)-pimarenely-15-one, ent- 8(14)-pimarene-15R,16-diol, stigmasterol and β-sitosterol (Chen *et al*., 2008). Tagalsins Q, R and U showed moderate antifeedant activity against the third instar larvae of Brontispa (Hu *et al*., 2010). Caspase-3 enzyme was activated by dolabr-4(17),15(16)-dien-3-one,

isopimar-8(14)-en-15,16-diol, isopimar-8(14)-en-16-hydroxy-15-one, lupeol, lup-20(29)-en-3β,28-diol and lup-20(29)-en-3β-hydroxy-28-oic acid isolated from the roots of marine mangrove *C. tagal* (Chacha, 2011).

2, 6-dimethoxy-p-benzoquinone isolated from *R.apiculata* was identified as an active constituent component against fungi, bacteria and boll weevils (Kokpol *et al*., 1993). Taraxerol and cinnamoyllupeol, two triterpenoids from the leaves and stems of *R. mangle* were found exhibit insecticidal activity towards *Cylas formicarius*, one of the most destructive pests of the sweet potato (Williams, 1999). Among the compounds isolated from the leaves of *R. stylosa*, taraxerol has been confirmed to have the abilities to inhibit the growth of Hela and BGC-823 while cis-careaborin could inhibit the growth of BGC-823 and MCF-7. Also, astilbin and rutin present in it were firstly reported to stimulate the proliferation of mice splenic lymphocytes markedly in a dose-dependent manner (Yang *et al*., 2008). The compounds, (-)-epicatechin, (-)-Catechin, 3-O-acetyl(-)-epicatechin, 3,7-O-acetyl(-)-epicatechin, (+)-afzelechin, Cinchonain 1b and proanthocyanidin B2 isolated from the same plant displayed DPPH radical scavenging activity comparable to that of the positive control butylated hydroxytoluene (BHT). Proanthocyaninidin B2 showed the strongest activity with IC_{50} 4.3μg/ml, 4 times greater than the positive control, BHT (IC_{50} 18.0 μg/ml). The antioxidant flavan-3ol glycosides from *R.stylosa* showed an increase in their radical scavenging activities with increase in number of catechol moieties present in the molecules (Takara *et al*., 2008).

1.4 Chemotaxonomy

Chemical plant taxonomy or chemotaxonomy of plants may be defined as a scientific investigation of the potentialities of chemical characters for the study of problems of plant taxonomy and plant phylogeny. Chemical characters can serve as guides for classification. Varying interpretation and evaluation of morphological characters very often result in disagreement regarding classification. In such instances characters other than morphological ones are considered. Generally anatomical, embryological, palynological and cytological characters are considered first (Hegnauer, 1967). Sometimes they produce convincing evidence and sometimes they fail to do so. In such situations chemical characters may become very useful for taxonomic classification. At present one important task of chemotaxonomy is to procure additional evidence in all cases of obscure relationships of plants. Thorough phytochemical investigations may result in a better understanding and a re-evaluation of all available facts. Chemical characters can be as valid in future for taxonomic work as are morphological ones. To reach this stage, however, our knowledge about plant metabolism and its resulting products still has to be considerably extended.

Plant species are composed of interbreeding populations of individuals. If a species has been highly successful and covers a large area at present, many of its populations become geographically and (or) ecologically separated. Gradually the gene pools of radiating populations may change and distinct topotypes or ecotypes may emerge. The latter may still be interfertile with all other populations of the species and clearly

represent only variants of one wide-spread species. If, however, by polyploidy or some other mechanism barriers to gene exchange between the diverging entities have arisen or if clear cut morphological differences have evolved the matter of species delimitation becomes a delicate and difficult task. Many of the so called species aggregates have been taxonomically interpreted in different ways and nomenclature has often become complex and rather disappointing in such notoriously difficult groups. In this field of taxonomy, cytotaxonomical research has proved to be often successful. It may be an invaluable aid for an unambiguous identification of distinct entities and in many instances it has offered even a clue for a better understanding of the past history of such puzzlingly complex aggregates. Frequently past history gave rise to slightly differing metabolic patterns in members of a species aggregate. The study of their chemical constituents may therefore bring to light new characteristics helpful in identification. Thus, chemical characters serve as aids in unambiguous identifications of plants. They can also be used as aids to delimit taxa in such a manner that they really represent natural entities.

There are, however, many more aspects, which make the study of chemical characters at intraspecific and specific levels a very fascinating one. Besides being helpful with the identification of plant specimens it informs us about patterns of chemical variation within genera and aggregate species and it may ultimately demonstrate how one pattern of plant constituents evolved from a preceding one. Moreover, joint botanical and phytochemical studies may provide us with a better understanding of the biological and ecological meaning of distinct spectra of primary and secondary plant metabolites. A thorough knowledge in these fields is

essential for a judgement of the overall taxonomic implications of the overwhelming multitude on phytochemical patterns. To make the most appropriate use of chemical characters in plant taxonomy one has to realise clearly that several factors affect and restrict their taxonomic meaning. Some factors which limit the taxonomic value of chemical characters are parallelism, diversification and methods of documentation.

As per the available reports (Wu *et al*., 2008; Nebula *et al*., 2013) on the chemical constituents of mangrove plants of the three true mangrove genera belonging to the family *Rhizophoraceae*; *Bruguiera*, *Ceriops* and *Rhizophora*, the diterpenoid class kauranes exist in the genera *Bruguiera* and *Ceriops* but found to be absent in the genus *Rhizophora.* The genus *Bruguiera* is characterised by the presence of disulphides and polydisulphides which are unique to it. Thus they can be considered as significant chemotaxonamic markers of this genus. Also, it can be observed that ent-pimarane coexists with isopimarane in the genus *Bruguiera.* The presence of dolabranes only in the genus *Ceriops* makes it a significant chemotaxonomic marker of it. Similarly, labdane found only in the genus *Rhizophora* can be seen as the significant chemotaxonomic marker of that particular genus.

Further extensive investigation is needed to identify and classify the chemical constituents of mangrove plants to build up a firm and through basement for the chemotaxonomic studies of these versatile plants. Among the chemical constituents, flavonoids, alkanes and fatty acids can be used as potential chemotaxonomic markers in addition to their bioactive potentials.

1.4.1 Flavonoids

Among the various phytochemicals as the secondary metabolites of plants, phenolic compounds are the common ones and frequently present in the plant kingdom. Phenolic constituents exhibit several bioactivities such as antimicrobial, antioxidant, antiviral, anti-inflammatory (Bravo, 1998). Flavonoids are the most important and most studied phenolic phytochemicals that are widely distributed in plants.

Flavonoids are a large family of over 4000 secondary plant metabolites called C6–C3–C6 phenolics, which are classified in three groups, depending on the nature of the C3 fragment and the type of the heterocyclic ring, as follows: 1) chromone derivatives (flavones, flavonols, flavanones, and flavanonols); 2) chromane derivatives (catechines and antocyanidines); and 3) flavonoids with open propane chain (chalcones) and with a furane ring (aurones) (Harborne, 1994). From all of these, flavones, flavonols, and flavanones are the most abundant in the plant kingdom and their skeleton is given in Fig. 1.3. Substitution in the positions 3, 5, 6, 7, 8, 2', 3', 4', 5', and 6' gives all the compounds from these groups, with hydroxylation, methoxylation, and glycosylation being the most common substitution. Thousands of various flavonoids with various substitution patterns are recognised today as free flavones, flavonols, and flavanones, i.e., aglycones, and as flavonoid glycosides, which consist of flavonoid, nonsugar component aglycone, connected to the sugar moiety (mostly monosaccharides and disaccharides). Bonding to sugars makes flavonoids soluble in water and enables their easy transport within plants. Many flavonoids are present in plant tissues in relatively high concentrations as sugar conjugates. Among the more

ubiquitous flavonoids over 50 different glycosides have been identified (Hertog *et al*., 1992).

Flavonoids are a remarkable group of plant metabolites belonging to radical-scavenging antioxidants which scavenge radicals to inhibit chain initiation and break chain propagation. Flavonoids are almost ubiquitous in plant foods (vegetables, cereals, legumes, fruits, nuts, etc.) and beverages (wine, cider, beer, tea, cocoa, etc.). The presence of flavonoids in plant foods is largely influenced by genetic factors and environmental conditions. Other factors such as germination, degree of ripeness, variety, processing, and storage also influence the content of plant phenolics (Bravo, 1998). Flavonoids and other polyphenols are partially responsible for sensory and nutritional qualities of plant foods. The astringency and bitterness of foods and beverages depends on the content of polyphenols. Flavonoids are chelators of metals and inhibit the Fenton and Haber-Weiss reactions, which are important sources of active oxygen radicals (Shahidi *et al*., 1992). In addition, flavonoids retain their free radical scavenging capacity after forming complexes with metal ions (Afanas'ev *et al*., 1989). Interest in food phenolics has increased owing to their role as antioxidants, antimutagens, and scavengers of free radicals and their implication in the prevention of pathologies such as cancer and cardiovascular disease.

No other class of secondary product has been credited with so many—or such diverse—key functions in plant growth and development. Many of these tasks are critical for survival, such as attraction of animal vectors for pollination and seed dispersal, stimulation of *Rhizobium* bacteria for nitrogen fixation, promotion of pollen tube growth, and the resorption of

mineral nutrients from senescing leaves while others provide a competitive edge to plants that grow under suboptimal environments (Andersen and Markham, 2006). Flavonoids, for example, are known to enhance tolerance to a variety of abiotic stressors, they are employed as agents of defence against herbivores and pathogens, and they form the basis for allelopathic interactions with other plant species. They are involved in production of autumn's colours and photoprotection of leaf's cell. The flavonoids are evidently extremely useful to plants, and it is not surprising, therefore, that species from all orders of the plant kingdom, from the basal liverworts to the most advanced angiosperms, invest significant amounts of metabolic energy into the production of these compounds.

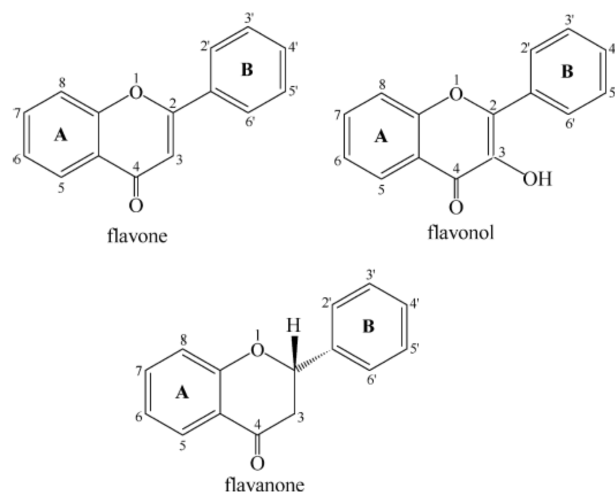


Fig. 1.3 Structure of flavone, flavonol and flavanone

Most flavonoids, however, have very restricted distributions within the plant kingdom, many occurring in only one genus or even species (Markham, 2012). They are already proved as potentially important markers for taxonomic studies due to its characteristics such as structural variability,

chemical stability, ubiquitous occurrence and easy and rapid identification (Joshi, 2008). Moreover, the flavonoids are also used to solve the problems of plant identification where flowering and fruit development does not occur frequently (Joshi *et al*, 2004).

1.4.2 Alkanes

n-Alkanes are saturated hydrocarbons, also known as 'paraffins'. They are composed only of two elements: carbon and hydrogen, connected by simple bonds. The size of the alkanes is defined by a number of inter-connected C atoms. The most important commercial sources of alkanes are natural gas and naphtha. Additionally, alkanes are tar, asphalt and biogas components. Plants typically produce a range of n-alkanes, commonly with a strong odd-over-even predominance and one or two dominant chain lengths (Eglinton and Hamilton, 1963, 1967). Alkane biosynthesis is genetically controlled. Genetic changes result in a decreased production of waxes or reduction of content of some of the components, and they might even lead to a change of wax crystal structure.

Most represented alkanes in majority of plants are n-C_{29} (n-nonacosane) and n-C_{31} (n-hentriacontane), sometimes constitutes as much as 90% of the total wax composition. Terrestrial plants are characterised by strong domination of odd n-alkanes in range C_{25}–C_{35}, while aquatic plants are typified by C_{23} (n-tricosane) and C_{25} (n-pentocosane) abundance. Long chain (C_{21} to C_{37}) n-alkanes are among the most long-lived and widely utilised terrestrial plant biomarkers. Because they are straight-chain hydrocarbons lacking functional groups, n-alkanes are especially stable and long-lived molecules that can survive in the fossil record for tens of millions

of years (Eglinton and Logan, 1991; Peters *et al*., 2005). n-alkanes occur in both modern and fossil leaves, in soils, paleosols, and fluvial sediments and in both lacustrine and marine sediments (Bush and McInerney., 2013). Long chain n-alkanes have great potential to inform us on past terrestrial ecosystems and environments, but their interpretation as paleo-proxies requires a strong understanding of variations in n-alkane production both within and between modern plants.

Apart from the taxonomic relevance, the alkane content of the plants can be used to assess their importance as a source of novel products. Biologically produced alkanes represent potential renewable alternatives to petroleum-derived chemicals. Hydrocarbons of plant origin can have profound use in food as well as pharmaceutical industry. Alkanes are used as a constituent of various polishes, candles, varnishes, sealing waxes, electrical insulators, carbon papers, cosmetic and other wax containing preparations (Stoker, 2012). The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores have been reviewed by Dove and Mayers, 1991. n-alkanes as faecal markers, have been widely investigated for estimation of diet composition. Generally, diet composition of free-ranging ruminants is not as complex as the plant communities of the rangeland they graze. Therefore, Dove and Mayes (2005) suggested that microscopic methods of faecal cuticle analysis or observational measurements may provide a good indication of whether an animal is consuming an individual plant species, and the combination of such qualitative procedures with plant wax marker methods would give a more reliable estimate of diet composition for animals in complex plant communities.

1.4.3 Fatty Acids

Fatty acids are the constituents of all plant cells, where they function as membrane components, storage products, metabolites, and as a source of energy (Wada *et al*., 1994. Also, they are important nutrient substances and metabolites in living organisms (Chen and Chaung, 2002). Furthermore, their biological specificity, and the fact that they are transferred from primary producers to higher trophic levels without change, make fatty acids suitable for use as biomarkers (Parrish *et al*., 2000). Several previous studies have used fatty acids as biomarkers for bacteria, diatoms, dinoflagellates, zooplankton, macroalgae and vascular plants (Alfaro *et al*., 2006).

The use of the fatty acid biomarker technique to determine the fate of the sources of organic matter (from green plants and microbial producers) relies on the ability to find the compounds that exclusively characterise these sources. These biomarkers can then be used to trace the origin and trajectory of organic matter in the ecosystem. However, these biomarkers have limitations. For example, some fatty acid markers may be metabolised and transformed once consumed by the animal, and only relative rather than absolute amounts may be measured. Thus, biomarkers should be used with caution or in conjunction with other quantitative techniques, such as stable isotopes.

Apart from the use of fatty as biomarkers and chemotaxonomic markers, they serve as a main source of energy for human beings and other animals. According to the report on polyunsaturated fatty acids in the food chain in US, Polyunsaturated fatty acids (PUFAs) contribute ≈7% of total energy intake and 19–22% of energy intake from fat in the diets of adults, a

level that is within recommended intakes for both men and women (Kris-Etherton *et al.*, 2000). Linoleic acid (18:2ω6) is the major PUFA, comprising 84–89% of the total PUFA energy, whereas α-linolenic acid (ALA; 18:3ω3) contributes 9–11% of the total PUFA energy (equivalent to 1.1–1.6 g/d) in the diets of the adult population.

1.5 Aim and scope of the present study

Mangrove vegetation is an important coastal ecosystem associated with tidal / mud flats and back water systems. Mangroves exist under stressful conditions and serve as a bridging ecosystem between freshwater and marine systems. These plants have specially adapted their own morphological structures and physiological mechanisms to the harsh natural surroundings. Pneumatophores, stilt roots and buttresses, salt-excreting glands in the leaves, and viviparous propagules are some of the several highly specialised adaptations of this group. The path of photosynthesis in mangroves is different from other glycophytes. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis. As they survive under extreme conditions of salinity, temperature, tides and anoxic soil conditions they may have chemical compounds, which protect them from these destructive elements. Even though extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens there is a gap in investigations to identify the metabolites responsible for their bioactivities.

In India, the states like West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Andaman and Nicobar Islands, Kerala, Goa, Maharashtra, and

Gujarat occupy vast area of Mangroves. Among the mangrove species found in the Kerala coast *Rhizophoraceae* mangroves are very common. The *Rhizophoraceae* family of true mangrove plants is the most populated and contains widely distributed species. All these plants are characterised by the presence of aerial roots and viviparous germination. *Rhizophoraceae* mangroves are ranked as “major elements of mangroves” as they give the real shape of this unique and interesting ecosystem and these mangrove species most productive and typical ecosystem of World renowned. They mostly grow in the intertidal silty clay and loam soil, frequently inundated with high saline tidal seawater or brackish water; their salt resistance efficiencies are very high for several morphological and anatomical features of these true mangrove species.

It was found that the *Rhizophoraceace* mangrove extracts exhibit several bioactive properties. Various parts of these mangroves are used in ethnomedicinal practices. Even though extracts from these mangroves possess therapeutic activity against humans, animal and plant pathogens, the specific metabolites responsible for these bioactivities remains to be elucidated. Thorough phytochemical investigation can achieve the validity of ethnomedicines as well as apply the use of mangrove plants in the development of new drugs. Such studies can pave a firm base for their use in biomarker and chemotaxonomic studies as well as for the better management of the existing mangrove ecosystem. There is a gap of information towards the chemistry of *Rhizophoraceae* mangroves from Kerala. Our knowledge about the metabolic products of these mangroves still has to be considerably extended in order to aid better classification, utilisation and management of these plants of immense potentials.

Five mangrove plants of family *Rhizophoraceae; Bruguiera cylindrica* and *Bruiguiera gymnorrhiza* belonging to the genus *Bruguiera*;, *Kandelia candel*,of the genus *Kandelia*; *Rhizophora mucronata* and *Rhizophora apiculata* of the genus *Rhizopora* which are commonly found in Kerala, were chosen for the present study. The plant materials were collected from in and around Kochi. The leaves and bark of the plants were the plant parts considered for this study.

The present work aims to:

- Assess the elemental, isotopic, mineral and biochemical composition of the selected mangrove plant parts for the basic chemical characterisation of each species.
- Quantify the content of food Flavonoids and their relevance towards chemotaxonomy
- To study the variations in fatty acid profiles of common *Rhizophoraceae* mangrove species found in Kochi.
- Quantification of alkanes in the leaves and bark of mangrove plants to understand the intraspecific as well as interspecific variations.
- To identify the potent mangrove species for the extraction of bioactive metabolites for future drug development as well as to provide chemical backup for the ethnopharmaceutical practices.

References

Abeysinghe, P. D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. *Indian journal of pharmaceutical sciences*, *72*(2), 167- 172.

Achamadi, S., Gustini S., Elvin, T. C., and Richard, W. H. (1994). Catechin-3-0-rhamnoside Chain Extemder Units in Polymeric Procyanidins from Mangrove Bark. *Phytochemistry*, 35 (1), 217–219.

Afanas' ev, I. B., Dcrozhko, A. I., Brodskii, A. V., Kostyuk, V. A., & Potapovitch, A. I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical pharmacology*,*38*(11), 1763-1769.

Afzal, M., Mehdi, F. S., Siddiqui, Z. S., and Shaukat, S. S. (2005). Use of *Rhizophora mucronata Poir* against the growth of some atmosphericfungal allergens. *International Journal of Biology and Biotechnology (Pakistan)*, 2 (4), 941-945.

Agoramoorthy, G., Chen, F. A., Venkatesalu, V., Kuo, D. H., and Shea, P. C. (2008). Evaluation of antioxidant polyphenols from selected mangrove plants of India. *Asian Journal of Chemistry*, *20*(2), 1311-1322.

Alarcon-Aguilara, F. J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C. C., and Flores-Saenz, J. L. (1998). Study of the anti-hyperglycemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology*, *61*(2), 101-110.

Alfaro, A. C., Thomas, F., Sergent, L., and Duxbury, M. (2006). Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science*, *70*(1), 271-286.

Alikunhi, N. M., Kandasamy, K., Manoharan, C., & Subramanian, M. (2012). Insulin-like antigen of mangrove leaves and its anti-diabetic activity in alloxan-induced diabetic rats. *Natural product research*, *26*(12), 1161-1166.

Andersen, Ø.M. and Markham, K.R. (2006). Chemistry, Biochemistry and Applications, PP.397-425.

Anjaneyulu, A. S., and Rao, V. L. (2001). Rhizophorin A, a novel secolabdane diterpenoid from the Indian mangrove plant Rhizophora mucronata. *Natural product letters*, *15*(1), 13-19.

Anjaneyulu, A. S., and Rao,V.L. (2002). Ceriopsins A–D, diterpenoids from *Ceriops decandra*. *Phytochemistry*, *60*(8), 777-782.

Anjaneyulu, A. S., Anjaneyulu, V., and Rao, V. L. (2002b). New beyerane and isopimarane diterpenoids from Rhizophora mucronata. *Journal of Asian natural products research*, *4*(1), 53-60.

Anjaneyulu, A. S., Rao, V. L., Lobkovsky, E., and Clardy, J. (2002a). Ceriopsin E, a New Epoxy ent-Kaurene Diterpenoid from *Ceriops decandra* 1. *Journal of natural products*, *65*(4), 592-594.

Anjaneyulu, A.S.R, and Rao, V. L. (2003). "Ceriopsins F and G, diterpenoids from *Ceriops decandra*." Phytochemistry, 62(8), 1207–1211.

Arivuselvan, N., Silambarasan, D., Govindan, T., and Kathiresan, K. (2011). Antibacterial activity of mangrove leaf and bark extracts against human pathogens. *Advances in Biological Research*, *5*(5), 251-254.

Bandaranayake, W. M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management*, *10*(6), 421-452.

Banerjee, D., Chakrabarti, S., Hazra, A. K., Banerjee, S., Ray, J., & Mukherjee, B. (2008). Antioxidant activity and total phenolics of some mangroves in Sundarbans. *African journal of biotechnology*, *7*(6), 805-810.

Bao, S. Y., and Lin, W. H. (2006). Compounds from marine mangrove plant Bruguiera sexangula var. rhynchopetala. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica*, *31*(14), 1168-1171.

Bao, S., Deng, Z., Fu, H., Proksch, P., and Lin, W. (2005). Diterpenes and Disulfides from the Marine Mangrove Plant *Bruguiera sexangula var. rhynchopetala*. *Helvetica chimica acta*, *88*(10), 2757-2763.

Bao, S., Ding, Y., Deng, Z., Proksch, P., and Lin, W. (2007). Rhyncosides A-F, phenolic constituents from the Chinese mangrove plant *Bruguiera sexangula var. rhynchopetala*. *Chemical and Pharmaceutical Bulletin*, *55*(8), 1175-1180.

Basha, S.C. (1992). Mangroves of Kerala- A fast disappearing asset, *Indian forester*. 120(2), 175-189.

Baskaran, R., and Mohan, P. M. (2012). In vitro antibacterial activity of leaf extracts of Rhizophora mucronata L. against multi drug resistant Vibrio spp. isolated from marine water Lobster's larvae hatcheries. *Indian Journal of Geo-Marine Sciences*, *41*(3), 218.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, *56*(11), 317-333.

Bunt, J., S. (1992). How can fragile ecosystems best be conserved? In: Hsu¨ KJ, Thiede J (Eds). Use and misuse of the seafloor (Dahlem workshop reports: environmental science research report 11). Wiley, Chichester, pp. 229-242.

Bush, R. T., and McInerney, F. A. (2013). Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. *Geochimica et Cosmochimica Acta*, *117*, 161-179.

Chacha, M. (2011). "Terpenoids from the Roots of Ceriops Tagal Induces Apoptosis Through Activation of Caspase-3 Enzyme." *International Journal of Biological and Chemical Sciences* 5, 402–409.

Chandrasekaran, M., Kannathasan, K., Venkatesalu, V., and Prabhakar, K. (2009). Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant Staphylococcus aureus. *World Journal of Microbiology and Biotechnology*, *25*(1), 155-160.

Chantrapromma, S., Fun, H. K., Pakhathirathien, C., Karalai, C., and Chantrapromma, K. (2007). 7-Glycoloyl-2-hydroxy-1, 4b, 7, 10a-tetramethyl-4a, 4b, 5, 6, 7, 8, 8a, 9, 10, 10a-decahydrophenanthren-3 (4H)-one. *Acta Crystallographica Section E: Structure Reports Online*, *63*(2), 459-461.

Chen, J. D., Feng, D. Q., Yang, Z. W., Wang, Z. C., Qiu, Y., and Lin, Y. M. (2008). Antifouling metabolites from the mangrove plant Ceriops tagal.*Molecules*, *13*(2), 212-219.

Chen, J. D., Yi, R. Z., Lin, Y. M., Feng, D. Q., Zhou, H. C., and Wang, Z. C. (2011). Characterization of Terpenoids from the Root of Ceriops tagal with Antifouling Activity. *International journal of molecular sciences*, *12*(10), 6517-6528.

Chen, S. H., and Chuang, Y. J. (2002). Analysis of fatty acids by column liquid chromatography. *Analytica Chimica Acta*, *465*(1), 145-155.

Chumkaew, P., Kato, S. and Chantrapromma, K. (2005). A new triterpenoid ester from the fruits of Bruguiera parviflora. *Chemical & pharmaceutical bulletin*, 53(1), 95–96.

Clough, B. (2013) Continuing the Journey amongst Mangroves. ISME Mangrove Educational Book Series No. 1. International Society for Mangrove Ecosystems (ISME), Okinawa, Japan, and International Tropical Timber Organization (ITTO), Yokohama, Japan.PP5.

Das, A. K., Rohini, R., and Hema, A. (2009). Evaluation of Anti-diarrhea activity of Rhizophora mucronata bark extracts. *The Internet Journal of Alternative Medicine*, *7*(1).

Dittmar, T., Hertkorn, N., Kattner, G., and Lara, R. J. (2006). Mangroves, a major source of dissolved organic carbon to the oceans. *Global Biogeochemical Cycles*, *20*(1),

Dove, H., and Mayes, R. W. (2005). Using *n*-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Ruminant Research*, *59*(2), 123-139.

Duke, N.C. (1992). Mangrove floristics and biogeography. In “Tropical Mangrove Ecosystems” (A.I. Robertson and D.M. Alongi, eds), pp.63-100. American Geophysical Union, Washington DC., USA.

Eglinton G. and Hamilton R. J. (1963). The distribution of alkanes. In Chemical Plant Taxonomy (ed. T. Swain). Academic Press, London.

Eglinton G. and Hamilton R. J. (1967). Leaf epicuticular waxes. *Science* 156, 1322–1335.

Eglinton G. and Logan G. A. (1991) Molecular preservation. Phil. Trans. Roy. Soc. London B 333, 315–328.

FAO, 2007. The world's mangroves 1980-2005. *Forestry Paper* No. 153, Rome, 77 p.

Fun, H. K., Pakhathirathien, C., Chantrapromma, S., Karalai, C., and Chantrapromma, K. (2006). 7-Ethenyl-1-[(Z)-hydroxymethylidene]-4b, 7, 10a-trimethylperhydrophenanthren-2-one. *Acta Crystallographica Section E: Structure Reports Online*, *62*(12), 5539-5541.

Ganguly, S. N., and A. Sarkar. (1978). "Gymnorhizol, a New Triterpene Alcohol from Bruguiera Gymnorhiza." Indian Journal of Chemistry Section B 16: 742–744.

Gao, M. Z., Yuan, X. Y., Cheng, M. C., Xiao, H. B., and Bao, S. X. (2011). A new diterpenoid from Rhizophora apiculata. *Journal of Asian natural products research*, *13*(8), 776-779.

Gazim, Z. C., Rezende, C. M., Fraga, S. R., Svidzinski, T. I. E., and Cortez, D. A. G. (2008). Antifungal activity of the essential oil from Calendula officinalis L.(Asteraceae) growing in Brazil. *Brazilian Journal of Microbiology*, *39*(1), 61-63.

Ghosh, A., Misra, S., Dutta, A. K., and Choudhury, A. (1985). Pentacyclic triterpenoids and sterols from seven species of mangrove. *Phytochemistry*, *24*(8), 1725-1727.

Gopalan, U. K., Vengayil, D. T., Udayavarma, V. P., & Krishnankutty, M. (1983). The shrinking backwaters of Kerala. *Journal of the Marine Biological Association of India. Cochin*, *25*(1), 131-141.

Han, L., Huang, X. S., Sattler, I., Fu, H. Z., Grabley, S., and Lin, W. H. (2007). Two new constituents from mangrove Bruguiera gymnorrhiza. *Journal of Asian natural products research*, *9*(4), 327-331.

Han, L., Huang, X., Sattler, I., Dahse, H. M., Fu, H., Grabley, S., and Lin, W. (2005a). Three new pimaren diterpenoids from marine mangrove plant, Bruguiera gymnorrhiza. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *60*(9), 705-707.

Han, L., Huang, X., Sattler, I., Dahse, H. M., Fu, H., Lin, W., and Grabley, S. (2004). New Diterpenoids from the Marine Mangrove *Bruguiera gymnorrhiza*. *Journal of natural products*, *67*(9), 1620-1623.

Han, L., Huang, X., Sattler, I., Moellmann, U., Fu, H., Lin, W., & Grabley, S. (2005b). New Aromatic Compounds from the Marine Mangrove *Bruguiera gymnorrhiza*. *Planta medica*, *71*(02), 160-164.

Harborne, J. B. (1994)Ed. The Flavonoids, Advances in Research Since 1986; Chapman and Hall: London.

Hegnauer, R. (1967). Chemical characters in plant taxonomy: some possibilities and limitations. *Pure and Applied Chemistry*, *14*(1), 173-188.

Hertog, M. G., Hollman, P. C., & Venema, D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, *40*(9), 1591-1598.

Hogg, R., and Gillan, F., T. (1984). Fatty Acids, Sterols and Hydrocarbons in the Leaves from Eleven Species of Mangrove. *Phytochemistry*, *23* (1), 93–97.

Homhual, S., Bunyapraphatsara, N., Kondratyuk, T., Herunsalee, A., Chaukul, W., Pezzuto, J. M., Fong, H.H., and Zhang, H. J. (2006b). Bioactive dammarane triterpenes from the mangrove plant *Bruguiera gymnorrhiza*. *Journal of natural products*, *69*(3), 421-424.

Homhual, S., Zhang, H. J., Bunyapraphatsara, N., Kondratyuk, T. P., Santarsiero, B. D., Mesecar, A. D., Herunsalee, A., Chaukul, W., Pezzuto, J., M., and Fong, H. H. (2006a). Bruguiesulfurol, a new sulfur compound from *Bruguiera Gymnorrhiza*. *Planta Medica, 72* (3), 255–260.

Hong, L. S., Ibrahim, D., Kassim, J., and Sulaiman, S. (2011). Gallic Acid : An anticandidal compound in hydrolysable tannin extracted from the barks of *Rhizophora apiculata blume*. *Journal of Applied Phramaceutical Science, 01* (06), 75–79.

Hossain, H., Moniruzzaman, S., Nimmi, I., Kawsar, H., Hossain, A., Islam, A., and Jahan, I. A. (2011). Anti-inflammatory and antioxidant activities of the ethanolic extract of Ceriops decandra (Griff.) Ding Hou bark. *Oriental Pharmacy and Experimental Medicine*, *11*(4), 215-220.

Hsu, F.-L., Nonaka, G.-I., and Nishioka, I. (1985). Tannins and related compounds. XXXI. Isolation and characterization of proanthocyanidins in *Kandelia candel* (*L.*) *Druce.Chemical & Pharmaceutical Bulletin*, *33*(8), 3142-3152.

Hu, W. M., Li, M. Y., Li, J., Xiao, Q., Feng, G., and Wu, J. (2010). Dolabranes from the Chinese mangrove, *Ceriops tagal*. *Journal of Natural Products*, *73*(10), 1701-1705.

Huang, X. Y., Wang, Q., Liu, H. L., Zhang, Y., Xin, G. R., Shen, X., Dong, M., L., and Guo, Y. W. (2009). Diastereoisomeric macrocyclic polydisulfides from the mangrove *Bruguiera gymnorrhiza*. *Phytochemistry*, *70*(17), 2096-2100.

Ingole, B. 2005. Indian ocean coasts, coastal ecology. Encyclopedia of Coastal Science. pp 446-554.

Jennerjahn, T. C., and Ittekkot, V. (2002). Relevance of mangroves for the production and deposition of organic matter along tropical continental margins.*Naturwissenschaften*, *89*(1), 23-30.

Joshi, K. (2008). Chemotaxonomic investigation of Cotylelobium species (Dipterocarpaceae) using flavonoid analysis. *Scientific World*, *6*(6), 24-26.

Joshi, K., Chavan, P., Warude, D., and Patwardhan, B. (2004). Molecular markers in herbal drug technology. *Current Science*, *87*, 159-165.

Juncosa, A. M., & Tomlinson, P. B. (1988). Systematic comparison and some biological characteristics of Rhizophoraceae and Anisophylleaceae. *Annals of the Missouri Botanical Garden*,75, 1296-1318.

Kabaru, J. M., and Gichia, L. (2009). Insecticidal activity of extracts derived from different parts of the mangrove tree Rhizophora mucronata (Rhizophoraceae) Lam. against three arthropods. *African Journal of Science and Technology*,*2*(2), 44-49.

Karalai, C., and Laphookhieo, S. (2005). Triterpenoid esters from *Bruguiera cylindrica*. *Australian journal of chemistry*, *58*(7), 556-559.

Kathiresan, K. (2000). A review of studies on Pichavaram mangrove, southeast India. *Hydrobiologia*, *430* (1-3), 185-205.

Kathiresan, K. (2003). How do mangrove forests induce sedimentation?. *Revista de biologia tropical*, *51*(2), 355-360.

Kathiresan, K., and Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in marine biology*, *40*, 81-251.

Kokpol, U., Chavasiri, W., Chittawong, V., and Miles, D. H. (1990). Taraxeryl cis-p-hydroxycinnamate, a novel taraxeryl from Rhizophora apiculata. *Journal of Natural Products*, *53*(4), 953-955.

Kokpol, U., Chavasiri, W., Chittawong, V., Bruce, M., Cunningham, G. N., and Miles, D. H. (1993). Long chain aliphatic alcohols and saturated carboxylic acids from heartwood of *Rhizophora apiculata*. *Phytochemistry*, *33*(5), 1129-1131.

Kris-Etherton, P. M., Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R. L., Zhao,G., and Etherton, T. D. (2000). Polyunsaturated fatty acids in the food chain in the United States. *The American journal of clinical nutrition*, *71*(1), 179S-188S.

Kuete, V. (2010). Potential of Cameroonian plants and derived products against microbial infections: A review. *Planta Medica, 76*(14), 1479-1491.

Laphookhieo, S., Karalai, C., and Ponglimanont, C. (2004b). New sesquiterpenoid and triterpenoids from the fruits of Rhizophora mucronata. *Chemical and pharmaceutical bulletin*, *52*(7), 883-885.

Laphookhieo, S., Karalai, C., Ponglimanont, C., and Chantrapromma, K. (2004a). Pentacyclic triterpenoid esters from the fruits of *Bruguiera cylindrica*. *Journal of natural products*, *67*(5), 886-888.

Li, D. L., Li, X. M., and Wang, B. G. (2008). Pentacyclic triterpenoids from the mangrove plant *Rhizophora stylosa*. *Natural product research*, *22*(9), 808-813.

Li, D. L., Li, X. M., Peng, Z. Y., and Wang, B. G. (2007). Flavanol derivatives from *Rhizophora stylosa* and their DPPH radical scavenging activity.*Molecules*, *12*(5), 1163-1169.

Li, Liang, Cai-Guo Huang, Chang-Yun Wang, and Yue-Wei Guo. 2010. “Sexangulic Acid, a New Cytotoxic Triterpenoid from the Chinese Mangrove Bruguiera Sexangula.” Natural Product Research 24 (11) (July): 1044–1049.

Littleton, J., Rogers, T., and Falcone, D. (2005). Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation. *Life sciences*, *78*(5), 467-475.

Loder, J. W., and Russell, G. B. (1969). Tumour inhibitory plants. The alkaloids of *Bruguiera sexangula* and *Bruguiera exaristata* (*Rhizophoraceae*). *Australian Journal of Chemistry*, *22*(6), 1271-1275.

Markham, K., R. (2012). Flavones, flavonols and their glucosides. In: *Methods in Plant Biochem*istry, Vol1, APL, 197.

Mohanan, C.N., 1997. Mangroves. In : Ed., Thampi, K.B., Nayar, N.M., and Nair, C.S., *National Resources of Kerala*. WWF- India, Thiruvanathapuram, Kerala. PP 149-158.

Nabeel, M. A., Kathiresan, K., & Manivannan, S. (2010). Antidiabetic activity of the mangrove species Ceriops decandra in alloxan-induced diabetic rats.*Journal of diabetes*, *2*(2), 97-103.

Nascimento, G., F., Locatelli, J., Freitas, P., C., and Silva, G., L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology, 31*(4), 247-256.

Naskar, K., and Mandal, R. (1999). *Ecology and biodiversity of Indian mangroves*. Daya Books Naskar, K. and R. Mandal. 1999. Ecology and Biodiversity of Indian Mangroves. Daya Publishing House, Delhi, India. pp. 386-388.

Nebula, M., Harisankar, H. S., & Chandramohanakumar, N. (2013). Metabolites and bioactivities of Rhizophoraceae mangroves. *Natural Products and Bioprospecting*, *3*(5), 207-232.

Odum, E., P. (1971). *Fundamentals of Ecology*. Philadelphia: W.B. Saunders Company and Toppan Company, pp. 574 - 575.

Ouyang, X.,W., Xia-Chang W., Qing-Xi Yue, and Li-Hong Hu. 2010. “A New Dolabrane- Type Diterpene from Ceriops Tagal.” *Natural Product Communications* 5 (1): 9–12.

Pakhathirathien, C., Karalai, C., Ponglimanont, C., Subhadhirasakul, S., & Chantrapromma, K. (2005). Dammarane Triterpenes from the Hypocotyls and Fruits of Ceriops t agal. *Journal of natural products*, *68*(12), 1787-1789.

Panda T., 2010, Preliminary study of ethno-medicinal plants used to cure different diseases in coastal district of Orissa, India, British Journal of Pharmacology and Toxicology 1(2), 67-71

Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., Pulchan, K., Ramos, C. (2000). Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky, P. (Ed.), The Handbook of Environmental Chemistry, Part D, Marine Chemistry. Springer, Berlin, Heidelberg, pp. 193-233.

Peters K. E., Walters, C., C., and Moldowan, J., M. (2005) The Biomarker Guide. Cambridge University Press, Cambridge, Berlin, Heidelberg, UK. pp. 193e233.

Ponglimanont, C., and Thongdeeying, P. (2005). Lupane-triterpene esters from the leaves of Ceriops decandra (Griff.) Ding Hou. *Australian journal of chemistry*, *58*(8), 615-618.

Premanathan, M., Kathiresan, K., and Nakashima, H. (1999). Mangrove halophytes: A source of antiviral substances. *South Pacific Study*, *19*(1-2), 49-57.

Premanathan, M.; Kathiresan, K.; Nakashima, H. 1999. Mangrove halophytes: A source of antiviral substances, South Pacific Study, 19, 49–57

Rahim, A. A., Rocca, E., Steinmetz, J., Jain Kassim, M., Sani Ibrahim, M., and Osman, H. (2008). Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. *Food chemistry*, *107*(1), 200-207.

Ram, T. A. and Shaji, C.S. (2013). Diversity and Distribution of Mangroves in Kumbalam Island of Kerala, India, Journal Of Environmental Science, Toxicology And Food Technology, 4 (4), 18-26.

Rao, B., V., Rao, C. V., Subrahmanyam, C., Jairaj, M. A. (2005). Chemical constituents of Rhizophora mucronata of Andaman and Nicobar Islands. *Journal of Indian Chemical Society*, 82 (2), 155–157.

Rates, S.M.K., 2001. Plants as source of drugs, Toxicon 39, 603–613

Ravikumar, S., Gnanadesigan, M., Suganthi, P., & Ramalakshmi, A. (2010). Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. *International Journal of Medicine and Medical Sciences*, *2*(3), 94-99.

Richter, A., Thonke, B. and Popp, M. (1990). "1D-1- *0*-methyl-*muco*-inositol in *Viscum Album* and members of the *Rhizophoraceae*." *Phytochemistry*, *29*(6), 1785-1786.

Rohini, R. M., and Das, A. K. (2010). Triterpenoids from the stem bark of Rhizophora mucronata. *Natural product Research*, *24*(2), 197-202.

Saenger, P. 2002. Mangrove Ecology, Silviculture and Conservation. Kluwer Academic publishers, Dordrecht, The Netherlands. pp. 11-18.

Salae, A. W., Chantrapromma, S., Fun, H. K., & Ponglimanont, C. (2007). ent-Kaur-16-ene-13, 19-diol. *Acta Crystallographica Section E: Structure Reports Online*, *63*(4), 1899-1901.

Saxena, E., and Garg, H. S. (1994). Apiculol, 1β–hydroxy–13–epimanoyl Oxide from Roots of Rhizophora apiculata Mangrove. *Natural Product Letters*, *4*(2), 149-154.

SFR, 2009. Forest Survey of India 2007. India State of Forest Report, Dehradun, pp. 27-31.

SFR, 2011. Forest Survey of India 2011. India State of Forest Report, Dehradun.

Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants.*Critical Reviews in Food Science & Nutrition*, *32*(1), 67-103.

Singh, A. K., Ansari, A., Kumar, D., & Sarkar, U. K. (2012). Status, Biodiversity and Distribution of Mangroves in India: An overview. *Marine Biodiversity, Uttar Pradesh State Biodiversity Board*, 59-67.

Singh, G., Ramanathan, A., L. and Prasad, M., B., K. (2005). Nutrient cycling in mangrove ecosystem: A brief overview. *Journal of Ecology and Environmental Science, 30*(3), 231-244.

Spalding, M. (1997). The global distribution and status of mangrove ecosystems. International News Letter of Coastal Management-Intercoast Network, Special edition 1, 20-21.

Stoker, H. S. (2012). *General, organic, and biological chemistry*. Cengage Learning.

Subrahmanyam, C., Rao, B. V., Ward, R. S., Hursthouse, M. B., and Hibbs, D. E. (1999). Diterpenes from the marine mangrove Bruguieragymnorhiza. *Phytochemistry*, *51*(1), 83-90.

Suganthy, N., Pandian, S. K., & Devi, K. P. (2009). Cholinesterase inhibitory effects of Rhizophora lamarckii, Avicennia officinalis, Sesuvium portulacastrum and Suaeda monica: mangroves inhabiting an Indian coastal area (Vellar Estuary). *Journal of enzyme inhibition and medicinal chemistry*, *24*(3), 702-707.

Suma, K.P., Distribution of mangrove vegetation and associated algal flora in Vypeen block. M Phil dissertation, University of Kerala, Thiruvananthapram, 1995

Sur, T. K., and Pandit, S. (2004). Hypoglycemic Activities of a Mangrove Plant *Rhizophora apiculata Blume*. *Natural Product Sciences*, *10*(1), 11-15.

Takara, K., Kuniyoshi, A., Wada, K., Kinjyo, K., and Iwasaki, H. (2008). Antioxidative flavan-3-ol glycosides from stems of *Rhizophora stylosa*. *Bioscience, Biotechnology, and Biochemistry*, *72*(8), 2191-2194.

Tomlinson, P.B. (1986). The Botany of Mangroves. Cambridge, London, New York: Cambridge University Press. 414 pp.

Wada, H., Gombos, Z., and Murata, N. (1994). Contribution of membrane lipids to the ability of the photosynthetic machinery to tolerate temperature stress.*Proceedings of the National Academy of Sciences*, *91*(10), 4273-4277 .

Wang, X. C., Ouyang, X. W., and Hu, L. H. (2010). Three new lupane-type triterpenes from Ceriops tagal. *Journal of Asian Natural Products Research*,*12*(7), 576-581.

Wei, S. D., Zhou, H. C., and Lin, Y. M. (2010). Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. *International Journal of Molecular Sciences*, *11*(10), 4080-4093.

Williams, L. A. D. (1999). *Rhizophora mangle (Rhizophoraceae)* triterpenoids with insecticidal activity. *Naturwissenschaften*, *86*(9), 450-452.

Wu, J., Xiao, Q., Xu, J., Li, M. Y., Pan, J. Y., & Yang, M. H. (2008). Natural products from true mangrove flora: source, chemistry and bioactivities. *Natural Product Reports*, *25*(5), 955-981.

Yang, S., Lin, P., and Tsuneo, N. (1997). Ecology of mangroves in Japan. *Journal of Xiamen University*, *36*(3), 471-477.

Yang, X. H., Li, H. B., Chen, H., Li, P., and Ye, B. P. (2008). Chemical constituents in the leave of Rhizophora stylosa L and their biological activities.*Yao xue xue bao= Acta Pharmaceutica Sinica*, *43*(9), 974-978.

Zhang, L. L., Lin, Y. M., Zhou, H. C., Wei, S. D., and Chen, J. H. (2010). Condensed tannins from mangrove species *Kandelia candel* and *Rhizophora mangle* and their antioxidant activity. *Molecules*, *15*(1), 420-431.

Zhang, Y., Deng, Z., Gao, T., Proksch, P., and Lin, W. (2005a). Tagalsins A–H, dolabrane-type diterpenes from the mangrove plant,*Ceriops tagal*.*Phytochemistry*, *66*(12), 1465-1471.

Zhang, Y., Lu, Y., Mao, L., Proksch, P., and Lin, W. (2005b). Tagalsins I and J, Two Novel Tetraterpenoids from the Mangrove Plant, *Ceriops tagal*. *Organic letters*, *7*(14), 3037-3040.

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Chapter 2

MATERIALS AND METHODS

Contents

2.1 Sampling location
2.2 Plant materials
2.3 Analytical methodology

2.1 Sampling location

Mangrove plants were collected from three locations in Kochi, Southwest coast of India the according to the availability of the species. The sampling locations are shown in Fig. 2.1. The mangroves, *B. cylindrica, B. gymnorrihzha* and *R. mucronata* were collected from Kerala University of Fisheries and Ocean Sciences (formerly Fisheries Station, Kerala Agriculture University), Puthuvypu. It is a mangrove nursery maintained by Kerala University of Fisheries and Ocean Sciences. It is located close to the sea and is almost undisturbed except by the research activities carried out at the station and is also free from sewage dumping. A lot of developmental pressures, including the proposed gas thermal plant, are threatening the very existence of these mangroves. Heavy developmental activities after the construction of the bridges connecting the mainland are threatening the existence of mangrove patches in the adjoining areas as well.

The mangrove *K. candel* was collected from is situated on the eastern side of the Vembanad ecosystem having several mangrove and shell fish fauna. It has been observed that much mangrove vegetation have been lost from the area due to man-made encroachments. *R. apiculata* was collected from Aroor. Aroor is a dwindling mangrove site with low plant density. As per already reported data, the hydrographic/sediment characteristics of these sites were found to be similar in nature. (Ratheesh Kumar 2011, Meera and Nandan, 2010, Rini, 2002).

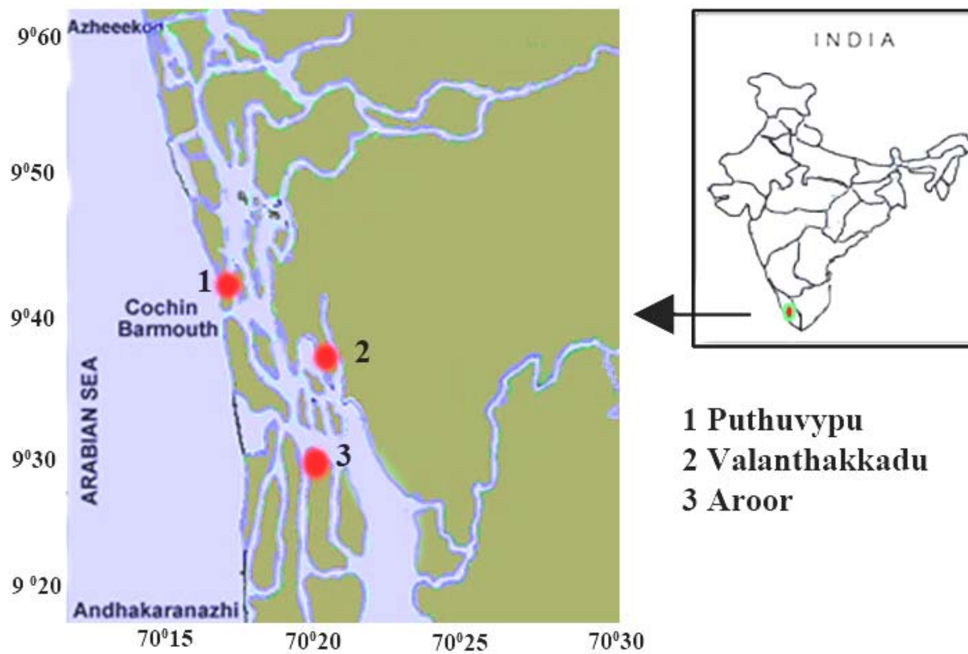

Fig. 2.1 Map of Kochi showing sampling locations

2.2 Plant materials

Mangrove plant samples were collected in August 2011during the monsoon period, the details of which are given in Table 2.1. Leaves and bark of five mangrove plants belonging to the *Rhizophoraceae* family of true mangroves were collected and transported to the laboratory.

Table 2.1 Details of mangrove plants selected for the present study

Species	Genus	Family	Order	Location
Bruguiera cylindrica	*Bruguiera*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi
Bruguiera gymnorrizha	*Bruguiera*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi
Kandelia candel	*Kandelia*	*Rhizophoraceae*	*Malghiales*	Valanthakkad, Kochi
Rhizophora apiculata	*Rhizophora*	*Rhizophoraceae*	*Malghiales*	Aroor
Rhizophora mucronata	*Rhizophora*	*Rhizophoraceae*	*Malghiales*	Puthuvypu, Kochi

All the plants (Fig. 2.2) were identified by Dr. Khaleel K.M. (Former Director, School of Enviromental Studies, Kannur University), Principal, Sir Sayeed College, Kannur. Voucher specimens (BCP8/2011, BGP8/2011, KCV8/2011, RAA8/2011, RMPP8/2011) were kept in at Inter University Centre for Marine Biotechnology, Cochin University of Science and Technology.

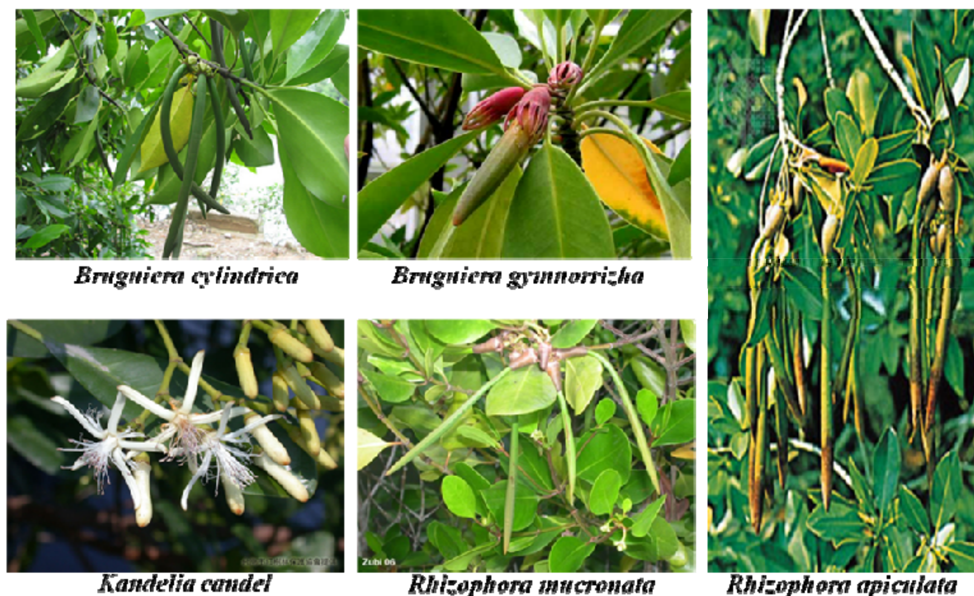

Fig. 2.2 Mangroves plants collected for the present study

2.3 Analytical methodology

The collected leaves and barks of the mangrove plants were washed with water and dried in an incubator at 40°C. Dried samples were ground to produce fine homogenous powders using an electric blender. Dried plant parts were stored in desiccators until analysis. The dried samples were either used directly or extracted using appropriate solvent for the determination of various parameters.

2.3.1 Macro and micronutrient composition

The elemental carbon, hydrogen, nitrogen and sulphur in the dried plant parts were determined using Vario EL III CHNS Analyser. Total phosphorous was estimated using vanadomolybdophosphoric acid method (Bhargava and Raghupathi, 2005).

Minerals (micro and macronutrients) present in the plant parts such as magnesium, iron, copper, zinc, manganese, cobalt and heavy metals, lead, cadmium in the plant part were estimated using Flame AAS (Perkin Elmer-311 0) after digestion using (di-acid mixture (1:5 HCIO3:HNO3) (AOAC, 1995). Accuracy of the analytical procedure was checked using standard reference material. Triplicate analysis of BCSS-1 showed a good accuracy and the recovery rate ranged between 82.7 % for Mn and 103.9 % for Zn (Table 2.2).

Table 2.2 Analysis of Standard reference material for heavy metals (BCSS-1)

Metal	Certified Value	Obtained concentration (n=3)
Co (µg g^{-1})	11.4±2.1	10.67±2.68
Cr (µg g^{-1})	123±1.4	112±0.65
Cu (µg g^{-1})	18.5±2.7	18.2±0.25
Fe (%)	4.7±0.14	4.64±0.41
Mg (%)	2.44±0.23	2.32±0.36
Mn (µg g^{-1})	229±15	189.47±10.75
Ni (µg g^{-1})	55.3±3.6	49.16±2.01
Pb (µg g^{-1})	22.7±3.4	24.9±0.08
Zn (µg g^{-1})	119±12	123.64±2.51

2.3.2 Isotopic analysis

The carbon and nitrogen isotope values were measured at the Marine Stable Isotope Lab (MASTIL) at National Centre for Antarctic & Ocean

Research, Goa, India using an Isoprime Stable Isotope Ratio Mass Spectrometer in continuous-flow mode coupled with an EA (Isoprime, Vario Isotope Cube). The external precisions on $\delta^{13}C$ and $\delta^{15}N$ are ±0.04‰ and ±0.07‰ (1σ standard deviation) respectively obtained by repeatedly running Cellulose (IAEA-CH-3) & Ammonium sulphate (IAEA-N1) standards (n=21). $\delta^{13}C$ values are reported with respect to V-PDB and $\delta^{15}N$ values are reported with respect to air N_2. The reference standard used for normalising to V-PDB and air N_2 scale are Cellulose (IAEA-CH-3) & Ammonium sulphate (IAEA-N1).

2.3.3 Biochemical Composition

Spectrophotometric methods were employed for the determination of biochemical compounds in plant parts. Proteins (PRT) analyses were carried out following the procedure of Lowry *et al*., (1951) with albumin as the standard. Total carbohydrates (TCHO) were analysed using phenol sulphuric acid method (BeMiller, 2010) and low molecular weight carbohydrates were analysed as per Popp, 1984 using glucose as the standard. Total lipids (LPD) were according to Cheng et. al, 2011 using cholesterol as the standard. All the analyses were carried out on triplicates and the average value was reported.

Dried subsamples were ashed at 500^0C in a muffle furnace for 5hr to determine the ash percentage (de Lacerda *et al*., 1986). The polysaccharide concentration in leaves was calculated from the values of total carbohydrates (TCHO) and low molecular weight carbohydrates (LWMC).

Polysaccharide concentration= TCHO-LMWC mg/g dw

Also, the calorific values of the mangrove plant parts were determined as follows:

$$\text{Calorific value} = \frac{5.65 \times \text{PRT} + 9.45 \times \text{LPD} + 4.20 \times \text{TCHO}}{100}$$

Where, PRT is the total protein content, LPD is the total lipid concentration and TCHO is the total carbohydrate content expressed in percentage to plant material (Dare and Edwards, 1975).

2.3.4 Total phenolics and flavonoids

The 100mg of air dried, finely ground leaves and bark samples were extracted with 25ml 80% methanol at 55^0C for 2 hours, cooled, made upto the volume, filtered and used for total phenol content and total flavonoid and DPPH free radical scavenging activity analysis.

The total phenolic content (TPC) of the dried plant parts were determined with the Folin-Ciocalteau assay (Atanassova *et al*., 2011). An aliquot (1 ml) of extracts or a standard solution of gallic acid (20, 40, 60, 80 and 100mg/l) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was also prepared using distilled water. 1ml of the 1:1 Folin Ciocalteus phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 mixture. The solution was diluted to 25 ml with distilled water and mixed well. After incubation for 30 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with an UV-VIS double beam Spectrophotometer,

Anelytikajena. The data for the total phenolic contents of mangrove leaves and bark were expressed as milligrams of gallic acid equivalents (GAE) per gram dry mass (mg GAE/g dw). All samples were analysed in duplicates.

Colorimetric aluminum chloride method was used for flavonoid determination (Ghasemi *et al*., 2009). Briefly, 0.5 mL solution of each plant extracts in 80% methanol were separately mixed with 1.5 mL of 80% methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Anelytikajena UV/Visible spectrophotometer. Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 20 to 100 mg ml-1 in methanol.

2.3.5 DPPH free radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was studied (Mensor *et al*., 2001). 100µl of plant extract or standard were taken in different test tubes and 5 ml of reagent solution (0.006 gm of DPPH in 100 ml methanol) was added to each test tube. The test tubes were incubated for 30 minutes to complete the reaction. The absorbance of the solutions were measured at 517nm using a UV/Visible spectrophotometer (Genesis, Anelytikajena) against blank The percentage (%) inhibition activity was calculated from the equation: $[(A_0 – A_1)/A_0] \times 100$. Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard.

2.3.6 Quantification and identification of flavonoid agylcons

The quantitative and qualitative analysis of five food flavonoids, myricetin, quercetin, kaempferol, luteolin and apigenin was carried out using LCUV-MS (Hertog *et al.*, 1992; Chua *et al.*, 2011).

(i) Extraction and Hydrolysis

The extracts for HPLC analysis were prepared as per (Hertog *et al.* 1992). 40 ml of 62.5% aqueous methanol containing 2g/l TBHQ was added to 5mg of air dried sample material. To this extract was added 10ml of 6M HCl with careful mixing. The extraction solution thus consisted of 1.2M HCl in 50% aqueous methanol (v/v). After refluxing at 90^0C for 2hours with regular swirling, the extract was allowed to cool and subsequently made upto 100ml with methanol. Approximately 2ml was filtered through a 0.45µm filter for organic solvents prior to injection.

(ii) Flavonoid standards

The flavonoid standards of myricetin, quercetin, kaempferol, luteolin and apigenin (Sigma Aldrich) were dissolved in methanol to a concentration of 500 mg/l and stored at 4^0C. Standard stock solutions were diluted in 20ml of 62.5% aqueous methanol to which 2g/l of TBHQ was added. To this solution was added 5ml of 6M HCl and the solution was subsequently made up to 50ml with methanol.

(iii) LCUV-MS

Quantification of flavonoids was performed on a Shimadzu Prominent Liquid Chromatograph. Chromatographic separations were performed

on a Supelco C-18 (25cm x 4.6mm, 5μm) column under ambient temperature. The HPLC system was of connected to LC20AD pump. The peaks were detected using the UV-Visible detector SPD-20A at 370nm. The working solutions were injected onto the column which was previously equilibrated with eluent for 60 minutes. The mobile phase consisting of methanol and 0.1% formic acid in low pressure gradient mode (0-5 min, 50% B; 5-11 min, 50-45%B; 11-25 min, 45% B; 25-26 min, 45-50%B, 26-40 min 50% B) with a flow rate of 0.4ml min^{-1} was used.

Liquid chromatography (LC)- Mass spectrometry (MS) analysis was performed with Shimadzu LCMS 2020 connected to Shimadzu Prominent Liquid Chromatograph equipped with ESI source (nitrogen gas flow rate 2.5 l min^{-1}). The mass spectra were acquired from m/z 200–700. All mass spectrometric data were acquired in negative ionisation mode. The detector voltage was maintained at 1.15KV with an interface temperature of 350^0C. The scan speed was 3000 μs^{-1}. Data acquisition and data processing were performed using the software provided by the manufacturer. The scan mode of enhanced mass spectra (EMS) was used to screen the sample profile. Single ion mode was used to determine the characteristic ions and to confirm the presence of agylcon peaks.

The results of reverse phase HPLC chromatogram with detecting at UV- 370 nm showed the released agylcons, namely myricetin, quercetin, luteolin, kaempferol and apigenin in the aqueous methanol mangrove leaves and bark extracts after hydrolysis. The retention time of the agylcons under investigation in the hydrolysed mangrove extracts

were identical those of their corresponding standards under the same conditions. Calibration plots of peak area against concentration for all analytes were obtained by linear regression analysis with an average of at least three data points per concentration (five concentration points).

Peak identity of the samples was confirmed by comparing the spectrum of each peak with the corresponding standard spectrum and the m/z values. Peaks were only quantified if they matched the above mentioned criteria. Quantification was based on peak area.

2.3.7 Extraction and isolation of a flavonoid from R. mucronata

(i) Extraction and separation

The fresh leaves of R. mucronata (3kg) were pulverised and were extracted by maceration in cold 95% methanol (2 l x 3) for 10 days. The extracts were combined and partially evapourated under reduced pressure at 40^0C to remove methanol. The resultant aqueous phase was defatted with 1L hexane (250x4) and the aqueous phase is further extracted with three portions of 1.5L ethyl acetate. After that, the ethyl acetate fraction was concentrated in vacuo, the pooled fraction (4g) was further fractioned using silica column chromatography after adsorbing onto silica.

A portion of the ethyl acetate extract (3.5 g) was presorbed on silica gel and applied on a 150-g silica gel column (3.5 cm diameter X 30 cm length). elution done with chloroform:ethyl acetate mixture (8:2 and 1:1) (Metwally *et al.* 2010).The polarity was increased using 5% increments of methanol upto 20% methanol. Fourty four fractions of 250 ml in each were collected, screened chromatographically using solvent

system chloroform-ethyl acetate-methanol in the ratios (8:2:1) and (8:2:2) and the fractions showing similar separation were combined to get 7 fractions (FA-FG). As the TLC showed single spot for the fraction FF (Fr 20-30), it is washed with ethylacetate and centrifuged to remove the residue. The supernantant was filtered, dried and redissolved in methanol and analysed using HPLC.

(ii) HPLC analysis of fractions

10µl of the fraction was injected to Supelco C-18 (25cm x 4.6mm, 5µm) column of Shimadzu UFLC prominent under ambient temperature, connected to LC20AD pump. The peaks were detected using the UV-Visible detector SPD-20A at 370nm. The mobile phase used consisted of 1:1 methanol: 0.5% phosphoric acid having a pH 2.4 with a flow rate of 1ml/min. According to the results of the HPLC analysis the fraction was subjected to semi-preparative separation using HPLC.

(iii) Semipreparative separation of active components

The fraction FF with ethyl acetate was subjected to semi-preparative separation using preparative Enable C-18G column (25cm x 10mm, 10µm) column under ambient temperature. The Shimadzu UFLC Prominent was of connected to LC20AD pump. The peaks were detected using the UV-Visible detector SPD-20A at two wave lengths 280nm and 370nm. The mobile phase consisting of methanol and water in low pressure gradient mode (0-5 min, 50% B; 5-11 min, 50-45%B; 11-25 min, 45% B; 25-26 min, 45-50%B, 26-40 min 50% B) with a flow rate of 1.5 ml min^{-1} was used. The peaks were collected and MS spectrum was taken.

(iv) LCMS analysis

The Fr1 obtained after semipreparative separation was loaded on to Supelco C-18 (25cm x 4.6mm, 5µm) at ambient temperature attached to MS (Shimadzu LCMS 2020) using the mobile phase consisting of methanol and water in low pressure gradient mode (0-5 min, 50% B; 5-11 min, 50-45%B; 11-25 min, 45% B; 25-26 min, 45-50%B, 26-40 min 50% B) with a flow rate of 0.5 ml min^{-1} . The MS was equipped with ESI source (nitrogen gas flow rate 2.5 l min^{-1}). Working condition was in ESI negative mode.

(v) ^{1}HNMR analysis

^{1}HNMR analysis of the sample in $CDCl_3$ was carried out using 500 MHz NMR spectrometer (Bruker AMX 500).

2.3.8 Extraction and GC analysis of alkanes and fatty acids

(i) Extraction

The method described by Harvey (1994) was selected for the present study. Freeze dried plant tissue samples were extracted by shaking for 72 hrs with a mixture of dichloromethane: methanol (2:1 ratio). The extracts were combined and evaporated to dryness using rotary evaporation. The extracted residue was subjected to mild alkaline hydrolysis using 6% w/v KOH in methanol and refluxed for 4 hrs at 70^0C. The fraction containing neutral organic biomarkers were recovered with n-hexane, which was evaporated and kept for glass column chromatography. The remaining aqueous layer containing the fatty acid salts was acidified to pH 2 by adding 6M HCl and the fatty acids were extracted with dichloromethane.

The polar lipid fraction containing the fatty acids was evaporated to dryness using rotary evaporation and treated with 10 ml of 12% BF3in methanol (Sigma Aldrich), while heating at 70oC for 1hr to form the fatty acid methyl esters (FAMEs). The FAMEs were subsequently partitioned from the reaction solution into10ml of hexane. The hexane layer was evaporated to dryness and the extract was then re-dissolved in n-hexane for gas chromatographic analysis. The neutral fraction obtained by mild alkaline hydrolysis of the total lipid extract was separated into individual compound classes on silica gel viz. saturated hydrocarbons, aromatic hydrocarbons and polar compounds (Otto and Simoneit, 2001). The fractions were eluted with hexane, hexane: dichloromethane (1:1), dichloromethane and methanol, respectively, and dried under nitrogen.

(ii) Gas Chromatographic Analysis

a) Alkanes

The hydrocarbon fraction eluted with n-hexane using silica gel column was evaporated to 1ml under ultrahigh purity N2 prior to concentration determination on a Gas Chromatograph (Perkin Elmer Clarus GC 620) equipped with Flame Ionisation Detector (GC-FID) with a 30m×0.25mm i.d. DB-5 column, 0.25 µm film thickness. Oven temperature was held at 50°C for 5 min and then increased to 300°C at a rate of 3°C per min and held for 5 min. The injector temperature was kept at 260°C and the detector temperature was maintained at 325°C. Nitrogen was used as carrier gas with a flow rate of 2ml per min. Identification of individual compounds was achieved by comparison of GC retention times with those of standard compounds. Quantification was made based on the calibration with authentic standards of n- alkanes from C_7-C_{40} (Sigma Aldrich).

b) Fatty acids

FAME analysis was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) using a Perkin Elmer Clarus GC 620, with MS detector equipped with a non-polar HP ultra-double-fused silica capillary column (30 m, 0.32mm internal diameter, 0.25µm film thickness). Operating conditions were as follows: ion source of electron voltage 70eV kept at 200°C. Spectra were scanned from 50 to 600 m/z with a scan time of 1.50 s. Initially the temperature was increased from 50°C to 200°C at a rate of 2°C per min and held at 200°C for 5 min. Then the temperature was again increased from 200°C to 280°C at a rate of 10°C per min and held at 280°C for 10min. The detector was held at 290°C and helium was used as carrier gas. Full data acquisition was obtained with the use of MS turbo mass version 5.3.2. Quantification was achieved by calibration of FAMEs standards supplied by Sigma Aldrich (Supelco 37 Component FAME Mix, 18919-1AMP). Sample FAMEs were also injected under the above mentioned conditions and their concentrations were determined from the calibration plot.

2.3.9 Statistical analysis

All data were subjected to statistical analysis wherever necessary using 'Statistical Package for Social Sciences (SPSS), version-13'. One way analysis of variance (ANOVA), correlation analysis and factor analysis were done to find out the inter relations between different parameters. Statistical significance of the observed variations among species and plant parts were checked using ANOVA with species and plant part as source of

variation. Principal component analysis (SPSS 15.0) was done to find out the factors contributing to diversity among the mangrove plants. Factor loadings were considered significant if they were >0.50.

References

AOAC. 1995. Metals In plants. *Official methods of AnalysiJ of AOAC Imemational.* 16 th eds. Vol. J, AOAC International. USA.

Atanassova, M., Georgieva, S., and Ivancheva, K. (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology and Metallurgy*, *46*(1), 81-88.

BeMiller, J. N. (2010). Carbohydrate analysis. In *Food analysis* (pp. 147-177). Springer US.

Bhargava, B.,S., and Raghupathi, H.,B. (2005). Methods of Analysis of soils, Plants, Waters, Fertilizers and Organic Manures, Second edn, edited by Tondon H.L.S., Fertilizer Development and Consultation organization, New Delhi, 87-88.

Cheng, Y. S., Zheng, Y., and VanderGheynst, J. S. (2011). Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. *Lipids*, *46*(1), 95-103.

Chua, L. S., Latiff, N. A., Lee, S. Y., Lee, C. T., Sarmidi, M. R., and Aziz, R. A. (2011). Flavonoids and phenolic acids from *Labisia pumila* (Kacip Fatimah). *Food Chemistry*, *127*(3), 1186-1192.

Dare, P. J., and Edwards, D. B. (1975). Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. *Journal of Experimental Marine Biology and Ecology*, *18*(2), 89-97.

de Lacerda, L. D., Jose, D. V., de Rezende, C. E., Maria Cristina, F., Wasserman, J. C., & Martins, J. C. (1986). Leaf chemical characteristics affecting herbivory in a New World mangrove forest. *Biotropica*, 350-355.

Ghasemi, K., Ghasemi, Y., and Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci*, *22*(3), 277-281.

Harvey, H. R. (1994). Fatty acids and sterols as source markers of organic matter in sediments of the North Carolina continental slope. *Deep Sea Research Part II: Topical Studies in Oceanography*, *41*(4), 783-796.

Hertog, M. G., Hollman, P. C., and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of agricultural and food chemistry*, *40*(12), 2379-2383.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.

Meera, S., and Nandan, S. B. (2010). Water quality status and Primary productivity of Valanthakad Backwater in Kerala. *Indian Journal of Marine Sciences*, *39*(1), 105-113.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., and Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, *15*(2), 127-130.

Metwally, A. M., Omar, A. A., Harraz, F. M., and El Sohafy, S. M. (2010). Phytochemical investigation and antimicrobial activity of Psidium guajava L. leaves. *Pharmacognosy magazine*, *6*(23), 212-218.

Otto, A., and Simoneit, B. R. (2001). Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation, Saxony, Germany. *Geochimica et Cosmochimica Acta*, *65*(20), 3505-3527.

Popp, M. (1984). Chemical composition of Australian mangroves II. Low molecular weight carbohydrates. *Zeitschrift für Pflanzenphysiologie*, *113*(5), 411-421.

Ratheesh Kumar, C.S.(2011). Triterpenoids as biomarkers of mangrove organicmMatter in Cochin estuarine system, *PhD Thesis*, Cochin University of Science and Technology, Kochi, India.

Rini, S., 2002. Some biogenic compounds and their derivatives in selected mangrove ecosystems, *Ph.D. Thesis*, Cochin University of Science and Technology, Kochi, India.

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Chapter 3

MACRO AND MICRONUTRIENTS

Contents

3.1 Introduction
3.2 Results
3.3 Discussion
3.4 PCA and chemotaxonomic significance
3.5 Conclusions

3.1 Introduction

Plants take up and accumulate mineral nutrients in their tissue from soil solution and also from air in some exceptional cases. They also release some nutrients back into the surrounding medium. The major fraction of the fresh weight of living plant organs, i.e. those displaying an active metabolism, consists on average of 85-90% water. The dry substance of the plant body is mainly composed of the following elements: carbon (44.5 %), oxygen (42. %), hydrogen (6.5 %), nitrogen (2.5%), phosphorus (0.2%), sulphur (0.3%) and the alkali and alkaline-earth metals potassium (1.9%), calcium (1.0 %) and magnesium (0.2 %) (Markert, 1992). On the basis of their higher concentration in the plant body the nine elements mentioned above are also termed macroelements. In addition, there are also so-called microelements present in the plant organism in lower concentrations and vital for most plants. These elements are chlorine (2000 mg/kg dry

substance), silicon (1000 mg/kg), manganese (200 mg/kg), sodium (150 mg/kg), iron (150 mg/ kg), zinc (50 mg/kg), boron (40 mg/kg), copper (10 mg/kg), chromium (1.5 mg/kg), molybdenum (0.5 mg/kg) and cobalt (0.2 mg/kg) (Markert,1992). Both macro and microelements are plant nutrients vital for the growth and normal development of the plant and their function cannot be replaced by any other element. They are thus essential. Macro and microelements are therefore also known as macro and micronutrients.

Apart from the macro and micronutrients discussed above, a number of further chemical elements also occur in plants (Adriano 2001; Bodeck *et al*., 1988; Hamilton 1979 and 1980; Caroli *et al*., 1989). The elements fluorine, iodine, nickel, selenium, tin and vanadium are already regarded as essential for animal organisms. Further elements are under discussion including some which until recently were only considered from a toxicological point of view (e.g. cadmium and lead). There are currently indications that in a correspondingly low concentration these elements exercise metabolic functions in living organisms (Markert, 1992). Due to specific site conditions, element or organism specific accumulation processes frequently occur: sodium, bromine and chlorine are accumulated by many halophytes (Markert and Jayasekera 1987); copper, nickel, zinc, lead, cadmium and other heavy metals are taken up by metallophytes to an increased extent (Ernst 1974; Ernst and Joosse-Van Damme 1983). Admittedly, an accumulation is not to be equated with an increased physiological benefit from the element for the organism, indeed this probably often purely represents an adaptation to the respective site (Market 1992). The accumulation of nutrients by the plant is referred to as net uptake and is based on both influx and efflux. Nutrients, thus taken up by the plants are translocated to other plant parts. Also, plants

are able to take up elements via leaf surface both through stomata (gases) and through the cuticle (ions) (Marschner, 2012). With a defined nutrient, the uptake by plants depends on the reserves of the nutrient in the uptake medium and its availability.

Mangroves acquire a range of features which make them uniquely adaptable to their stressful environment, they are halophytic or salt tolerant, have aerial roots for gathering oxygen and seeds that geminate on the tree. Mangroves like other plants depend on the photosynthetic reduction of carbondioxide to form carbohydrates and other organic constituents necessary for growth and maintenance. They are known to synthesise more polyphenols and tannins in response to salinity and organic acid metabolism which vary with different species (Basak *et al*., 1996). Mangroves which grow in the saline environment must regulate ion uptake so as to maintain turgor, but at the same time protect sensitive metabolic sites from ion stress. They accumulate high concentration of inorganic ions which apparently function in osmoregulation of leaves and other tissues (Popp, 1984). The osmotic adjustment within the cells is apparently achieved by synthesis of compatible solutes, low molecular weight organic compounds which do not interfere with metabolism (Popp 1984; Popp *et al*., 1984). Different taxa have different mechanisms for coping with high salt concentrations.

Mangrove trees have more energy content than the other tropical rainforest trees (Golley,1969). Differences in elemental composition in plants can arise from numerous sources including environmental variation, biological and environmental changes, as well as environment- organism interactions (Garten *et al*., 1977). The mineral metabolism in mangroves is

also known to be influenced by many factors, such as soil, water and climatic conditions (Kotmire and Bhosale, 1979).

Eventhough the *Rhizophoraceae* mangroves have proven potentials to use as source of novel compounds for food, pharmaceutical and agricultural use, there is a gap of information towards the chemistry of *Rhizophoraceae* mangroves from Kerala. In this perspective, the basic knowledge of the elemental, mineral, isotopic, and biochemical compositions are of much importance towards asserting the toxic and non- toxic limits. These data can also form a firm base for their use in biomarker and chemotaxonomic studies as well as for the better management of the existing mangrove ecosystem. This chapter is an attempt to characterise the *Rhizophoraceae* mangroves found in Kerala in terms of the elemental, carbon and nitrogen isotopic and biochemical composition including phenolic content present in their leaves and bark.

3.2 Results

The concentration of micro and macronutrient elements along with the isotopic composition of the leaves and bark of mangrove plants are detailed in Tables 3.1 and 3.2.

3.2.1 Macronutrient elements

The macronutrient elements considered for the present study are C, H, N, S, P, Ka and Mg. The carbon content in the mangrove plants was observed in the range from 40.68% (*B. cylindrica* leaves) to 46.33% (*K. candel* bark). In the leaves of the mangrove plants under investigation, the carbon varied from 40.68% (*B. cylindrica*) to 45.02 (*K. candel*). The barks showed higher carbon content than the leaves of a species. The carbon

content in the barks of mangrove was observed to be maximum in the bark of *K. candel* (46.33%) and minimum value was observed in the bark of *B. cylindrica* (42.90%). The hydrogen in the mangrove plant parts varied from 5.54% (*R. mucronata* bark) to 7.65% (*B. cylindrica* leaves). The leaves of the mangrove plants showed hydrogen content in the range from 5.83% (*R. apiculata*) to 7.65% (*B. cylindrica*) while the bark showed values in the range from 5.54% (*R. mucronata*) to 6.58% (*R. apiculata*). The leaves of *B. gymnorrizha* showed the highest sulphur content (0.85%) while the lowest value for sulphur was obtained for the bark of *K. candel* (0.21%). The leaves of the mangroves showed a concentration in the range 0.48% (*K. candel*) to 0.85% (*B. gymnorrhizha*). In the barks of the mangroves lower concentrations of sulphur was observed. In the barks, its value ranged from 0.21% (*K. candel*) to 0.56% (*R. mucronata*).

The nitrogen content in the mangrove plant parts ranged from 0.70% (*R. mucronata* bark) to 1.9% (*B. cylindrica* leaves). Among the leaves alone, it varied from 1.29% (*R. mucronata*) to 1.9% (*B. cylindrica*) while in bark samples, the lowest value observed was 0.70% (*R. mucronata*). The bark of *B. cylindrica* was found to be the bark having highest nitrogen content (1.23%). Fig. 3.1 describes the distribution of phosphorus in the leaves and barks of mangrove plants. The phosphorus concentration in the present study falls in the range from 0.37±0.01 mg g^{-1} (*R. mucronata* bark) 1.39±0.01 mg g^{-1} (*K. candel* leaves). The phosphorus content was observed in the range 0.70±0.02 mg g^{-1} (*R. mucronata*) to 1.39±0.01 mg g^{-1} (*K. candel*) in the leaves of the mangroves while the bark showed concentrations from 0.37±0.01 mg g^{-1} (*R. mucronata*) to 1.15±0.04 mg g^{-1} (*B. cylindrica*).

Table 3.1 C, H, N, S and Isotopic composition of *Rhizophoraceae* mangroves

Plant species	Plant part	Carbon %	Hydrogen %	Nitrogen %	Suplur %	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
B. cylindrica	**Leaves**	40.68	7.65	1.90	0.72	-31.48	5.47
	Bark	42.90	6.44	1.23	0.40	-31.37	5.11
B. gymnorrizha	**Leaves**	44.30	7.00	1.35	0.85	-31.96	4.56
	Bark	43.71	6.50	0.81	0.51	-32.02	3.65
K. candel	**Leaves**	45.02	6.73	1.73	0.48	-29.32	5.98
	Bark	46.33	6.41	0.83	0.21	-29.75	5.00
R. apiculata	**Leaves**	41.31	6.98	1.67	0.58	-31.57	4.47
	Bark	44.90	6.58	0.82	0.31	-32.52	2.79
R. mucronata	**Leaves**	43.44	5.83	1.29	0.80	-30.49	4.85
	Bark	46.02	5.53	0.70	0.56	-30.56	3.69
Anova p- value	**Species wise**	0.122	0.038	0.039	0.001	0.004	0.019
	Part wise	0.061	0.033	0.001	0.000	0.213	0.009

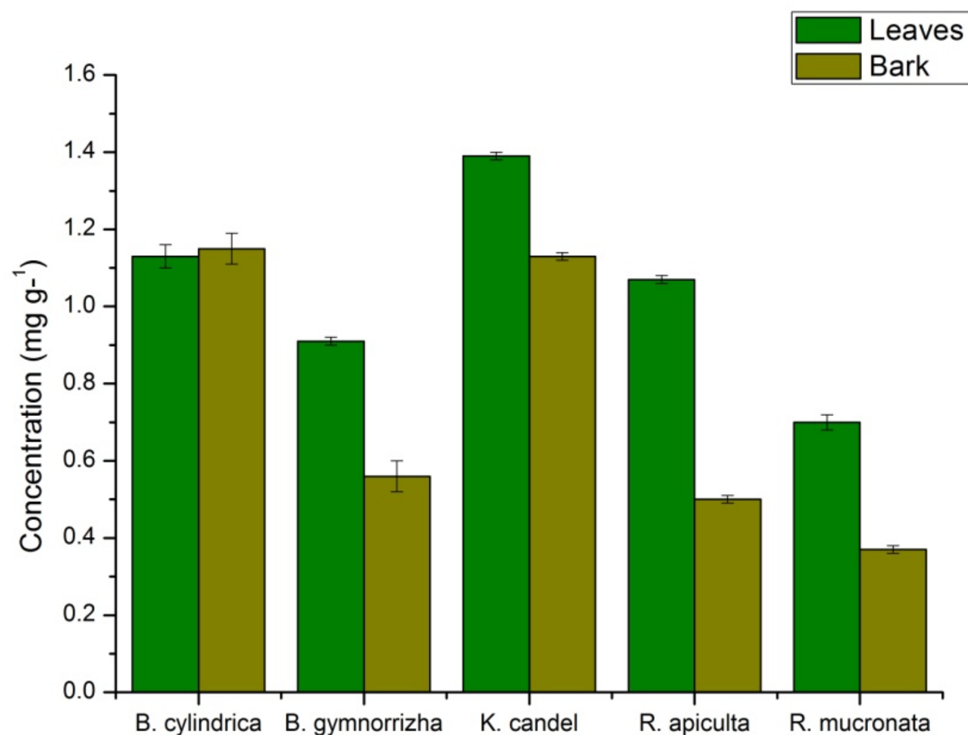

Fig. 3.1 Variation of phosphorus content in the leaves and bark *Rhizophoraceae* mangroves

The concentration of the macronutrient element (Table 3.2), potassium in the mangrove plant parts varied from 3.5±0.14 mg g^{-1}(*R. mucronata* bark) to 16.5±0.28 mg g^{-1}(*R. apiculata* leaves). Potassium concentration in the leaves ranged from 4.5±0.13 mg g^{-1}(*B. gymnorrizha*) to 16.5±0.28 mg g^{-1} (*R. apiculata*). The barks exhibited lower potassium content than the leaves and it ranged from 3.5±0.14 mg g^{-1} (*R. mucronata*) and a higher concentration of 8.50±0.07 mg g^{-1}(*K. candel*).

Table 3.2 Mineral composition in the leaves and bark of *Rhizophoraceae* mangroves along with ANOVA results

Elements	*B. cylindrica*		*B. gymnorrizha*		*K. candel*		*R. apiculata*		*R. mucronata*		Anova p- value	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Species wise	Part wise
Na ($mg\ g^{-1}$)	36±0.49	21.5±0.76	22.5±0.74	56.5±1.20	18.5±0.86	12±1.12	23±0.81	14±1.12	21±1.53	17±1.85	0.482	1.000
K ($mg\ g^{-1}$)	6.5±0.23	4.5±0.20	4.5±0.13	7±0.17	16.5±0.21	5±0.20	16.5±0.28	5±0.28	10.5±0.41	3.5±0.14	0.615	0.097
Mg ($mg\ g^{-1}$)	5.675±0.04	2.095±0.05	3.04±0.07	3.065±0.02	3.415±0.12	2.63±0.07	6.995±0.18	1.045±0.07	4.41±0.07	1.91±0.14	0.944	0.073
Fe ($mg\ kg^{-1}$)	65.65±0.83	159.75±0.35	1315±1.41	1680±0.78	211.30±0.57	58.40±0.55	50±0.14	91.30±1.07	112.70±0.28	2660±4.24	0.305	0.310
Cu ($mg\ kg^{-1}$)	4.7±0.18	3.8±0.03	2.45±0.06	2.75±0.11	4.7±0.07	4.9±0.16	2.6±0.11	2.9±0.14	3.25±0.04	3.5±0.16	0.014	0.904
Zn ($mg\ kg^{-1}$)	13.5±0.58	11±0.28	6.5±0.21	5.6±0.14	14.85±0.08	17.6±0.21	6.35±0.03	5.15±0.03	3.4±0.14	2.8±0.13	0.003	0.604
Mn ($mg\ kg^{-1}$)	55.85±1.23	32.2±0.42	51.25±0.47	15.5±0.42	235±1.41	110±1.51	94±0.28	ND	29.1±0.57	41.35±.54	0.084	0.098
Co ($mg\ kg^{-1}$)	5.425±0.11	1.225±.01	3.575±0.05	0.875±0.01	5.225±0.02	4.675±0.02	5.175±0.08	1.475±0.02	4.375±0.09	2.325±0.02	0.286	0.015
Ni ($mg\ kg^{-1}$)	6.925±0.11	8.8±0.17	3.15±0.08	8.45±0.07	4.825±0.16	2±0.06	4.675±0.12	6.5±0.10	4.4±0.07	4.1±0.10	0.409	0.431
Pb ($mg\ kg^{-1}$)	2±0.14	1.5±0.06	4±0.14	3±0.05	4.5±0.10	1±0.03	6±0.16	1.5±0.07	1±0.04	2.5±0.07	0.677	0.212
Cd ($mg\ kg^{-1}$)	0.1±0.003	ND	ND	0.4±0.004	0.55±0.014	ND	0.75±0.028	0.45±0.014	ND	ND	0.293	0.526

Results given as (concentration ± standard deviation)

Another macronutrient element, magnesium is one of the major macronutrient elements present in plants. The leaves of *R. apiculata* showed the highest mangnesium concentration (6.99±0.18 mg kg^{-1}) while its bark exhibited the lowest Mg content (1.05±0.07 mg kg^{-1}) among the plant components of this study. In the leaf samples, it ranged from 3.04±0.07 mg kg^{-1} (*B. gymnorrizha*) to 6.99±0.18 mg kg^{-1} (*R. apiculta*). In the barks of mangrove plants under investigation the concentration of magnesium ranged from 1.05±0.07 mg kg^{-1} (*R. apicult*a) to 3.07±0.02 mg kg^{-1} (*B. gymnorrizha*).

3.2.2 Micronutrient elements

The micronutrient elements analysed were Na, Fe, Cu, Zn, Mn and Co in the leaves and bark of the mangroves (Table 3.2). Sodium was found to be present in the leaves and bark in the range, 12±1.12 mg g^{-1} (*K. candel* bark) to 56.5±1.20 mg g^{-1}(*B. gymnorrizha* bark). In the leaves the highest sodium content was observed for *B. cylindrica* (36±0.49 mg g^{-1}) and lowest for *K. candel* leaves (18.5±0.86 mg g^{-1}). Among the bark samples investigated, *K. candel* exhibited lowest sodium accrual while *B. gymnorrizha* showed the highest (56.5±1.20 mg g^{-1}).

In the leaves and bark of the mangrove plants high concentrations of iron was found. It ranged from 2660±4.24 mg kg^{-1} (*R. mucronata* bark) to 50±0.14 mg kg^{-1} (*R. apiculata* leaves). Barks were found to accumulate more Fe than the leaves. In the bark samples it ranged from 58.40±0.55 mg kg^{-1} to 2660±4.24 mg kg^{-1} while the leaves showed iron concentration in the range 50±0.14 mg kg^{-1} to 1315±1.41 mg kg^{-1}. The presence of copper was detected in the range 2.45±0.06 mg kg^{-1} (*K. candel* bark) to 4.9±0.16mg kg^{-1} (*B. gymnorrizha* leaves). The leaves and bark exhibited a more or less

similar range of copper concentration; from 2.45±0.06 mg kg^{-1} (*B. gymnorrizha*) to 4.7±0.18 mg kg^{-1} (*B. cylindrica*) in the leaves and from 2.75±0.11 mg kg^{-1} (*B. gymnorrizha*) to 4.9±0.16 mg kg^{-1} (*K. candel*) in the bark. The distribution of zinc in the *Rhizophoraceae* mangrove plants under investigation was found to be in the range 2.8±0.13 mg kg^{-1} (*K. candel* bark) to 17.6±0.21 mg kg^{-1} (*R. mucronata* bark). The leaves showed a highest concentration of 14.85±0.08 mg kg^{-1} in K. *candel* while the lowest was found to be 3.4±0.14 mg kg^{-1} in *R.mucronata*. Among the bark samples, zinc concentration was found to be highest in *K. candel* (17.6±0.21 mg kg^{-1}) and lowest in *R. mucronata* (2.8±0.13 mg kg^{-1}).

The manganese concentration in the mangrove plants were observed between 15.5±0.42 mg kg^{-1} (*B. gymnorrizha* bark) and 235±1.41 mg kg^{-1} (*K. candel* leaves). In the leaves of the *Rhizophoraceae* mangroves the highest concentration was observed in *K. candel* (235±1.41mg kg^{-1}) while the lowest level was observed in *R. mucronata* (29.1±0.57 mg kg^{-1}). In the barks, it ranged from 110±1.51 mg kg^{-1} (*K. candel*) to 15.5±0.42 mg kg^{-1} (*B.gymnorrhizha*). It was observed that the cobalt concentration varied between 0.875±0.01 (*B. gymnorrizha* bark) to 5.425±0.1 (*B. cylindrica* leaves). Among the leaf samples the highest cobalt concentration was observed in the leaves of *B. gymnorrizha* (3.575±0.05) and the lowest was found in *B. cylindrica* leaves. The bark samples under present investigation showed a minimum cobalt concentration of 0.875±0.01 in the bark of *B. gymnorrizha* and a maximum concentration 4.675±0.02 in the bark of *K. candel*.

The two elements, Pb and Cd were also considered in the present study. Lead content was detected in the mangrove plant parts under investigation. The value ranges from 1±0.03mg/kg (*K. candel* bark) and 1±0.04 (*R. mucronata* leaves) to 6±0.16mg/kg (*R. apiculata* leaves). Among the leaves of mangrove plants under investigation *R. mucronata* leaves showed the lowest lead content (1±0.04 mg/kg) while the leaves of *R. apiculata* were found to have highest lead content (6±0.16 mg/kg). For the bark samples, the lead was found to be more concentrated in the bark of *B. gymnorrhizha* (3±0.05 mg/kg) while the *K. candel* bark accumulated lowest lead concentration (1±0.03 mg/kg). Low levels of cadmium were detected in a few samples. It ranged from below the level of machine detection in a few samples to 0.75±0.028 mg/kg (*R. apiculata* leaves). Cadmium was found to be present in the leaves of *B. cylindrica*, *K. candel* and *R. apiculata* as well as in the bark tissues of *B. gymnorrizha* and *R. apiculata*.

3.2.3 Isotopic composition

The values of $\delta^{13}C$ ranged from -32.52‰ (*R. apiculata* bark) to -29.32 ‰ (*K. candel* leaves) among the various plants of this study (Table 3.1). In the leaves, the maximum enrichment was found to be -29.32 ‰ (*K. candel)* and the most depleted was the leaves of *B. gymnorrizha* (-31.96‰). The bark showed a lowest value of $\delta^{13}C$ in *R. apiculata* (-32.52‰) and the enrichment showed a maximum of -29.75‰ in *K. candel*.

For $\delta^{15}N$, the maximum was found to be 5.98 (*K. candel* leaves) and the minimum was found to be 2.79 (*R. apiculata* bark). In the leaves, the observed range was from 4.47 (*R. apiculata*) to 5.98 (*K. candel*). The bark

samples showed $\delta^{15}N$ in the range from 2.79 (*R. apiculata*) to 5.47 (*B. cylindrica*).

3.2.4 Biochemical composition

The results of total carbohydrates (TCHO), Low molecular weight carbohydrates (LMWC), polysaccharides (PS), Total proteins (PRT), Total lipids (LPD) present in the leaves and bark in different mangrove plants under investigation along with their calorific value and ash content are tabulated in Table 3.3.

The concentration of total carbohydrates ranged from 124.55±2.09 mg g^{-1} (*R. apiculata* bark) to 198.17±3.03 mg g^{-1} (*B. cylindrica* bark). In the leaves of the mangroves, the value of TCHO varied between 125.90±1.04 mg g^{-1} (*B. gymnorrizha*) and 160.74±1.08 mg g^{-1} (*K. candel*) while the bark of the mangrove plants showed a variation from 124.55±2.09mg g^{-1} (*R. apiculata*) to 198.17±3.03 mg g^{-1} (*B. cylindrica*). Among the species under investigation, the LMWC concentrations varied from 52.67±2.03 mg g^{-1} (*B. gymnorrizha* leaves) to 177.82±2.55 mg g^{-1} (*K. candel* bark). The leaves of the mangrove plants showed LMWC in the range 52.67±2.03 mg g^{-1} (*B. gymnorrizha*) to 128.46±1.47mg g^{-1} (*R. mucronata*) whereas in the bark samples it ranged from 56.88±1.69mg g^{-1} (*B. gymnorrizha*) to 177.82±2.55mg g^{-1} (*K. candel*). The polysaccharide concentrations in the mangrove plants ranged between 17.36±0.19 mg g^{-1} (*K. candel* bark) and 112.55±2.06 mg g^{-1} (*B. cylindrica* bark). It varied between concentrations 29.49±0.75 mg g^{-1} (*R. mucronata*) and 83.40±1.86mg g^{-1} (*B. cylindrica*) in the leaves of the plants while the bark samples exhibited a concentration range between 17.36±0.19 mg g^{-1} (*K. candel*) and 112.56±2.06 mg g^{-1} (*B. cylindrica*).

Table 3.3 Biochemical compositions, ash% and leaf water content of *Rhizophoraceae* mangroves

Plant and Parts		Carbohydrates (mg g^{-1})	LMWC (mg g^{-1})	Polysaccharides (mg g^{-1})	Lipids (mg g^{-1})	Protein (mg g^{-1})	Calorific value (K Cal g^{-1})	Ash %	PRT/ CHO	LPD/ CHO	Leaf water content (%)
B. cylindrica	Leaves	152.80±0.37	68.09±2.22	83.40±1.86	39.28±0.43	13.33±0.49	1.09±0.13	13.48±0.70	0.09	0.26	77.29
	Bark	198.17±3.03	84.15±0.96	112.56±2.06	31.35±0.98	15.48±0.98	1.22±0.05	8.82±0.43	0.08	0.16	-
B. gymnorrizha	Leaves	125.9±1.04	52.67±2.03	74.13±1.27	48.01±0.48	17.14±1.29	1.08±0.02	9.08±1.28	0.14	0.38	73.43
	Bark	153.98±0.84	56.88±1.69	96.50±0.85	21.16±0.56	15.83±0.20	0.94±0.01	8.39±0.63	0.10	0.14	-
K. candel	Leaves	160.74±1.08	80.07±1.44	78.88±2.52	56.08±0.62	15.93±0.14	1.30±0.03	9.00±0.67	0.10	0.35	72.16
	Bark	194.05±0.95	177.82±2.55	17.36±.19	59.01±1.44	34.27±2.46	1.57±0.06	8.50±0.79	0.18	0.30	-
R. apiculata	Leaves	147.44±2.04	76.77±1.30	68.30±3.35	45.64±2.12	9.35±0.99	1.10±0.10	10.5±0.71	0.06	0.31	73.29
	Bark	124.55±2.09	84.2±2.07	40.36±1.2	17.47±1.12	21.81±0.80	0.81±0.01	8.90±1.17	0.18	0.14	-
R. mucronata	Leaves	157.42±2.22	128.46±1.47	29.49±0.75	45.19±1.69	15.95±1.07	1.18±0.07	8.89±1.10	0.10	0.29	73.29
	Bark	146.98±2.12	115.54±2.20	34.5±0.97	37.39±1.67	19.67±0.80	1.08±0.05	6.75±0.96	0.13	0.25	

The total lipid concentrations (LPD) among the mangrove plants varied from 17.47±1.12 mg g^{-1} (*R. apiculta* bark) to 59.01±1.44 mg g^{-1} (*K. candel* bark). In the leaves of the mangroves it ranged from 39.28 ± 0.43mg g^{-1} (*B. cylindrica*) to 56.08±0.62 mg g^{-1} (*K. candel*) whereas in the bark samples the its value ranged between 17.47±1.12 mg g^{-1} (*R. apiculata*) and 59.01±1.44 mg g^{-1} (*K. candel*). The total protein content (PRT) of the mangrove plants showed higher concentration in the leaves. The PRT varied from 9.35±0.99 mg g^{-1} (*R. apiculata* leaves) to 34.27±2.46 mg g^{-1} (*K. candel* bark). If the leaves samples alone is considered, the leaves of *R. apiculata* contained lowest protein content (9.35±0.99 mg g^{-1}) while the leaves of *B. gymnorrizha* showed highest PRT (17.14±1.29mg g^{-1}) . Among the barks, the bark of *K. candel* exhibited highest PRT (34.27±2.46 mg g^{-1}).

Using TCHO, PRT and LPD, the calorific value of the plant components were also calculated. The calorific value of the mangrove plants varied between 0.81 K Cal g-1 (*R. apiculata* bark) to 1.57 K Cal g-1 (*K. candel* bark). The leaves showed calorific values in the range 1.08±0.02 K Cal g^{-1} (*B. gymnorrizha*) to 1.30±0.03 K Cal g^{-1}(*K. candel*). The bark of the mangrove plants exhibited calorific value in the range 0.81±0.01K Cal g^{-1} (*R. apiculata*) to 1.57K Cal g^{-1}±0.06 (*K. candel*). The leaf water content varied in the range 72.16 (*K. candel*) to 77.29% (*B. cylindrica*). The ash content of the species varied between 6.75 % (*R. mucronata* bark) and 13.48% (*B. cylindrica* leaves). In the leaves of the mangrove plants it showed a variation from 8.9% (*R. mucronata*) to 13.48% (*B. cylindrica*). The bark samples exhibited ash content in the range 6.75% (*R. mucronata*) to 8.39% (*B. gymnorrizha*).

3.2.5 Total Phenol content and DPPH radical scavenging activity

The total phenol content (TPC) and the DPPH radical scavenging activity of the leaves and bark are depicted in Table 3.3. It was the bark samples exhibited high phenolic content as well as DPPH radical scavenging activity comparing to the leaves. In the bark samples the TPC ranged between 36.85±0.97 (*B. cylindrica*) to 91.36±2.89 (*R.mucronata*) whereas in leaves the lowest value observed was 129.29±2.76 (*R.apiculata*) and the highest was 74.95±1.02 (*R.mucronata*). The DPPH radical scavenging activity was found to be highest for the barks of Rhizophora mangroves; 96.11% and 95.68% for *R. apiculata* and *R. mucronata* respectively while the lowest DPPH activity was obtained for the leaves of *B. cylindrica.* In the leaves the radical scavenging activity ranged from 25.87 % (*B. cylindrica*) to a maximum of 95.95 (*R. mucronata*) whereas among the bark specimens, the percentage DPPH activity was obtained in the range 96.11% (*R. apiculata*) to 62.15% (*K. candel*).

Table 3.4 Total Phenolic content and DPPH radical scavenging activity of *Rhizophoraceae* mangroves

Plant name	Part examined	Total Phenolics (mg GAE g^{-1})	% DPPH activity
B. cylindrica	Leaves	20.23± 0.78	25.87
	Bark	36.85±0.971	82.40
B. gymnorrhizha	Leaves	45.12±0.99	87.37
	Bark	37.02±0.76	93.67
K. candel	Leaves	35.67±0.69	95.53
	Bark	129.29±2.76	62.15
R. apiculata	Leaves	11.47±0.08	31.99
	Bark	56.00±0.96	96.11
R.mucronata	Leaves	74.95±1.02	95.95
	Bark	87.265±1.53	95.68

3.3 Discussions

3.3.1 C, H, N, S, P composition

The global storage of carbon (C) in mangrove biomass is estimated to be 4.03 Pg C. The average rate of wood production is 12.08 Mg $ha^{-1}yr^{-1}$, which is equivalent to a global estimate of 0.16 Pg C yr^{-1} stored in mangrove biomass (Patil et. al., 2012). In the present work, the carbon content in the leaves of the mangrove plants was found to be lower than the corresponding bark samples. *B. cylindrica* showed the lowest carbon content while *K. candel* was found to exhibit highest carbon content among the five *Rhizophoraceae* mangroves under investigation. The carbon content was found to be in the order *B. cylindrica*<*R. apiculata*<*B. gymnorrizha*<*R. mucronata*<K. candel.

As per a pilot study on carbon sequestration of mangrove forest, the average percentage of carbon in leaf samples of mangroves from Mumbai Coast was found to be 36.95% (Patil et. al., 2012) which is lower than the values observed in this study. Mangroves are known to remove CO_2 from the atmosphere through photosynthesis. They fix greater amounts of CO_2 per unit area, than what the phytoplankton do in the tropical oceans (Kathiresan and Bingham, 2001). The mangroves have the capacity to accumulate and store carbon in the soil in large quantities. The present study indicates, the *Rhizophoraceae* mangroves from Kochi, thus contributes significantly to the carbon budget of this geographical location, *K. candel* being the major contributor. Because the mangroves fix and store significant amounts of carbon, their loss may have impact on global carbon budget and global warming.

In the present study, the bark of the mangroves showed comparatively lower percentage of hydrogen than the leaves. The genus *Bruguiera* showed higher hydrogen levels, followed by *Kandelia* and *Rhizophora* mangroves. *R. mucronata* showed lowest concentration of hydrogen in its bark and leaves than other mangrove plants under investigation. Similar to hydrogen levels, the nitrogen content also was observed to be higher in the leaves than their bark. Nitrogen and phosphorus availability influences primary production and growth in mangroves (Clough, 1992). The phosphorus levels was found to comparatively lower than N levels and found to be more concentrated in the leaves than in the bark. The sulphur content was found to least in K. candel leaves (0.48%) and bark (0.21%) while higher concentrations was observed in the leaves and bark of *B. gymnorrizha* (0.85% & 0.51%) and *R. mucronata* (0.80% & 0.56%). As observed for nitrogen and hydrogen, bark specimens showed lower sulphur percentage than the leaves. So it can be concluded that except carbon content, the macronutrient elements, H, N, P and S are more concentrated in the leaves and less in the bark of mangroves while C is more concentrated in their bark. No specific trend was observed according to genre. The elemental composition follows the order C>H>N>P>S.

The terrestrial vascular plants have C/N ratios more than 12 (Meyers 1994; Rumolo *et al*., 2011). The observed C/N ratios for the mangroves are in the range 21.43 to 65.23 (Fig. 3.1a). The basic reason for these higher C/N ratios is simply due to the carbohydrate-rich (e.g., cellulose)/ protein-poor nature (Meyers, 1997) of these plant components. Also, decreased nitrogen invested in leaves and a concomitant increase in the carbon:

nitrogen ratio of plant tissues can be attributed to adaptation of these plants to elevated levels of CO_2.

In this study, the C/N levels (Fig. 3.2a) were found to be higher in the bark samples than the leaves due to high carbon levels and lower N levels The lower C/N in the leaves suggests that the leaves have high nutrient quality compared to bark and significantly higher nutritional quality was observed in *B. cylindrica* leaves than the other mangrove plants in this study. Mfilinge *et al*., 2002 has reported higher N and lower C/N values for K. candel than *B. gymnorrizha* leaves in Okinawa, Japan indicating the higher nutritional quality of the former than latter. The current investigation is consistent with the aforesaid observation.

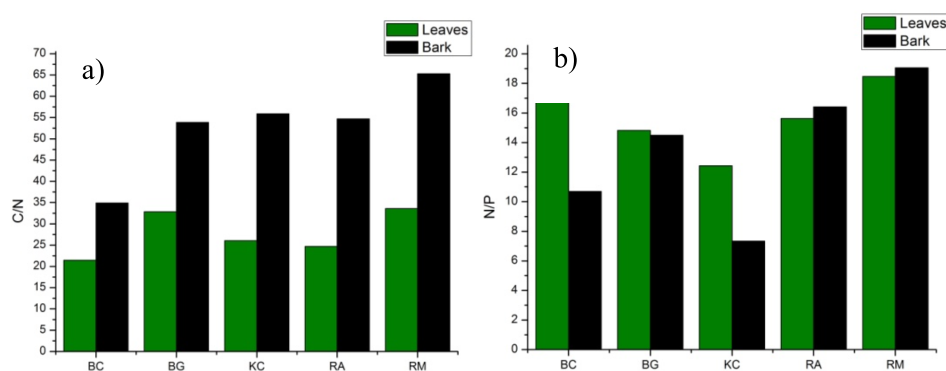

Fig. 3.2 Variation in a) C/N and b) N/P ratios in the leaves and bark *Rhizophoraceae* mangroves

Mangroves appear to be highly plastic in their responses to changes in nutrient availability, achieving high growth rates when nutrient limitations are relieved that are accompanied by associated reductions in nutrient-use efficiency and other nutrient conservation mechanisms (Reef *et al*.,2010).

The ratio N/P in plant tissue has also been used to infer N or P limitations to growth (Güsewell, 2004). Variation in leaf N/P, particularly where N/P is >32 (which is a global average for mangroves; Lovelock *et al*., 2007), indicates that P may limit growth in many mangrove habitats (e.g., Malaysia, Kenya, China, Puerto Rico, Venezuela, Victoria, Australia, Florida and Honduras; reviewed in Lovelock *et al*., 2007). The observed N/ P value for the *Rhizophoraceae* mangroves; 10.68-19.05 (Fig. 3.2b), thus suggest that P is not a limiting factor in the ecosystems of the present study. The lower C/P ratio and hence the enrichment of phosphorous in the mangroves sediments of Puthuvypu (Ratheesh Kumar, 2011) supports this observation.

3.3.2 Isotopic Composition

Carbon and nitrogen stable isotopic data from the primary producers in mangrove ecosystems are needed to investigate trophic links and biogeochemical cycling. Discrimination varies among plants using different photosynthetic pathways. The Calvin cycle (C3), Hatch–Slack cycle (C4) and Crassulacean acid metabolism (CAM) photosynthetic pathways differ so profoundly and so consistently (O'Leary 1981, 1988) that ecologists have used isotopic signatures in large-scale surveys of plant species (Teeri and Stowe 1976; Sage and Monson 1999). Mangroves belong to the group of plants which use C3 photosynthetic pathway. The carbon isotope composition of leaves *Rhizophoraceae* mangrove under the present investigation shows a range from -32.52‰ to-29.32 ‰ which matches well with the all ready established values of $\delta^{13}C$ for C3 plants (Smith and Epstein, 1971; Rao *et al*., 1994; Smallwood *et al*., 2003). The plant components of *K. candel* were enriched in ^{13}C relative to other *Rhizophoraceae* mangroves by 1 to 2.5‰.

The most depleted $\delta^{13}C$ was found in *B. gymnorrizha*. Lower isotopic values have been attributed to the contribution of CO_2 resulting from the decay of organic matter and respiration (Muzuka and Shunula, 2006). Stable carbon isotope composition also varies among plant tissues (O'Leary 1981). Some of this variation is due to differences among the chemical components of plant tissue. But in the present study, the leaves and bark specimens of same mangrove plant showed almost similar values for $\delta^{13}C$ (Fig. 3.3). The variability in $\delta^{13}C$ exhibited by C3 plants is primarily determined by variations in the concentration of CO_2 of the internal leaf space (Farqhuar *et al.*, 1982), primarily determined by the stomatal conductance to CO_2. A number of environmental factors can influence stomatal conductance, including salinity, humidity, soil moisture and temperature, which will subsequently influence stable isotope fractionation. The $\delta^{13}C$ values exhibited by *L. racemosa* from Florida and Belize are comparable to the average value for C3 plants (–27‰) and would therefore seem to show that the *L. racemosa* studied are not experiencing physiological stress that would cause enrichment in ^{13}C (Wooller *et al.*, 2003).

Although the use of natural abundance ratios of N in plants is not as well established as that of C isotope ratios, there is a great deal to be learned from a comparison of $\delta^{15}N$ among plants within an ecosystem, between plants and their source of N, and among plant components. Some key applications using $\delta^{15}N$ in plant tissues include assessing contributions of various N sources to plant N uptake in the field, including symbiotic nitrogen fixation and atmospheric deposition, the role of mycorrhizal infection, uptake of dissolved N, and the interpretation of $\delta^{15}N$ profiles in soils. The $\delta^{15}N$ of plants reflects the net effect of many processes including

the $\delta^{15}N$ of the source N, enzymatic fractionations within a plant, and plant-microbial interactions in soil (Dawson *et al*., 2002). The majority of terrestrial plants have $\delta^{15}N$ near 0‰ in temperate zones, however, different species growing in the same environment have been found to vary by as much as 10‰ (Handley and Scrimgeour, 1997). Mangrove trees, in general, have N isotopic compositions that reflect the overall nutrient status of the ecosystem (Fry *et al*., 2000). The present nitrogen stable isotope results are in accordance with various reported values that are greater than 4‰ and fall within the range of plants that obtain inorganic nitrogen directly from seawater (Chong *et al*., 2001; Bouillon *et al.,* 2002). In this study, the $\delta^{15}N$ is found to be more enriched in the leaves than the bark samples (Fig. 3.3) by an average of 1.02‰. Organ-specific loss of nitrogen, different patterns of nitrogen assimilation, and reallocation of nitrogen can cause intra-plant variation in $\delta^{15}N$. Also, the loss of NH_3 could enrich leaves in $\delta^{15}N$ (Shearer and Kohl, 1986). The positive $\delta^{15}N$ indicates the fertility of the sediments.

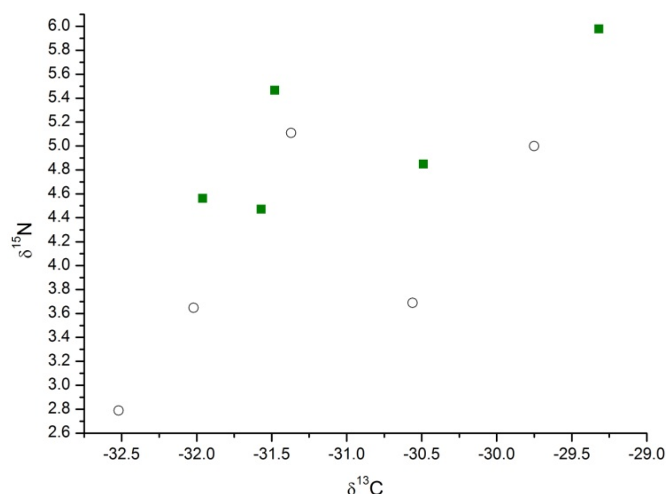

Fig. 3.3 The δ^{13}Cand δ^{15}N in the leaves (■) and bark (○) of *Rhizophoraceae* mangroves

3.3.3 Variations in mineral compositions

The plants belonging to *Rhizophoraceae* family exhibit relatively low concentration of metals due to the salt- exclusion mechanism that is operative in the species (Sarangi *et al*., 2002). These mangrove plants have a well developed ultra filtration mechanism (McMillan, 1974; Scholander, 1968). When wetland plants translocate metals from root tissue to aerial tissue, they are accumulated in leaves and stems. The degree of upward translocation is dependent on the species of the plant, the metal and a number of environmental conditions (Weis and Weis, 2004).

The variations of the macronutrient elements, Na, K and Mg in the leaves and bark of mangrove plants is shown Fig. 3.4. Due to specific site conditions, element- or organism- specific accumulation processes frequently is reported to occur in mangroves leading to the accumulation of minerals like sodium, bromine and chlorine (Markert and Jayasekera, 1987). The presence of sodium in five *Rhizhophoraceae* mangrove plants is being reported in this study. Sodium which is grouped under electrolytic elements (Sansoni and Iyengar, 1978) present in plants is required for the construction of specific physiological potentials and is important for maintain defined osmolytic conditions in the cell metabolism. Except *B. gymnorrizha*, all the other plants studied are found to accumulate sodium in their leaves than in the bark. *Bruguiera* gymnorrizha possessed higher sodium content in the bark (56.5±1.20 mg g^{-1}) than in the leaves (22.5±0.74 mg g^{-1}). The highest sodium content was found in the *bruguiera* plants; *B.cylindrica* and *B. gymnorrizha* having the sum of sodium concentrations in leaves and bark, 57.50 mg g^{-1} and 79 mg g^{-1} respectively. The next level was found in

rhizophora mangrove plants, *R. mucronata* (37 mg g^{-1}) and *R. apiculata* (38 mg g^{-1}) followed by *K. candel* (12 mg g^{-1}).

Potassium, one of the primary nutrients, regulates many metabolic processes required for growth, fruit and seed development. It increases disease tolerance and drought tolerance, regulates opening and closing of stomata. The genus *Rhizophora* as well as *Kandelia* exhibited higher potassium content than the *Bruguiera* plants. Except *B. gymnorrizha*, all the other four plants were found to have potassium levels high in their leaves. In *B. gymnorrizha*, the bark tissue displayed higher potassium content than the leaves. The leaves of *Rhizophora* mangroves possessed three times the amount potassium than the bark. *Rhizophora* species are salt- tolerant and can normally exist in soil salinities as high as 60‰ (Cintron *et al.*, 1978).

The salt tolerance results from the ability of the root membranes to exclude salts by developing a negative pressure in the plant conductive tissue due to leaf transpiration, a type of reverse osmosis process (Scholander *et al.*, 1965). Red mangroves exclude ions by this process (Scholander, 1968). It was shown that the this process was not an active transport mechanisms because electron transport uncoupling agents such as 2,4 dinitrophenol do not interfere with salt exclusion in *Rhizophora*. In a healthy tree with salt excluding capacity, the ratio Na/K would be smaller than in a tree unable to exclude salt effectively. The values of Na/K in the leaves of mangrove plants under the present study for *B. cylindrica*, *B. gymnorrizha*, *K. candel*, *R. apiculata* and *R. mucronata* are 5.54, 5, 1.2, 3.3 and 2 respectively indicating that the salt exclusion mechanism is efficiently operative in *K. candel* followed by *Rhizophora* species. *Bruguiera* showing highest values

of Na/K can be thus concluded to be the plants with least salt excluding capacity among the *Rhizophoraceae* plants under investigation. The values of Na/K given by Kotmire and Bhosale, 1979 for *R. mucronata* (4.5) and *B. gymnorrhizha* (14.1) also demonstrate that the former is better salt excluding species than latter. Page *et al*., 1985 has demonstrated that the Na/K ratio of leaf tissue is a readily measured and potentially useful sublethal indicator of oil stress for *Rhizophora* and for other salt excluding halophytes.

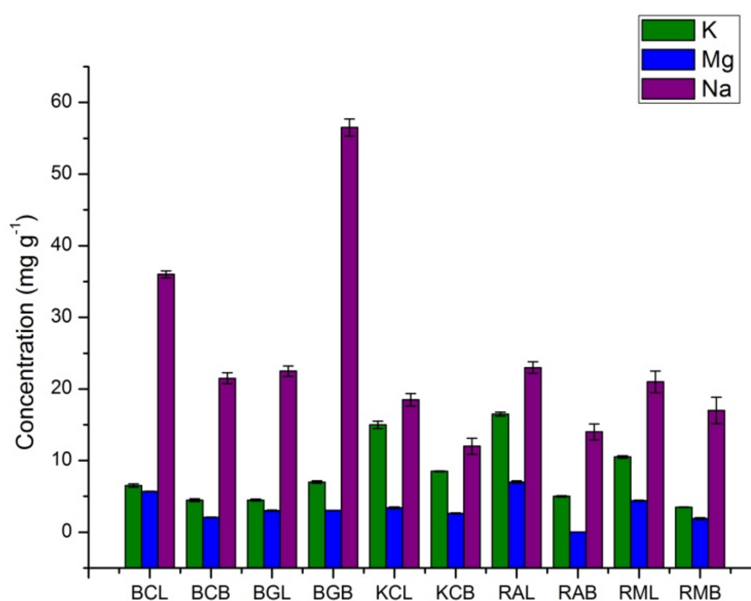

Fig. 3.4 Variations of Na, K, Mg in the mangrove leaves and bark

In general, halophytes have lower K/Na ratios (< 1.0) while glycophytes have higher K/Na ratios (>1.0) (Albert and Popp, 1977; O'Leary and Glenn, 1984). The K/Na values obtained in this study also fall in the aforementioned range for halophytes, ie; 0.15 for *B. cylindrica*, 0.22 for *B. gymnorrizha*, 0.066 for *K. candel*, 0.06 for *R. apiculata* and 0.10 for *R. mucronata*. Naidoo *et al.* (2002) found that true mangroves *A. marina*

and *Bruguiera gymnorrhiza* kept lower K/Na ratios in their leaves (0.1~ 0.25). This lower K/Na ratio is not attributed to the reduction in K+ uptake but apparently to the increase in Na+ uptake (Downton 1982; Parida *et al*., 2004). Maintenance of efficient uptake of K under higher saline habitats is a key feature of salt tolerance (Glenn *et al*., 1994; Niu *et al*., 1995).

Magnesium is a constituent of the chlorophyll molecule, which is the driving force of photosynthesis. It is also essential for the metabolism of carbohydrates (sugars). It is an enzyme activator in the symthesis of nucleic acids (DNA and RNA). It regulates uptake of the other essential elements, serves as a carrier of phosphate compounds throughout the plant, facilitates the translocation of carbohydrates (sugars and starches), and enhances the production of oils and fats (Wilkinson *et al*., 1990). In this work, among the five mangrove plants of the family *Rhizophoraceae, R. apiculata* leaves were found to be rich in magnesium content. At the same time its bark displayed the lowest Mg concentration. In *B. gymnorrizha*, it was observed that the Mg is uniformly distributed among the leaves and the bark. In rest of the plants leaves accumulate more Mg than their bark. In the *Rhizophora* mangroves studied, the difference observed in magnesium concentration between the leaves and the bark is very large when compared to the other genre studied, ie; *Bruguiera* and *Kandelia*.

The composition of micronutrient elements is depicted in Fig. 3.5. Iron is an essential micronutrient element for chlorophyll synthesis in plants and plants could not complete its survival cycles without it. But high density of iron in plant has toxicity effects. Its recommended value in plant tissue is about 50-500 mg/kg (Pendias and Pendias, 1994). However,

the results of the present study show considerably higher values of iron in the leaves and bark of the mangroves. Except *K. candel* all other plants show iron concentration more in the bark than in leaves. In case of *K. candel* the leaves are found to be iron rich than its bark. Among the leaves, the leaves of *B. gymnorrizha* (1315±1.41 mg kg^{-1}) and among the bark samples, the bark of *R. mucronata* (2880±4.24 mg/kg^{-1}) were found to be rich in iron content and these values are very higher than the recommended levels. Except these two plants under the present investigation, all others have Fe concentrations within the recommended levels. Thus, the iron rich mangrove plant was found to be *B. gymnorrizha* having high iron content in both leaves and bark. The results suggest that iron is taken up by the mangroves very efficiently. Higher values of iron have been reported earlier in the leaves of mangroves (Untawale *et al*., 1980; Kotmire and Bhonsale, 1979; Thomas and Fernandez, 1997; Ramos e Silva *et al*., 2006).

The physico-chemical characteristics of the soil have an influence on the nutritive value of plant organs (Assogbadjo *et al*., 2012). The soil characteristics of the area Puthuvypu show a higher iron concentration (Ratheesh Kumar, 2011). Certain seaweeds are reported to have metabolic systems in which it is capable of directly absorbing elements from the seawater (Norziah and Ching 2000). Mangroves are also reported to have the capacity to take up heavy metals from the environment (MacFarlane *et al*., 2007). May due to their specialised metabolic systems mangroves absorb elevated levels of iron from their iron rich environment and in the bark it is been stored as the amount of iron required for the normal growth of a plant is only 11 mg/100g of the dry tissue (Epstein, 1972).

Manganese is one of the microelement essential for plants and it activate several important enzymes, involved in chlorophyll formation, increases the availability of P and Ca. Manganese deficient plants will develop chlorosis between the veins of its leaves. The availability of manganese is partially dependent on soil pH. Recommended value of manganese in the plant tissue is about 100-500 mg kg^{-1} (Pendias and Pendias, 1994). In the present study manganese was found to be more concentrated in the leaves than in the bark of *B. cylindrica*, *B. gymnorrizha* and *K. candel* while it was found to be more concentrated in the bark than in the leaves of *R. apiculata* and *R. mucronata*. The levels of manganese in *Rhizophoraceae* mangroves selected for the present study was from 15.5±0.42 mg kg^{-1} (*B. gymnorrizha* bark) and 235.00±1.41 mg kg^{-1} (*K. candel* leaves). Thus in the current investigation, except in the two *Rhizophora* mangroves, the manganese levels were found to be more concentrated in the leaves than in the bark.

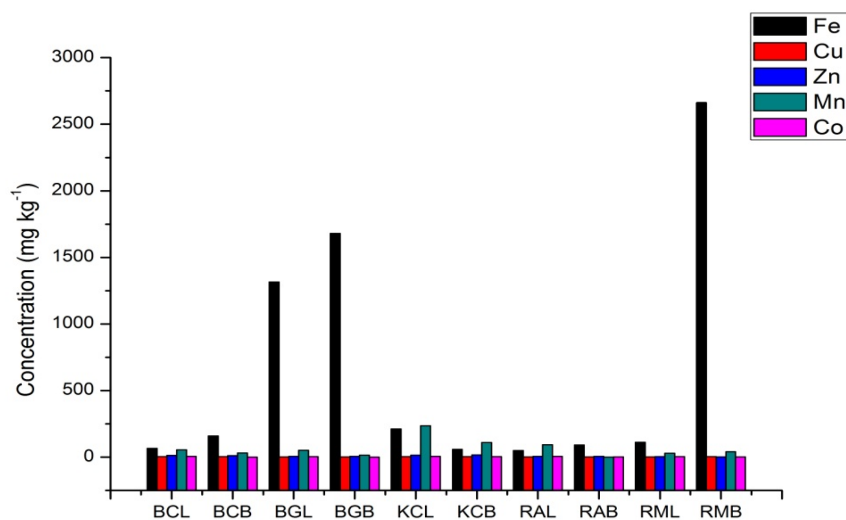

Fig. 3.5 Variation of microelements (Fe, Cu, Zn, Mn and Co) in the leaves and bark of mangrove plants.

While reporting the levels of manganese in the halophytes from two estuarine ecosystems on the central west coast of India, the *R. mucronata* and *B. gymnorrizha* leaves were reported to have its level at 80mg kg^{-1} and 120 mg kg^{-1} respectively (Kotmire and Bhosale, 1979). Manganese found in the Indian mangroves is fairly high when compared to the 5mg/100g dry weight value given by Epstein (1972). So it appears that manganese is taken up by the plants very efficiently. All the mangrove plant parts investigated contained manganese within the recommended level.

The micronutrient, zinc participates in chlorophyll formation, and also activates many enzymes. The deficiency of zinc in plants leads to chlorosis and stunted growth. The recommended value in plant tissue is about 20-100 mg kg^{-1} (Pendias and Pendias, 1994). In the present investigation on the elemental composition of *Rhizophoraceae* mangroves, zinc content was found to be highest in *K. candel*. The concentration of zinc as total of leaves and bark concentration is in the order KC>BC>BG>RA>RM. The Zn concentrations in all the plants were below recommended values. As observed for copper, the zinc concentration in the leaves and bark are very much similar, so that it can be said that it is uniformly distributed in the plant. In a study carried out by Khafaji *et al.*, 1993, the values for mineral composition of *R. mucronata* matches with the present results for *R. mucronata*.

Copper is an essential component of some plant enzymes. In the absence of copper these enzymes are inactivated. Like iron, copper is involved in redox reactions in the mitochondria and in the light reactions of photosynthesis. About 70% of copper in leaves is contained in the chloroplast of land plants (Wilkinson, 1994). The recommended value of it

in plant tissue is about 5-30mg kg^{-1} and more than 20-30 mg kg^{-1} have toxicity effects in plants (Pendias and Pendias, 1994). In the present study, the highest copper content was found in *K. candel*. The distribution of copper in the leaves and bark is found to be somewhat similar. There is no drastic difference in values of copper concentration in the leaves and the bark of the same plant. All the samples contained copper concentrations below the recommended value. Higher values for copper are reported in *B. gymnorrizha* by Thomas and Fernandez, 1997. Low concentrations of Cu and Zn have also been reported in the leaves of Kandelia candel in Taiwan (Chiu and Chou, 1991).

The toxic metals Pb and Cd were detected in the mangrove plants (Fig. 3.6). Lead is available for plants from soil and aerosol sources. Recommended value of it in plant is 30- 300 mg/kg (Rahmani *et al*., 2000). Amount of Pb in all the leaf and bark samples under study in this research were found to be below the recommended levels. The value of Mn, Cu, Zn and Pb were found be higher in *B. gymnorrhizha* collected from Kumarakom, Quilon and Veli in Kerala while the concentration of Fe was lower compared to the present values obtained for this plant (Thomas and Fernandez, 1997). In their studies high lead content was detected in the mangrove twigs.

Cadmium is an especially mobile element in the soil and is taken up by the plants primarily through the roots. Decisive for transfer into plants are cadmium levels, pH values and humus levels that determine the cadmium levels in the soil solution and plants availability to cadmium (Davami and Gholami, 2012). Plant and animals are needless from this element.

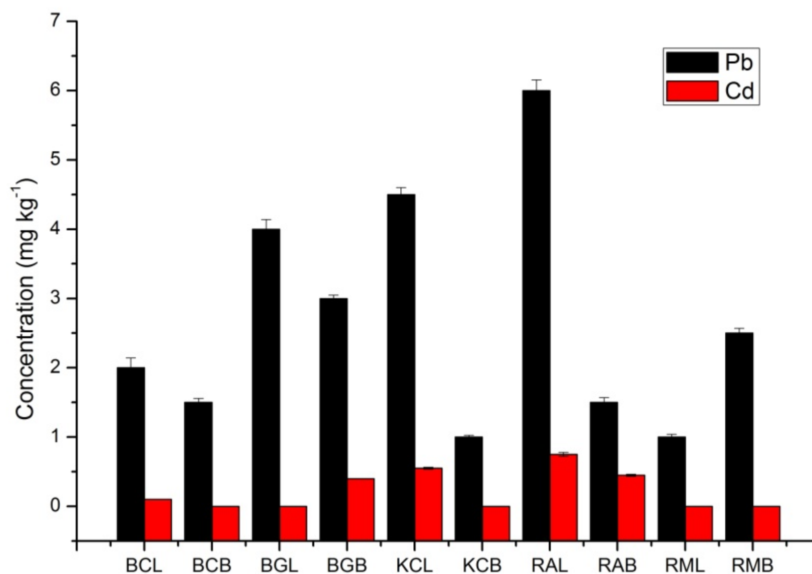

Fig. 3.6 Lead and Cadmium levels in the leaves and bark of *Rhizophoraceae* mangroves

In the this study, it was found that the leaves and bark of the mangrove plant *R. mucronata* is free from cadmium while it was found to be accumulated in the leaves and bark of *R. apiculata*. The leaves of *B. cylindrica* and K. candel were found to contain Cd while in their bark tissues it was not detected. The bark of *B. gymnorrizha* was found to accumulate cadmium whereas its leaves showed levels below the detection limits.

According to the specific environmental conditions accumulation processes specific to element or organism frequently occur in plants. An accumulation is not to be equated with an increase physiological benefit from the element for the organism; indeed this probably often purely represents an adaptation to the respective site (Markert, 1992). The reports of Utanwale *et al.*, 1980 suggests that the variation in the concentration of

some heavy metals in the leaves of seven mangrove vegetation from goa, revealed that maximum concentration of iron and manganese, occurs during the monsoon season without any significant toxic effect on the foliage of mangroves while other metals like copper, nickel, cobalt and lead showed somewhat uniform concentration patterns. In their studies, the micronutrient elements, iron and manganese concentration were found to be much higher. The present study also is in line with these results. This is probably because of more availability and accumulating capacity of mangroves for these metals while the lowest levels was observed in the barks of *R. apiculata*.

3.3.4 Biochemical composition

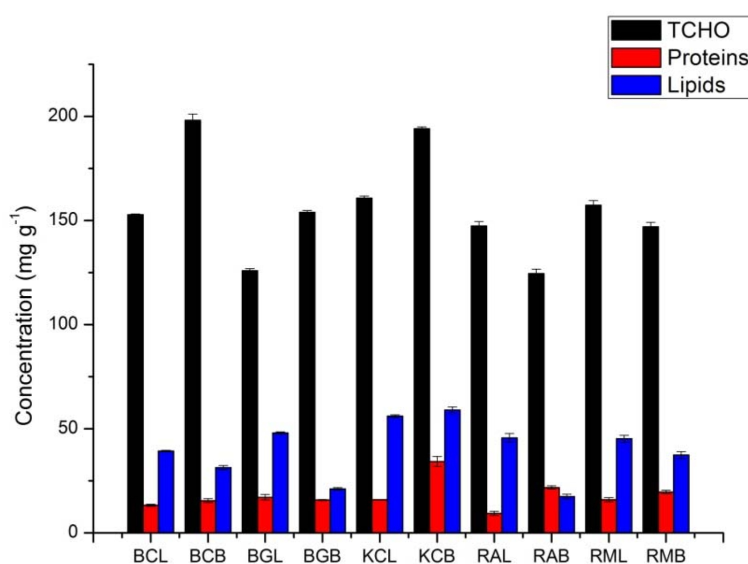

Fig. 3.7 Variations in total carbohydrates (TCHO), proteins and lipids in *Rhizophoraceae* mangroves

The major biochemical component in the selected *Rhizophoraceae* mangroves was carbohydrates (Fig. 3.7). The highest levels of total carbohydrates were found in *B. cylindrica* bark. Except for *Rhizophora*

mangroves, all other mangroves are found to have a higher total carbohydrate concentration in their bark while for *Rhizophora* mangroves the TCHO were found to be higher in the leaves than in the bark. The accumulation of carbohydrates may be due to reduction in their utilisation, either as a source of energy or for the formation of new cells and tissues or as osmolytes of the cells (Harish and Murugan, 2011).

The results of this investigation give an idea that, except for *Bruguiera* mangroves, the major portion of the total carbohydrates are in the form of low molecular weight carbohydrates (Fig. 3.8). They are found to be more concentrated in the bark samples. In *Bruguiera* mangroves considered for this study the levels of polysaccharides overwhelm the molecular weight sugars.

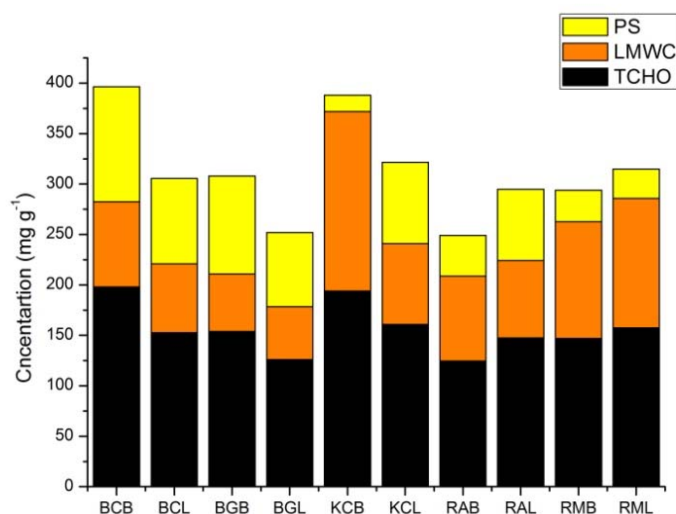

Fig. 3.8 Carbohydrates in the leaves and bark of *Rhizophoraceae* mangroves

In the leaves of *K. candel*, the levels of polysaccharides and low molecular weight carbohydrates are found to be present in equal proportion.

The preference for low molecular weight compounds in the mangroves specimen analysed may be the result of osmoregulation mechanisms operative in these plants in which low molecular weight compounds are produced to achieve the osmotic adjustment within the cells (Popp, 1984).

The lipid content was found to be highest in the *K. candel* and it was found to be accumulated more in the bark than in their leaves. But for all other species under this study, lipids are found in higher proportion in their leaves. Although the concentration of total lipids and fatty acids during winter and summer seasons in two mangroves *K. candel* and *B. gymnorrizha* collected from Okinawa island, Japan (Mfilinge et al., 2002) showed values for total lipids during both seasons (*B. gymnorrizha* 32.8 and 35.8 mg g^{-1} ; *K. candel* 50.8 and 41.6 mg g^{-1}) lower than the values obtained for total lipids in the current study,it supports the present investigation by the high lipid content of K. candel than *B. gymnorrizha* in both the seasons. The protein content obtained during this work was found to be lower than the total carbohydrates and lipids. It was found to be lower than the reported values in the leaves of *B. cylindrica* (13.26% dw), B. *gymnorrizha* (15.55% dw) and *R. apiculata* (16.87%dw) obtained from Orissa coast, India (Basak *et al*., 1998). In their study, they have reported that the *Rhizophoraceae* mangrove exhibit moderate to less amount of protein in leaf and the cause was attributed to the presence of polyphenols or tannins which suppresses the synthesis of protein at genomic level. The current results indicate that mangroves under investigation store protein more in their bark than in the leaves. The predominance of carbohydrates over protein in the plant components indicates that these mangrove detritus can contribute much to the sedimentary carbohydrates in the estuarine ecosystem, ie; Cochin

estuary, where preferential mineralisation of proteinaceous organic matter in surface sediments (Girish 2013) occur thereby intensifying the behaviour of the estuary as a detrital trap for the accumulation of aged organic matter.

Golley (1969) opined that mangrove trees have more energy content than the other tropical rain forest trees. In this study, *K. candel* being high in sugars, proteins and lipids was found to be species with high calorific value. The bark of the species exhibited high energy content than the leaves. *B. cylindrica* leaves were found to contain comparatively high water content in their leaves than other mangroves plants considered in this study. The *Rhizophoraceae* mangroves, *B. gymnorrizha*, *B. parviflora* and *R. mucronata* showed negligible seasonal changes in their moisture and ash content while studying the biochemical composition of mangrove foliage from Goa (Utanwale *et al*., 1978).

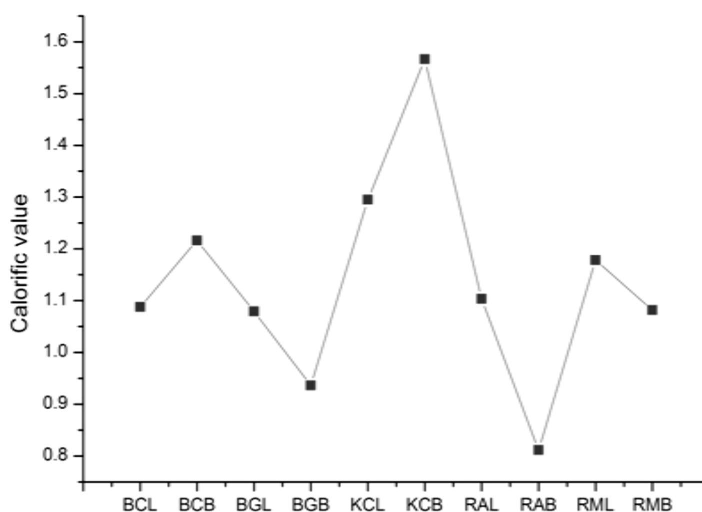

Fig. 3.9 Variations in calorific values in *Rhizophoraceae* mangroves

3.3.5 Total Phenolic content and DPPH radical scavenging activity

The variation of total phenolic content in the bark and leaves of the five mangrove plants *B. cylindrica* and *B. gymnorrhizha, K. candel, R. mucronata, R. apiculata,* are represented in Fig.3.10. The barks of the mangroves showed higher TPC than their leaves. The *Rhizophoraceae* mangroves are characterised by their heavy hardwood and tannin-rich bark. As such, they are widely valued for construction, fuel wood and tannin extraction (Ewel *et al*., 1998). The bark of *K. candel* showed highest TPC. *R. mucronata* is the mangrove having highest foliar TPC. DPPH has been widely used to evaluate the free radical scavenging activity of natural antioxidants (Jao and Ko, 2002). In the present study, the DPPH radical scavenging activity was found to be high in the bark than in the leaves (Table 3.4) which can be attributed to the higher phenolic content of the bark. *K. candel* is an exception from this general behaviour which shows a very high phenolic content in the bark but have high DPPH scavenging activity for the leaves. The reason for this may be due to differential response of these polyphenols to Folin-Ciocalteu reagent depending on the number of phenolic groups they possess (Singleton *et al*., 1999). All the mangrove components of this investigation are found to possess appreciable amount of radical scavenging chemicals. Similar observation is reported by Agooramorthy *et al*., 2008 for the mangroves from Pichavaram. The leaves of *K. candel*, the bark of *R. apiculata*, the leaves and bark of *R. mucronata* can be very good source for antioxidant materials.

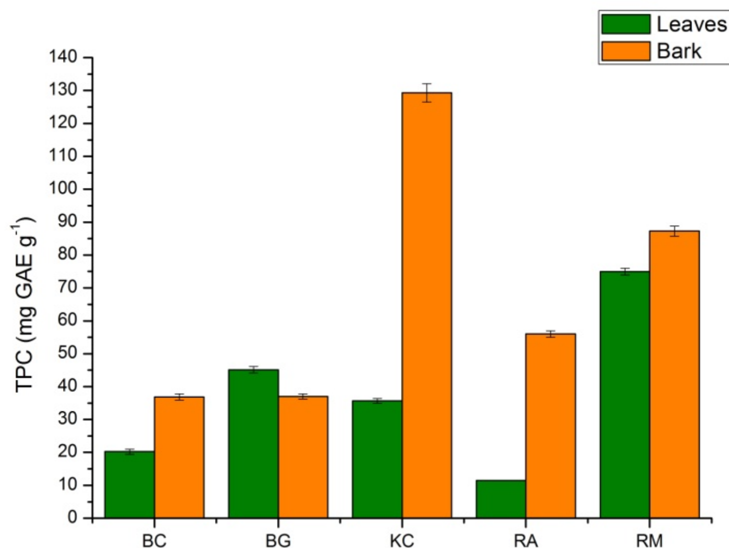

Fig. 3.10 Total phenolic content in the leaves and bark of *Rhizophoraceae* mangroves

Pearson correlation matrix for mangrove leaves (Table 3.5) and bark (Table 3.6) samples revealed differences in interrelationships existing among the minerals and biochemical parameters in these plants. In leaves, C exhibited significant negative correlation with Mg while a highly significant negative correlation was observed between C, Ni and polysaccharides of bark samples. H exhibited significant positive correlation with Co in the leaves while it exhibited a highly significant positive correlation was observed with ash%. S exhibited significant negative correlations with the metals such as Cd and Mn, while N and P showed significant positive correlation with Co and Zn respectively in the leaves of the mangrove plants. No significant correlations were observed for $\delta^{13}C$ of leaves whereas the Fe and Pb in the bark were found to significantly positively correlated with $\delta^{13}C$. The foliar $\delta^{15}N$ showed significant positive correlation with Cu

in the leaves and with P and total carbohydrates in the bark. Zn was exhibited significant positive correlation with P in both leaves and bark of mangroves.

Highly significant positive correlation of Na with ash% and highly significant negative correlation of Mg with total protein was recorded in the mangrove leaves. In the bark, Na and K were found to exhibit a significant positive correlation. Fe in the leaves showed a significant negative correlation with total carbohydrate content in the leaves and with ash% in the bark. The low molecular weight carbohydrates and polysaccharides in the leaves displayed significant negative correlation. In the bark, while Cu showed significant positive correlation with Mn, Co, LMWC and LPD, Mn displayed highly significant correlation with Co, LMWC and LPD. Co in the bark exhibited a significant negative correlation with Ni, significant positive correlation with lipids, proteins and total phenolic content. It also showed highly significant positive correlation with LMWC of bark. Ni which is showed negative correlation with Co was also found to exhibit negative correlation with protein and total phenolic content in the bark. Ni and Pb in the bark also showed a significant positive correlation with their polysaccharide content. In the bark samples significant negative correlations were observed between Pb, LMWC, PRT and TPC while highly significant positive correlations existed between LMWC and lipids, proteins and total phenolic content.

Table 3.5 Pearson correlation matrix for the nutrient and biochemical variables of mangrove leaves

	C	H	N	S	$\delta^{13}C$	$\delta^{15}N$	P	Na	K	Mg	Fe	Cu	Zn	Mn	Co	Ni	Pb	Cd	Ash	TCHO	LMWC	PS	LPD	PRT	TPC
C	1																								
H	-0.519	1																							
N	-0.519	0.748	1																						
S	-0.045	-0.132	-0.628	1																					
$\delta^{13}C$	0.554	-0.47	0.107	-0.611	1																				
$\delta^{15}N$	0.24	0.166	0.586	-0.553	0.749	1																			
P	0.067	0.534	0.796	-0.837	0.437	0.722	1																		
Na	-0.787	0.722	0.583	0.252	-0.5	0.104	0.048	1																	
K	0.085	-0.275	0.264	-0.876	0.602	0.233	0.473	-0.517	1																
Mg	-.894(*)	0.298	0.467	-0.25	-0.356	-0.295	0.014	0.444	0.334	1															
Fe	0.498	0.096	-0.518	0.545	-0.42	-0.358	-0.229	-0.208	-0.605	-0.654	1														
Cu	-0.037	0.276	0.696	-0.46	0.602	.954(*)	0.639	0.366	0.132	-0.085	-0.499	1													
Zn	0.003	0.602	0.828	-0.594	0.411	0.876	.900(*)	0.332	0.148	-0.104	-0.22	0.86	1												
Mn	0.464	0.056	0.438	-.879(*)	0.746	0.7	0.865	-0.431	0.685	-0.236	-0.192	0.507	0.682	1											
Co	-0.519	0.366	.879(*)	-0.748	0.354	0.568	0.656	0.377	0.568	0.611	-0.854	0.684	0.633	0.453	1										
Ni	-0.681	0.514	0.827	-0.283	0.082	0.536	0.392	0.784	0.028	0.518	-0.7	0.755	0.606	0.043	0.829	1									
Pb	0.055	0.31	0.243	-0.589	-0.091	-0.174	0.523	-0.353	0.541	0.221	0.141	-0.312	0.131	0.512	0.142	-0.302	1								
Cd	-0.122	0.14	0.473	-.885(*)	0.268	0.107	0.627	-0.301	.887(*)	0.485	-0.439	0.032	0.259	0.653	0.597	0.075	0.818	1							
Ash	-0.855	0.773	0.756	-0.005	-0.402	0.179	0.237	.962(**)	-0.272	0.6	-0.384	0.434	0.439	-0.25	0.594	0.872	-0.17	-0.034	1						
TCHO	-0.085	-0.293	0.399	-0.584	0.77	0.65	0.321	-0.021	0.604	0.233	-.887(*)	0.696	0.366	0.428	0.74	0.577	-0.276	0.306	0.118	1					
LMWC	0.09	-0.846	-0.403	0.055	0.481	0.007	-0.481	-0.316	0.301	0.065	-0.543	0.04	-0.436	-0.16	0.052	0.005	-0.549	-0.133	-0.328	0.638	1				
PS	-0.167	.897(*)	0.759	-0.43	-0.138	0.391	0.81	0.39	-0.011	0.061	0.144	0.378	0.781	0.467	0.39	0.35	0.529	0.358	0.491	-0.196	-.880(*)	1			
LPD	0.851	-0.34	-0.147	-0.542	0.688	0.375	0.468	-0.826	0.529	-0.589	0.208	0.085	0.244	0.819	-0.105	-0.511	0.458	0.405	-0.745	0.121	-0.025	0.107	1		
PRT	0.781	-0.35	-0.508	0.422	0.311	0.281	-0.186	-0.293	-0.479	-.961(**)	0.574	0.134	0.032	0.037	-0.617	-0.398	-0.481	-0.685	-0.491	-0.166	0.084	-0.209	0.381	1	
TPC	0.574	-0.831	-0.809	0.531	0.302	-0.08	-0.658	-0.437	-0.262	-0.606	0.214	-0.155	-0.505	-0.288	-0.611	-0.455	-0.662	-0.648	-0.621	0.068	0.674	-0.817	0.153	0.734	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Table 3.6 Pearson correlation matrix for the chemical variables of mangrove bark

	C	H	N	S	$\delta^{13}C$	$\delta^{15}N$	P	Na	K	Mg	Fe	Cu	Zn	Mn	Co	Ni	Pb	Cd	Ash	TCHO	LMWC	PS	LPD	PRT	TPC
C	1																								
H	-0.485	1																							
N	-0.745	0.431	1																						
S	-0.267	-0.598	-0.186	1																					
$\delta^{13}C$	0.624	-0.466	-0.123	-0.228	1																				
$\delta^{15}N$	-0.119	0.057	0.611	-0.298	0.673	1																			
P	-0.212	0.443	0.722	-0.621	0.44	.898(*)	1																		
Na	-0.541	0.234	-0.046	0.535	-0.426	-0.164	-0.241	1																	
K	-0.408	0.676	-0.043	-0.027	-0.444	-0.157	-0.005	0.818	1																
Mg	-0.128	0.081	0.005	0.161	0.367	0.498	0.312	0.655	0.57	1															
Fe	0.192	-0.794	-0.559	.886(*)	0.089	-0.323	-0.702	0.379	-0.118	0.225	1														
Cu	0.464	-0.081	0.178	-0.598	.891(*)	0.797	0.746	-0.569	-0.377	0.238	-0.371	1													
Zn	0.144	0.412	0.382	-0.782	0.602	0.798	.911(*)	-0.311	0.05	0.364	-0.678	0.868	1												
Mn	0.588	-0.144	-0.068	-0.503	.938(*)	0.705	0.601	-0.393	-0.218	0.425	-0.187	.950(*)	0.81	1											
Co	0.797	-0.164	-0.283	-0.608	0.862	0.45	0.413	-0.568	-0.302	0.168	-0.218	.883(*)	0.706	.937(*)	1										
Ni	-.960(**)	0.409	0.558	0.409	-0.788	-0.153	-0.063	0.603	0.429	0.011	-0.039	-0.691	-0.397	-0.783	-.930(*)	1									
Pb	-0.233	-0.359	-0.383	.902(*)	-0.336	-0.441	-0.69	0.783	0.358	0.356	0.845	-0.696	-0.741	-0.504	-0.584	0.402	1								
Cd	-0.268	0.529	-0.28	0.039	-0.852	-0.786	-0.526	0.485	0.672	-0.18	-0.07	-0.785	-0.47	-0.699	-0.55	0.454	0.339	1							
Ash	-0.507	.975(**)	0.572	-0.658	-0.426	0.146	0.543	0.064	0.502	-0.05	-.888(*)	0.006	0.464	-0.124	-0.144	0.404	-0.506	0.403	1						
TCHO	-0.176	0.149	0.643	-0.33	0.618	.995(**)	.924(*)	-0.11	-0.072	0.525	-0.376	0.769	0.819	0.678	0.416	-0.099	-0.443	-0.722	0.23	1					
LMWC	0.786	-0.232	-0.218	-0.588	.886(*)	0.485	0.432	-0.66	-0.43	0.085	-0.218	.909(*)	0.69	.929(*)	.989(**)	-.926(*)	-0.627	-0.636	-0.182	0.442	1				
PS	-.976(**)	0.359	0.708	0.389	-0.499	0.211	0.215	0.629	0.41	0.295	-0.042	-0.413	-0.139	-0.501	-0.759	.924(*)	0.349	0.153	0.365	0.26	-0.751	1			
LPD	0.618	-0.263	-0.068	-0.441	.973(**)	0.704	0.564	-0.47	-0.351	0.352	-0.124	.958(*)	0.753	.989(**)	.932(*)	-0.806	-0.499	-0.78	-0.222	0.664	.942(*)	-0.526	1		
PRT	0.776	0.07	-0.327	-0.769	0.663	0.274	0.365	-0.554	-0.143	0.056	-0.39	0.767	0.697	0.821	.950(*)	-.879(*)	-0.657	-0.287	0.072	0.261	.917(*)	-0.795	0.787	1	
TPC	.905(*)	-0.324	-0.453	-0.483	0.846	0.294	0.214	-0.58	-0.375	0.092	-0.05	0.788	0.535	0.871	.976(**)	-.987(**)	-0.467	-0.514	-0.316	0.248	.969(**)	-0.86	.882(*)	.918(*)	1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

3.4 PCA and Chemotaxonomic significance

The result of the principle component analysis of the elemental, mineral and phytochemical constituents of the leaves and bark of the five *Rhizophoraceae* mangroves are presented in Table 3.7.

Table 3.7 Factor loadings of PCA analysis of elemental, mineral and biochemical components of the leaves of *Rhizophoraceae* mangroves

Parameters	Leaves				Bark			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
C	-0.982	0.106	-0.096	-0.127	0.948	-0.121	-0.226	-0.188
H	0.382	-0.089	0.22	0.893	-0.239	-0.07	0.915	0.319
N	0.424	0.273	0.695	0.512	-0.649	0.603	0.418	-0.202
S	0.015	-0.868	-0.481	-0.123	-0.475	-0.179	-0.847	0.158
P	-0.14	0.55	0.599	0.565	0.015	0.835	0.549	0.006
Na	0.659	-0.535	0.364	0.384	-0.466	-0.171	-0.175	0.85
K	0.032	0.941	0.194	-0.275	-0.202	-0.247	0.362	0.876
Mg	0.95	0.311	0.001	-0.042	0.058	0.473	-0.161	0.864
Fe	-0.548	-0.369	-0.6	0.452	-0.013	-0.212	-0.957	0.199
Cu	-0.075	-0.035	0.992	0.093	0.6	0.765	0.179	-0.15
Zn	-0.137	0.155	0.81	0.548	0.401	0.708	0.57	0.114
Mn	-0.471	0.708	0.48	0.217	0.736	0.669	0.055	0.087
Co	0.497	0.453	0.737	0.061	0.905	0.405	0.112	-0.067
Ni	0.589	-0.119	0.791	0.117	-0.967	-0.139	0.122	0.177
Pb	-0.004	0.793	-0.335	0.509	-0.357	-0.369	-0.692	0.508
Cd	0.202	0.965	0.067	0.15	-0.244	-0.847	0.333	0.334
Ash	0.743	-0.286	0.445	0.409	-0.28	0.028	0.952	0.123
TCHO	0.116	0.323	0.772	-0.535	0.008	0.973	0.2	0.112
LMWC	0.024	0.002	0.136	-0.99	0.866	0.454	0.075	-0.194
PS	0.041	0.197	0.303	0.932	-0.927	0.228	0.066	0.291
LPD	-0.81	0.584	0.043	0.013	0.726	0.687	-0.037	-0.024
PRT	-0.845	-0.522	0.062	-0.097	0.918	0.194	0.345	-0.039
TPC	-0.525	-0.44	-0.144	-0.714	0.955	0.269	-0.044	-0.114

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The PCA of leaves shows that four components accounting for a total variance of 100% among the leaves of five mangrove plants. The PC1, PC2, PC3 and PC4 accounted for 26.274, 25.614, 24.986 and 23.126% of the total variation respectively. High significant positive loadings of Mg as well as high significant negative loadings for C, LPD and PRT characterise PC1. This identifies the photosynthesis process in which the production of energy molecules and their transport as well as the accumulation of Mg contents as the major processes taking place in the leaves. PC2 showed significant positive loadings for K and Cd as well as high significant negative loading for S whereas high positive loadings for Cu and Zn characterise PC3. PC4 showed significant positive loading for PS and high negative loading for LMWC. These observations explain the processes involved in the accumulation and depletion taking place in the plants. The processes leading to the accumulation of Cu, Zn, synthesis of PS and reduction in LMWC contributes towards diversity in the foliar composition among species.

The PCA of barks shows that four components accounting for a total variance of 100% among the leaves of five mangrove plants. The PC1, PC2, PC3 and PC4 accounted for 38.42, 25.36, 22.78 and 13.45% of the total variation respectively. PC1 is characterised by the high positive loadings for C, Cu, Zn, Mn, Co, LMWC, PRT and TPC and high significant negative loadings for Ni and PS. PC2 shows high positive loadings for P and TCHO as well as high significant negative loading for Cd. High positive loadings for H and ash as well as high negative loading for Fe are recorded in PC3. High positive loadings of Na, K and Mg characterise PC4. In this study, the PCA of the bark reveals the various processes involved so to bring about difference in chemical patterns in plants. PCA of bark identifies that the

storage and accumulation of major micro and nutrient elements, phenolic compounds and the products of photosynthesis as the major function of the bark.

The basic data of the micro and macro nutrient elements in the leaves and bark can suggest the chemical character of the *Rhizophoraceae* mangrove plant parts of this particular area, Kochi. It was found that all the minerals studied; Na, K,, Mg, Fe, Cu, Zn, Mn, Co and Ni, are found to be present in the mangroves under investigation. The higher iron accumulation capacity of RM bark and BG leaves is what makes them distinct with respect to the mineral composition. The absence of Cd in the leaves and bark of *R. mucronata* can be considered as a distinct feature of this plant.

The hierarchical cluster analysis of data of mineral and biochemical constituents in the leaves and bark using SPSS version 10 was carried out to obtain a dendrogram for species distances was obtained (Fig. 3.11a). The similarity matrix indicates that the highest similarity is between the leaves of BC, RA and RM which in turn show similarity with KC. BG leaves were found to be chemically distinct from others. This combined system could discriminate the five mangrove plants of different genera. The leaves of BC is chemically more similar to the two *Rhizophora* mangroves than BG of the same genus. The hierarchical cluster analysis of the bark data revealed that these five mangrove species fell into two groups (Fig.3.11b) in which the bark of RM and BG showing distinct chemical character from the other three bark samples.

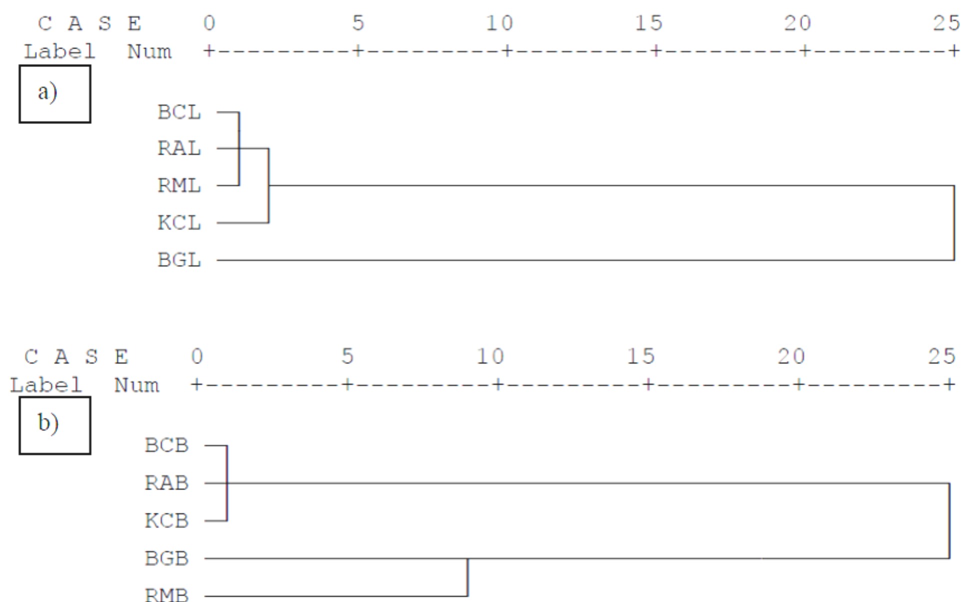

Fig. 3.11 Clustering of data of a) leaves and b) mineral, isotopic and biochemical parameters using Euclidean distance of five *Rhizophoraceae* mangroves

3.5 Conclusion

K. candel was found to exhibit highest carbon content among the five *Rhizophoraceae* mangroves under investigation. Except the carbon content, the macronutrient elements, H,N, P and S are more concentrated in the leaves and less in the bark of mangroves while C is more concentrated in their bark. No specific trends were observed according to genera. The carbon isotope composition of leaves of the *Rhizophoraceae* mangrove under the present investigation matches well with the already established values of $\delta^{13}C$ for C3 plants. The plant components of *K. candel* were enriched in ^{13}C relative to other *Rhizophoraceae* mangroves by 1 to 2.5‰. The most depleted $\delta^{13}C$ was found in *B. gymnorrizha*. Except

B. gymnorrizha, all the other plants studied are found to accumulate sodium in their leaves than in the bark. The genus *Rhizophora* as well as *Kandelia* exhibited higher potassium content than the *Bruguiera* plants. The values of Na/K in the leaves of mangrove indicate that the salt exclusion mechanism is efficiently operative in *K. candel* followed by *Rhizophora* species. *Bruguiera* showing highest values of Na/K can be thus concluded to be the plants with least salt excluding capacity among the *Rhizophoraceae* plants under investigation. *R. apiculata* leaves were found to be rich in magnesium content while the iron rich mangrove plant was found to be *B. gymnorrizha*. All the mangrove plant parts investigated contained manganese within the recommended level. Zinc content was found to be highest in *K. candel*. In the present study, the highest copper content was found in *K. candel*. Amount of Pb in all the leaf and bark samples under study in this research were found to be below the recommended levels In the this research study, it was found that the leaves and bark of the mangrove plant *R. mucronata* is free from cadmium while it was found to be accumulated in the leaves and bark of *R. apiculata*. The leaves of *B. cylindrica* and K. candel were found to contain Cd while in their bark tissues it was not detected. The bark of *B. gymnorrizha* was found to accumulate cadmium whereas its leaves showed levels below the detection limits.

Except for *Rhizophora* mangroves, all other mangroves are found to have a higher total carbohydrate concentration in their bark. The present study gives an idea that, except for *Bruguiera* mangroves, the major portion of the total carbohydrates are in the form of low molecular weight carbohydrates. Being high in sugars, proteins and lipids, *K. candel* was found to be species with high calorific value. PCA identifies that the

transport of photosynthetic products and minerals as the major processes differentiating the mangrove plants while the storage and accumulation of the photosynthetic products and minerals are significant in barks. The leaves of the two *Bruguiera* plants exhibited distinct chemical character with respect to the minerals and biochemical parameters plants, *B. cylindrica* being more similar to *Rhizophora* mangroves. K. candel showed similarity to the *Rhizophora* mangroves, but stayed different form that of *Bruguiera* plants. Thus, measuring the chemical parameters mangrove leaves and bark can assist in an effective application of taxonomic approach towards their use in various economic and ecological services.

References

Adriano, D. C. (2001). *Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals*. Springer.

Agoramoorthy, G., Chen, F. A., Venkatesalu, V., Kuo, D. H., and Shea, P. C. (2008). Evaluation of antioxidant polyphenols from selected mangrove plants of India. *Asian J Chem*, *20*(2), 1311-1322.

Albert, R., and Popp, M. (1977). Chemical composition of halophytes from the Neusiedler Lake region in Austria. *Oecologia*, *27*(2), 157-170.

Assogbadjo, A. E., Chadare, F. J., Kakaï, R. G., Fandohan, B., and Baidu-Forson, J. J. (2012). Variation in biochemical composition of baobab (*Adansonia digitata*) pulp, leaves and seeds in relation to soil types and tree provenances. *Agriculture, Ecosystems & Environment*, *157*, 94-99.

Basak, U. C., Das, A. B., and Das, P. (1996). Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangrove. *Bulletin of Marine Science*, *58*(3), 654-659.

Basak, U. C., Das, A. B., and Das, P. (1998). Seasonal changes in organic constituents in leaves of nine mangrove species. *Marine and freshwater research*, *49*(5), 369-372.

Bodek, I., Lyman, W. J., Reehl, W. F., and Rosenblatt, D. H. (Eds.). (1988). *Environmental inorganic chemistry: properties, processes, and estimation methods*. New York:: Pergamon press.

Bouillon, S., Koedam, N., Raman, A., and Dehairs, F. (2002). Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests.*Oecologia*, *130*(3), 441-448.

Caroli, S., Iyengar, G. V. and Muntau, H. (eds.) 1989. Bioelements: health aspects. Annali Dell'Instituto Superiori Di Sanita, 25, 373-378.

Chiu, C. Y., and Chou, C. H. (1991). The distribution and influence of heavy metals in mangrove forests of the Tamshui estuary in Taiwan. *Soil Science and Plant Nutrition*, *37*(4), 659-669.

Chong, V. C., Low, C. B., and Ichikawa, T. (2001). Contribution of mangrove detritus to juvenile prawn nutrition: a dual stable isotope study in a Malaysian mangrove forest. *Marine Biology*, *138*(1), 77-86.

Cintrón, G., Lugo, A. E., Pool, D. J., and Morris, G. (1978). Mangroves of arid environments in Puerto Rico and adjacent islands. *Biotropica*, *10*(2), 110-121.

Clough, B. F. (1992). Primary productivity and growth of mangrove forests. *Tropical Mangrove Ecosystems*, 41, 225–249.

Davami, A. H., and Gholami, A. (2012). Biomonitoring of Heavy Metals in Environment by Button Mangrove Plant. International Conference on Nuclear Energy, Environmental and Biological Sciences Bangkok (Thailand).

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., and Tu, K. P. (2002). Stable isotopes in plant ecology. *Annual review of ecology and systematics*, 507-559.

Downton, W. J. S. (1982). Growth and osmotic relations of the mangrove Avicennia marina, as influenced by salinity. *Functional Plant Biology*, *9*(5), 519-528.

Epstein, E. (1972). *Mineral nutrition of plants: principles and perspectives*, Pp, 412.

Ernst, W. (1974). *Schwermetallvegetation der erde* (p. 863). Stuttgart.: Gustav Fischer Verlag, Stuttgart, 194 pp.

Ernst, W. H., and Joosse-van Damme, E. N. (1983). *Umweltbelastung durch Mineralstoffe: biologische Effekte*. Stuttgart: Fischer. 234 pp.

Ewel, K., Twilley, R., and Ong, J. (1998). Different kinds of mangrove forests provide different goods and services. *Global Ecology & Biogeography Letters*,*7*(1), 83-94.

Farquhar, G. D., O'leary, M. H., and Berry, J. A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology*, *9*(2), 121-137.

Fry, B., Bern, A. L., Ross, M. S., and Meeder, J. F. (2000). $\delta^{15}N$ Studies of Nitrogen Use by the Red Mangrove,*Rhizophora mangle* L. in South Florida. *Estuarine, Coastal and Shelf Science*, *50*(2), 291-296.

Garten Jr, C. T., Gentry, J. B., and Sharitz, R. R. (1977). An analysis of elemental concentrations in vegetation bordering a southeastern United States coastal plain stream. *Ecology*, 979-992.

Gireesh Kumar, T.R. (2013). Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach, PhD. Thesis, Cochin University of Science and Technology, Kochi, India.

Glenn, E. P., Olsen, M., Frye, R., Moore, D., and Miyamoto, S. (1994). How much sodium accumulation is necessary for salt tolerance in subspecies of the halophyte Atriplex canescens?. *Plant, Cell & Environment*, *17*(6), 711-719.

Golley, F. B. (1969). Caloric value of wet tropical forest vegetation. *Ecology*, 517-519.

Güsewell, S. (2004). N: P ratios in terrestrial plants: variation and functiona significance. *New phytologist*, *164*(2), 243-266.

Hamilton, E. I. (1980). The need for trace element analyses of biological materials in the environmental sciences. In: International Atomic Energy Agency, (ed.), Element analysis of biological materials - current problems and techniques with special reference to trace elements. *Technical Report Series No. 197*, Wien, 39-54.

Hamilton, E. I. 1979. The chemical elements and man. John Wiley, New York, 578 pp.

Handley, L. L., and Scrimgeour, C. M. (1997). Terrestrial plant ecology and δ^{15} N natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Advances in Ecological Research, 27*, 133–212.

Harish, S. R., and Murugan, K. (2011). Biochemical and Genetical Variation in the Mangrove Associate (L) Gaertn. Under Different Habitats of Kerala. *Asian Journal of Experimental Biological Sciences, 2*(4): 553-561.

Jao, C. L., and KO, W. C. (2002). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fisheries Science*,*68*(2), 430-435.

Kathiresan, K., and Bingham, B. L. (2001). Biology of Mangroves and Mangrove Ecosystems. *Advances in Marine Biologgy*, *40*, 81–251.

Khafaji, A. K., El-Hassan, A. M., Mandura, A. S., Al-Hachim, G. H., and El-Nakkadi, A. M. N. (1993). Phytochemical studies on mangrove and the possibility of using it as fodder. *Journal of King Abdulaziz University, Marine Sciences*, *4*, 181-195.

Kotmire, S. Y., and Bhosale, L. J. (1979). Some aspects of chemical composition of mangrove, leaves and sediments. *Mahasagar*, *12*(3), 149-154.

Lovelock, C. E., Feller, I. C., Ball, M. C., Ellis, J., and Sorrell, B. (2007). Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecology Letters*, *10*(12), 1154-1163.

MacFarlane, G. R., Koller, C. E., and Blomberg, S. P. (2007). Accumulation and partitioning of heavy metals in mangroves: a synthesis of field-based studies.*Chemosphere*, *69*(9), 1454-1464.

Markert, B. (1992). Presence and significance of naturally occurring chemical elements of the periodic system in the plant organism and consequences for future investigations on inorganic environmental chemistry in ecosystems.*Vegetatio*, *103*(1), 1-30.

Markert, B. and Jayasekera, R. (1987). Elemental composition of different plant species. *Journal of plant nutrition*. 10, 7, 783-794.

Marschner, H. (2012). *Marschner's mineral nutrition of higher plants* (Vol. 89). P. Marschner (Ed.). Academic press.

McMillan, C. (1974). Salt tolerance of mangroves and submerged aquatic plants. In: Reimold, R. J., Queen, W. H. ed. *Ecology of Halophytes*, New York, Academic Press. Pp 379-390.

Meyers, P. A. (1997). Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. *Organic geochemistry*, *27*(5-6), 213-250.

Mfilinge, P., Atta, N., and Tsuchiya, M. (2002). Nutrient dynamics and leaf litter decomposition in a subtropical mangrove forest at Oura Bay, Okinawa, Japan.*Trees*, *16*(2-3), 172-180.

Muzuka, A. N., and Shunula, J. P. (2006). Stable isotope compositions of organic carbon and nitrogen of two mangrove stands along the Tanzanian coastal zone.*Estuarine, Coastal and Shelf Science*, *66*(3), 447-458.

Naidoo, G., Tuffers, A. V., and von Willert, D. J. (2002). Changes in gas exchange and chlorophyll fluorescence characteristics of two mangroves and a mangrove associate in response to salinity in the natural environment. *Trees*,*16*(2-3), 140-146.

Niu, X., Bressan, R. A., Hasegawa, P. M., and Pardo, J. M. (1995). Ion homeostasis in NaCl stress environments. *Plant physiology*, *109*(3), 735.

Norziah, M. H., and Ching, C. Y. (2000). Nutritional composition of edible seaweed *Gracilaria changgi. Food Chemistry*, *68*(1), 69-76.

O'Leary, J. and Glenn, E. 1994. Global distribution and potential for halophytes. In: Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Land. pp. 7–17. Squires, V. R. and Ayoub, A., Eds., Kluwer Academic, Netherlands.

O'Leary, M. H. (1981). Carbon isotope fractionation in plants. *Phytochemistry*, *20*(4), 553-567.

O'Leary, M. H. (1988). Carbon isotopes in photosynthesis. *Bioscience*, 328-336.

Page, D. S., Gilfillan, E. S., Foster, J. C., Hotham, J. R., and Gonzalez, L. (1985). Mangrove leaf tissue sodium and potassium ion concentrations as sublethal indicators of oil stress in mangrove trees. In *International Oil Spill Conference* (Vol. 1985, No. 1, pp. 391-393). American Petroleum Institute.

Parida, A. K., Das, A. B., and Mohanty, P. (2004). Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: Differential changes of isoforms of some antioxidative enzymes. *Journal of plant physiology*, *161*(5), 531-542.

Patil, V., Singh, A., Naik, N., Seema, U., and Sawant, B. (2012). Carbon sequestration in mangroves ecosystems. *Journal of Environment Research and Development*, *7*(1), 576– 583.

Pendias, K. A., and Pendias, H. (1984). Trace elements in soils and plants. CRC Press, Boca Raton.

Popp, M. (1984). Chemical composition of Australian mangroves II. Low molecular weight carbohydrates. *Zeitschrift für Pflanzenphysiologie*, *113*(5), 411-421.

Popp, M., Larher, F., and Weigel, P. (1984). Chemical composition of Australian mangroves III. Free amino acids, total methylated onium compounds and total nitrogen. *Zeitschrift für Pflanzenphysiologie*, *114*(1), 15-25.

Rahmani H., Karbasi M. and Haj Rasooli S.(2000). Lead contamination of plants by vehicles in the range of some major roads of Iran. *Journal of Ecology*, 26, 77-83.

Ramos e Silva, C. A., Da Silva, A. P., and De Oliveira, S. R. (2006). Concentration, stock and transport rate of heavy metals in a tropical red mangrove, Natal, Brazil. *Marine Chemistry*, *99*(1), 2-11.

Rao, R. G., Woitchik, A. F., Goeyens, L., Van Riet, A., Kazungu, J., and Dehairs, F. (1994). Carbon, nitrogen contents and stable carbon isotope abundance in mangrove leaves from an east African coastal lagoon (Kenya). *Aquatic Botany*,*47*(2), 175-183.

Ratheesh Kumar, C.S.(2011). Triterpenoids as biomarkers of mangrove organicmMatter in Cochin estuarine system, PhD Thesis, Cochin University of Science and Technology, Kochi, India.

Reef, R., Feller, I. C., and Lovelock, C. E. (2010). Nutrition of mangroves. *Tree Physiology*, *30*(9), 1148-1160.

Sage, R.F., Li, M. and Monson, R.K. (1999). The taxonomic distribution of C4 photosynthesis. In: *C4 Plant Biology* (Eds R.F. Sage and R.K. Monson), pp. 551–581. Academic Press, San Diego.

Sansoni, B., and Iyengar, V. (1978). *Sampling and sample preparation methods for the analysis of trace elements in biological material.* Kernforschungsanlage Juelich GmbH (Germany, FR). Zentralabteilung fuer Chemische Analyse.

Sarangi, R. K., Kathiresan, K., and Subramanian, A. N. (2002). Metal concentrations in five mangrove species of the Bhitarkanika, Orissa, east coast of India. *Indian journal of marine sciences*, *31*(3), 251-253.

Scholander, P. F. (1968). How mangroves desalinate seawater. *Physiologia Plantarum*, *21*(1), 251-261.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., and Hammel, H. T. (1965). Sap pressure in vascular plants negative hydrostatic pressure can be measured in plants. *Science*, *148*(3668), 339-346.

Shearer, G., and Kohl, D. H. (1986). N2-fixation in field settings: estimations based on natural 15N abundance. *Functional Plant Biology*, *13*(6), 699-756.

Singleton, V.L., Orthofer, R., Raventos, L.R.M., (1999). Analysis of total polyphenols and other oxidation substrates and antioxidants by means of Folin – Ciocatleu reagent. *Methods of Enzymology*, 299, 152-178.

Smallwood, B. J., Wooller, M. J., Jacobson, M. E., and Fogel, M. L. (2003). Isotopic and molecular distributions of biochemicals from fresh and buried Rhizophora mangle leaves. *Geochem. Trans*, *4*(7), 38-46.

Smith, B. N., and Epstein, S. (1971). Two categories of 13C/12C ratios for higher plants. *Plant physiology*, *47*(3), 380-384.

Teeri, J. A., and Stowe, L. G. (1976). Climatic patterns and the distribution of C4 grasses in North America. *Oecologia*, *23*(1), 1-12.

Thomas, G., and Fernandez, T. V. (1997). Incidence of heavy metals in the mangrove flora and sediments in Kerala, India. In *Asia-Pacific Conference on Science and Management of Coastal Environment* (pp. 77-87). Springer Netherlands.

Untawale, A. G., Bhosle, N. B., Dhargalkar, V. K., Matondkar, S. G. P., and Bukhari, S. S. (1978). Seasonal variation in major metabolities of mangrove foliage. *Mahasagar*, *11*(1-2), 105-110.

Untawale, A. G., Wafar, S., and Bhosle, N. B. (1980). Seasonal variation in heavy metal concentration in mangrove foliage. *Mahasagar*, *13*(3), 215-223.

Weis, J. S., and Weis, P. (2004). Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environment international*, *30*(5), 685-700.

Wilkinson, R. E. (Ed.). (1994). *Plant-environment interactions*. Marcel Deccar, New York.Pp. 559.

Wilkinson, S. R., Welch, R. M., Mayland, H. F., and Grunes, D. L. (1990). Magnesium in plants: uptake, distribution, function, and utilization by man and animals. In Sigel, H., and Astrid, S.ed. *Metal ions in Biological Systems*, 26, 34-55.

Wooller, M., Smallwood, B., Jacobson, M., and Fogel, M. (2003). Carbon and nitrogen stable isotopic variation in Laguncularia racemosa (L.)(white mangrove) from Florida and Belize: implications for trophic level studies. *Hydrobiologia*, *499*(1-3), 13-23.

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Chapter 4

FLAVONOIDS

Contents

4.1 Introduction
4.2 Results
4.3 Discussions
4.4 Flavonoids from R. mucronata
4.5 Conclusions

4.1 Introduction

Flavonoids are widely distributed in plant foods and therefore important constituent of human as well as animal food. Their concentration varies from plant to plant or even in different organs of the same plant and geographical location (Anderson and Markham, 2010; Dinelli *et al*., 2006; Justesen and Knethsen, 2001). Many plants are considered to be excellent sources of flavonoids that could be used, not only to preserve foods, but also to contribute to a healthy diet (Justesen and Knethsen, 2001). Dietary flavonoids are considered to be even more powerful antioxidants than vitamins C and E (Sokol-Letowska *et al*., 2007).

Flavonoids possess many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The

configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability (Heim *et al*., 2002; Pandey *et al*., 2012). The B ring hydroxyl configuration is the most significant determinant of scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilising them and giving rise to a relatively stable flavonoids radical (Cao *et al*., 1997).

Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging ROS; and (3) upregulation or protection of antioxidant defences (Kumar and Pandey, 2013). Free metal ions enhance ROS formation by the reduction of hydrogen peroxide with generation of the highly reactive hydroxyl radical. Due to their lower redox potentials flavonoids (Fl-OH) are thermodynamically able to reduce highly oxidizing free radicals (redox potentials in the range 2.13–1.0 V) such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation (Fig. 4.1 a). Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation. Quercetin in particular is known for its iron-chelating and iron-stabilising properties. Trace metals bind at specific positions of different rings of flavonoid structures. The binding sites are shown in Fig. 4.1 b.

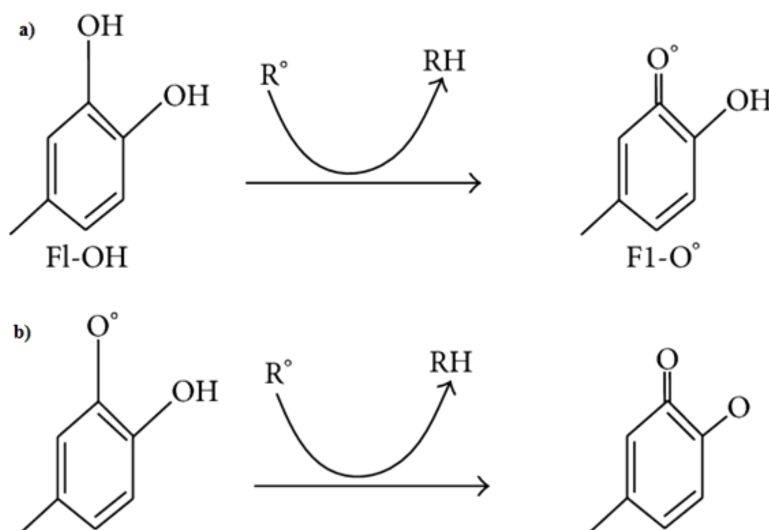

Fig. 4.1. (a) Scavenging of ROS (free radical,R^0) by flavonoids (Fl-OH) and (b) binding sites for trace metals where indicates metal ions.

Food derived flavonoids, especially; flavonols (kaempeferol, quercetin and myricetin) and flavones(apigenin and luteolin) are of particular importance and are reported to exhibit multiple biological functions such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, anti-thrombotic, anti-oxidant, cardioprotective and vasodilatory effects (Shahidi *et al.*, 1992; Manach *et al.*, 2005) and have significant vitamin C sparing activity, with myricetin being one of the most active (Miean and Mohamed, 2001). Vegetables, fruits, and beverages are the main dietary sources of these flavonols and flavones (Hertog *et al.*, 1992, 1993). The structures of apigenin, luteolin, myricetin, quercetin and kaempferol are given in Fig. 4.2.

4.1.1 Myricetin

Myricetin which is chemically, 3, 5, 7-trihydroxy-2-(3, 4, 5-trihydroxyphenyl)-4-chromenone is a naturally occurring flavonol. It is

found in many grapes, berries, fruits, vegetables, herbs, as well as other plants. Walnuts are a rich dietary source. Trace amounts can be found as glycosides (Miean and Muhammed., 2001). It is one of the phenolic compounds present in red wine (Maggiolini *et al*., 2005). In vitro research suggests that myricetin in high concentrations can modify LDL cholesterol such that uptake by white blood cells is increased. Myricetin plays a major role as an antioxidant (Gordon *et al*., 1998). A Finnish study correlated high myricetin consumption with lowered rates of prostate cancer (Knekt *et al*., 2002) and it can reduce the risk of pancreatic cancer (Nothlings *et al*., 2007).

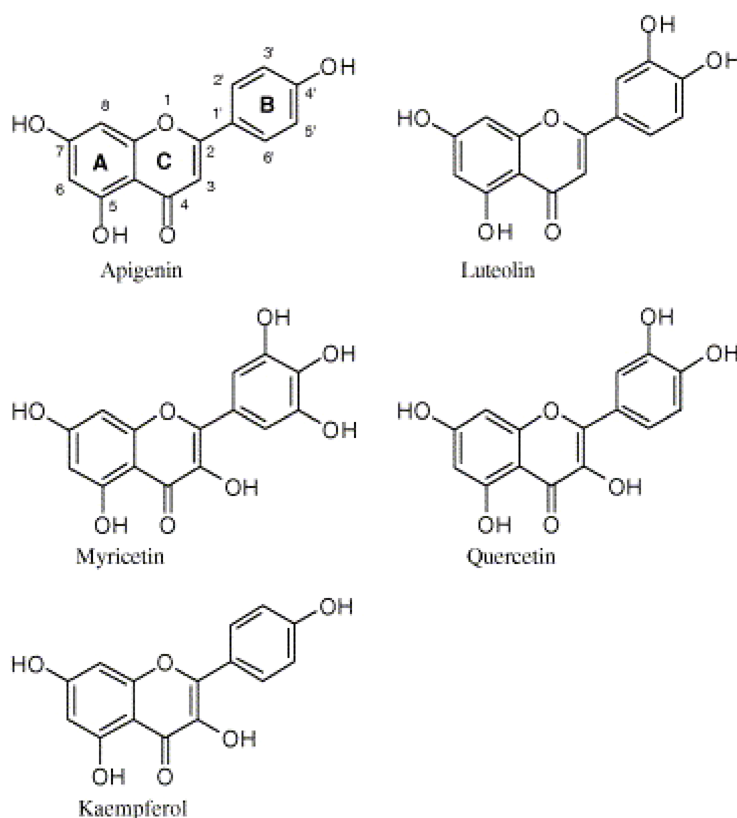

Fig.4.2 Structures of apigenin luteolin, myricetin, quercetin and kaempferol

Myricetin increases cell viability and decreased cell apoptosis and has the therapeutic value for preventing β-cell death. It serves as severe acute respiratory syndrome coronavirus (SARSCoV) chemical inhibitors (Yu *et al*., 2012) and also possesses significant analgesic activity and was found to increase insulin sensitivity and normalise blood glucose level.

4.1.2 Quercetin

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) is a widely distributed flavonoid of the class flavonol. It is found in many plants and foods, such as red wine, berries, onions, green tea and apples. Quercetin is the most common flavonol in fruits. It is a naturally occurring polar auxin transport inhibitor (Fischer *et al*., 2001). Quercetin is used for treating conditions of the heart and blood vessels and high cholesterol (Pace-Asciak *et al*., 1995; Arai *et al*., 2000). It is also used for diabetes, cataracts, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, viral infections, chronic fatigue syndrome (CFS) and for treating chronic infections of the prostate (Sabitha and Panneerselvam, 2013).

4.1.3 Kaempferol

Kaempferol is a flavonol which is chemically 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one and found in a variety of plants and plant-derived foods. Many kaempferol-containing plants have antioxidant activity regulated by the ability of kaempferol to lower cellular superoxide while its anti-inflammatory activity and antimicrobial activity makes it an attractive natural medicine (Calderon-Montaño, 2011) Kaempferol seems to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein

and the formation of platelets in the blood (Kong *et al*., 2013). Studies have also confirmed that Kaempferol acts as a chemopreventive agent which means that it inhibits the formation of cancer cell (Abdul-Jalil *et al*., 2010).

4.1.4 Luteolin

Luteolin, 2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone, is one of the most common flavonoids present in edible plants and in plants used in traditional medicine to treat a wide variety of pathologies. This flavone and its glycosides are widely distributed in the plant kingdom; they are present in many plant families and have been identified in Bryophyta, Pteridophyta, Pino phyta and Magnoliophyta and the main dietary sources of luteolin include, for instance, carrots, peppers, celery, olive oil, peppermint, thyme, rosemary and oregano (Lopez-Lazaro, 2009). The desmutagenicity of luteolin was similar to that of galangin and stronger than that of quercetin (Samejima *et al*., 1995). One possible desmutagenic mechanism of flavonoids is thought to be scavenging of the radicals before they damage the DNA.

4.1.5 Apigenin

Apigenin is a flavonoid belonging to the flavone structural class and chemically known as 4', 5, 7,-trihydroxyflavone. Apigenin is abundantly present in common fruits and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings (Patel *et al*., 2007). For centuries, apigenin has been utilised as a traditional or alternative medicine. For example, passion flower, which contains high levels of apigenin, has been used effectively to treat asthma, intransigent insomnia, Parkinson's disease, neuralgia, and shingles (Patel *et al*., 2007).

Although a number of studies on the flavonol and flavone contents of plant sources have been reported from different countries, the compositional data are still insufficient, which necessitates the need to investigate more and more materials for the search of credible and beneficial natural antioxidants as well as to aid the chemical characterisation the plants. There is gap of information regarding the flavonols and flavone contents of mangrove plants. The mangrove plants of the family *Rhizophoraceae* show comparatively higher antioxidant capacity which can be attributed to their higher phenolic content (Arivuselvan *et al*., 2011; Agooramoorthy *et al*., 2008). Also, they are source of potent antiviral substances (Premanathan *et al*., 1999). This chapter is an effort to quantify and qualify five common antioxidant food flavonoids; three major flavonols (kaempferol, quercetin, and myricetin) and two major flavones (luteolin and apigenin) in the leaves and bark of five mangrove plants; *B. cylindrica*, *B. gymnorrizha*, *K. candel*, *R.mucronata* and *R. apiculata* belonging to *Rhizophoraceae* family using High performance liquid chromatography. All the strategic plant materials have not yet been investigated and quantified for the specific flavonols (kaempeferol, quercetin, myricetin) and flavones (apigenin and luteolin). So, the present work would be informative and novel with regard to the quantification of these specific flavonoids and plant materials. Such study is valuable for researchers in providing a base line data for future detailed characterisation of other phenolics in these and related plants. The total flavonoids from these plants were also assayed. Furthermore, isolation of a bioactive component from *R.mucronata* was attempted.

4.2 Results

4.2.1 Total Flavonoids

Total flavonoid content (TFC) as determined by Aluminium chloride assay, is reported as quercetin equivalents per gram of dry weight of the sample (mg QE g^{-1}) (Table 4.1). The highest value for TFC was found to be in the leaves of *K. candel* (10.68±0.27 mg QE g^{-1}) whereas the lowest value was observed for *R.apiculata* bark (2.06±0.03 mg QE g^{-1}). Also *R.apiculata* leaves exhibited lowest TFC (5.51±0.35) among the leaves samples under investigation.

Table 4.1 Total flavonoid content (TFC) of the leaves and bark of Rhizophoraceae mangroves

Plant name	Part examined	TFC (mg QE g^{-1})
B. cylindrica	Leaves	7.28±0.36
	Bark	2.62±0.06
B. gymnorrhizha	Leaves	5.59±0.26
	Bark	2.13±0.29
K. candel	Leaves	10.49±0.25
	Bark	3.29±0.10
R. apiculata	Leaves	5.52±0.35
	Bark	2.07±0.03
R.mucronata	Leaves	7.23±0.53
	Bark	3.36±0.19

4.2.2 Flavonoid agylcons

The flavonoid agylcons, myricetin, quercetin, luteolin, kaempferol and apigenin contents were determined using LCUV-MS. In negative electrospray ionisation mode in the presence of mobile phase, standard quercetin,

kaempferol, myricetin, apigenin and luteolin formed a strong deprotonated molecule $[M-H]^-$ at m/z 301; 285; 317; 269 and 285, respectively (Table 4.2).

Table 4.2 HPLC retention time and m/z of flavonoid aglycons

Flavonoid aglycon	Retention time (In Minutes)	m/z $[M-H]^-$
Myricetin	12.1	317
Quercetin	16.6	301
Kaempferol	27.9	285
Luteolin	19.5	285
Apigenin	31.2	269

The flavonoid aglycons in the mangrove leaves and bark extracts on dry weight basis are presented in Table 4.2. The variations of the agylcons in the mangrove are as follows:

B. cylindrica

In the leaves and bark *B. cylindrica* the flavonoid found in higher concentration was quercetin (93.7±2.03 mg kg^{-1} and 13.96±2.03 mg kg^{-1} respectively). All the three flavonols and the flavone, luteolin are detected in this species. While luteolin was detected only in the bark, apigenin was not at all detected in this plant. The total concentration of the food flavonoids analysed is 2.9% of the TFC in leaves and 1.25% of the TFC in the bark of *B. cylindrica*.

Table 4.2 Results from quantitative and qualitative analysis of flavonoid agylcons (in mg kg $^{-1}$) of dry weight) from the leaves and bark of *Rhizophoraceae* mangroves.

Plant name	Plant part	Myricetin	Quercetin	Luteolin	Kaempferol	Apigenin
B. cylindrica	Leaves	29±0.78	93.7±2.03	30.5±.89	56±1.09	ND
	Bark	4.8±0.10	13.96±0.02	ND	9±0.14	ND
B. gymnorrizha	Leaves	803±19.09	404±12.12	ND	28±0.84	ND
	Bark	219±5.57	11±0.33	ND	16±0.48	ND
K. candel	Leaves	192±3.76	3570±107.1	ND	1463±39.89	1264±27.92
	Bark	ND	182±5.46	ND	26±0.56	116±3.09
R. apiculata	Leaves	ND	1079±32.37	ND	166±3.98	358±8.74
	Bark	77±1.31	23.7±0.71	ND	ND	ND
R.mucronata	Leaves	59.8±1.59	1960±58.8	ND	585±14.55	ND
	Bark	66±0.2	237±7.11	ND	26±0.49	ND

ND: Not detectable

B. gymnorrizha

B. gymnorrizha showed higher concentration of myricetin with leaves having 803±19.09mg kg $^{-1}$ and bark having 219±5.57 mg kg^{-1}. Quercetin levels were found to be lower than myricetin concentration. Both flavones were not detected in this plant but all the three flavonols were present in *B. gymnorrizha*. Thus in the leaves the five agylcons occur 27% of the total flavonoid concentration and 15% of the TFC in bark.

K. candel

Out of the five flavonoids analysed, four flavonoids were found to be present in the leaves whereas only three were found in the bark. Luteolin was not detected in this plant while myricetin was detected only in its leaves. The flavonoid agylcons form 69% of TFC in leaves and 13% of TFC in bark of *K. candel*. The highest concentration was observed for quercetin followed by kaempferol . The presence of apigenin in relatively higher concentration was also noticed in this plant.

R. apiculata

The leaves of *R. apiculata* showed the presence of flavonols- quercetin and kaempferol- and the flavone, apigenin. The flavonol, myricetin and the flavone, luteolin was not detected in the leaves. In the bark only myricetin and quercetin was detected. Quercetin was found to be the flavonoid present in highest concentration (1079±32.37 mg kg^{-1}) in the leaves whereas myricetin (77±1.31 mg kg^{-1}) dominated in the bark of *R. apiculata*. The flavonoid agylcons form 38% of the TFC in leaves and 6% of TFC in bark.

R.mucronata

The two flavones under investigation were found to be below the detection limits. The flavonoid content is dominated by quercetin both in the leaves and the bark; 1960±58.8 mg kg^{-1} and 237±14.55 mg kg^{-1} respectively. It can be inferred from the results that the five flavonoids form 42% of TFC in leaves and 10% of TFC in bark.

4.3 Discussion

4.3.1 Flavonoid distribution in the plants and prospects in bioactivity studies

The contents of flavonols, especially quercetin derivates, appear to increase with increased light irradiation, whereas temperature has a less prominent effect on the total flavonol content. However, there is some evidence that low temperature would increase the quercetin: kaempferol ratio (Jakkola and Hohtola, 2010). But under the same climatic regime, the variations in the flavonoid content can be considered as the genetic nature of the plants.

The total flavonoids are found to be more concentrated in the leaves than in the bark (Fig. 4.3). In plants, flavonoids play significant the role in photoprotection, UV protection and to manage heat stress as well as water stress (Andersen and Markham 2010; Agati and Tattini 2010). Marinova *et al.*, 2005 has reported that in some of the vegetables such as spring onion, leeks and beans the green parts showed higher phenolic as well as flavonoid content which has been credited to the fact that change in the colouring of the plant is a process associated with the redistribution of phenolics and flavonoids. So the more accumulation of flavonoids in the leaves of

mangroves of this study can be attributed to the more exposure of the leaves to solar radiation than the bark.

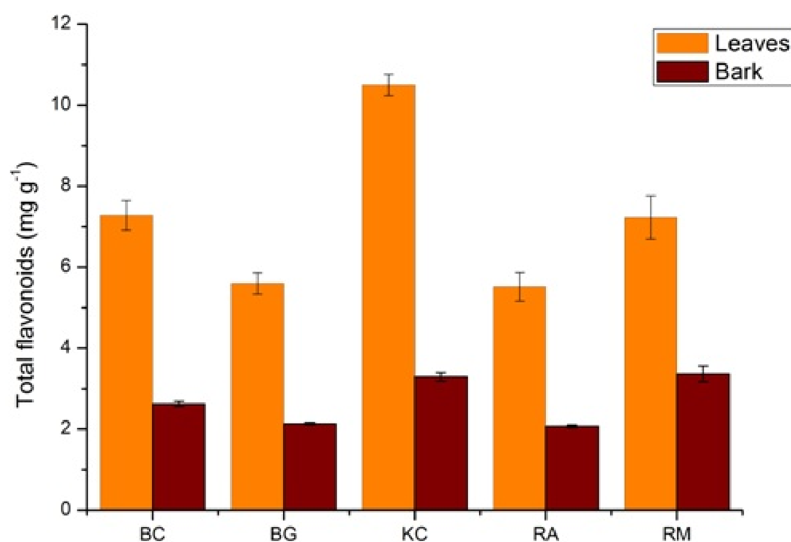

Fig. 4.3 Variation of total flavonoids in leaves and bark of *Rhizophoraceae* mangroves

It is evident from the results that the flavonoid agylcons are more concentrated in the leaves of the mangroves than in the bark. The TFC values of leaves and bark also supports this observation. As the formation of flavonoids is light-dependent, flavonoids occur predominantly in the leaves, and growing plants in glass houses reduces the flavonoid content (Lugasi *et al*., 2003). As the results for total phenolics (chapter 4) indicate, the bark is rich in phenolics. The bark of mangroves are reported to be rich sources of tannin (Nurulhuda *et al*., 1990). So it can be inferred that the bark of mangrove plants are rich in phenolics other than flavonoids. Jaakola *et al*., 2004 examined the activation of flavonoid biosynthesis by solar radiation in bilberry (Vaccinium myrtillus) leaves. The flavonol quercetin, various

anthocyanins glycosides, and the hydroxycinnamic acids were shown collectively to play a predominant role in the defense against high solar radiation; all of these compounds increased markedly with greater exposure to sunlight. In red onions, higher concentrations of quercetin occur in the outermost rings (Smith *et al*., 2003). So, the increased levels of flavonoids observed in the leaves of mangroves than their barks may be attributed to the increased exposure of the leaves to solar radiation than the barks.

The percentage of flavonoid agylcons to the TFC in the mangrove plants of the genus *Bruguiera* is shown in Fig. 4.4. Among the plants of this study, *B. cylindrica* is the species found to possess the least concentration of the food flavonoids. Apigenin was not detected in B. cylindrica but this is the only plant of this study in which luteolin is present. The dominance of flavonols over flavones is a noticeable feature of *B. cylindrica*. Various combinations of flavones and flavonols have been shown to exhibit synergism. Kaempferol and luteolin show synergistic effect against herpes simplex virus (HSV) (Amoros *et al*., 1992). The presence of luteolin along with kaempferol in the leaves of *B. cylindrica* suggests the possibility of such a synergism in its extracts which can impart bioactive properties. The fruits, leaves and roots of *B. cylindrica* are traditionally used for the treatment of hepatitis (Bandaranayake, 1998). The bark of *B. cylindrica* is used to stop hemorrhage and applied to malignant ulcers (Basu and Kirtikar, 1999).

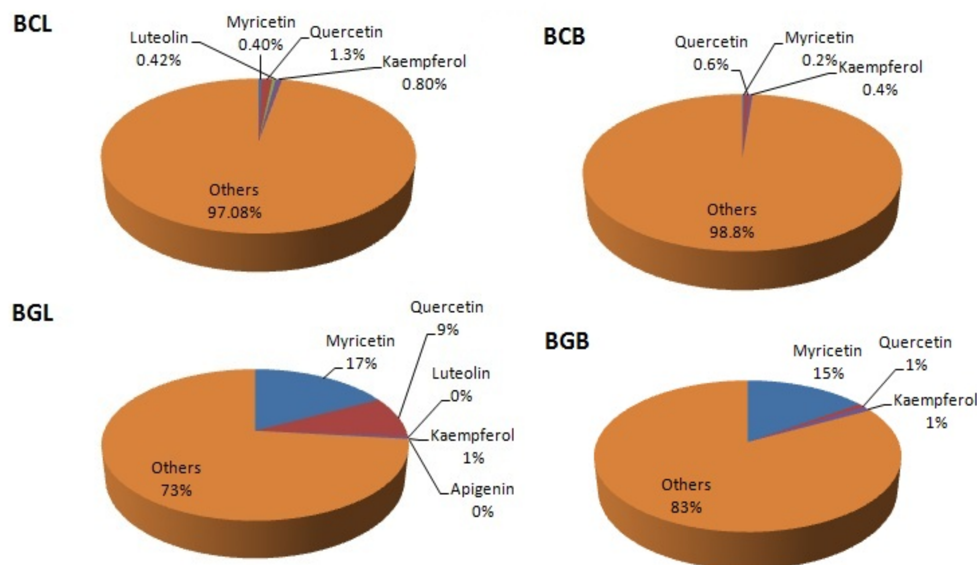

Fig. 4.4 Flavonoid agylcons in the leaves and bark of *Bruguiera* mangroves

Natake *et al*. (1989) found that sage decreased the Trp-P-2 mutagenicity and Samejima *et al*. (1995) reported that the compound of sage responsible for this effect was luteolin. Study by Sadhu *et al*., 2006) revealed the presence of two flavonoids luteolin and luteolin 7-O-β-glucoside from the dried powdered leaf of *Sonneratia caseolaris* from Sundarban mangroves of Bangladesh possessing antioxidant activity. The presence of luteolin in *B. cylindrica* thus reveals its relevance towards using it as medicinal source.

The leaves, barks and fruits of *B. gymnorrizha* are used as traditional medicine for different purposes by the endogenous people. The bark of *B. gymnorrizha* is used to treat burns in the Solomon Islands, malaria in Cambodia, cure fish poisoning in Marshall Islands, treat diarrhoea and fever in Indonesia while its leaves are used to control blood pressure in India

(Bandaranayake, 1998).Only the flavonols were found to be present in *B. gymnorrizha*. It is found to be the species having highest myricetin concentration among the plants analysed. The myricetin content in the leaves and bark of *B. gymnorrizha* was found to exceed the quercetin content of these tissues. *B. gymnorrhizha* possessing appreciably high concentration of myricetin than other dietary sources; fruits and vegetables (Lugasi *et al*., 2003), can be derived as a reliable source of this compound. The bark of *B. gymnorrizha* was found to be the flavonoid rich bark (Fig. 4.5) in the present study.

K. candel is the richest source of antioxidant food flavonoids in this study with the highest percentage of the flavonoid agylcons studied (Fig. 4.5). This can be related to the difference in stress responsive mechanism among the plants. The highest levels of quercetin, kaempferol and apigenin express the activation of the oxidative stress as well as the UV protection mechanism in this plant as dihydroxy B ring substituted flavonoids (flavonols) have a greater antioxidant capacity, while their monohydroxy B ring substituted counterparts (apigenin) have greater ability to absorb UV-wavelengths (Kumar and Pandey, 2013). So the extract from this plant may have greater antioxidant capacity as well as high UV- absorbing property. It has also been reported that the flavonoids chrysin, acacetin, and apigenin prevent HIV-1 activation via a mechanism that probably involves inhibition of viral transcription (Critchfield *et al*., 1996). Also, it has been reported that consumption of onions and/or apples, two major sources of the flavonol quercetin, is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast. So, the higher contents of these

flavonoids in *K. candel* imply its prospective role in prevention of these diseases.

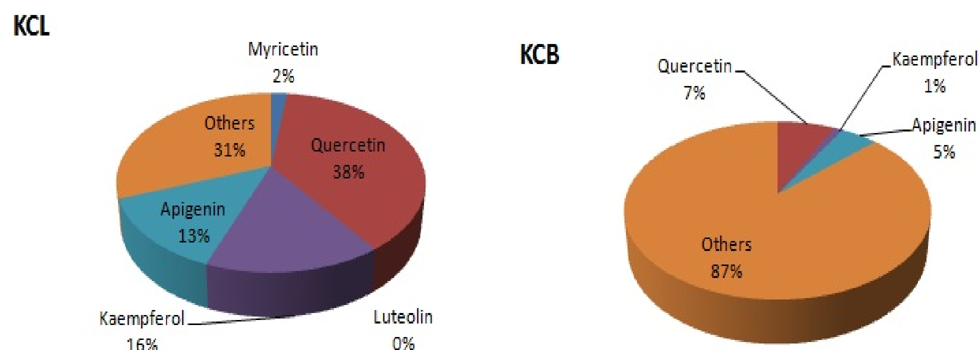

Fig. 4.5 Flavonoid agylcons in the leaves and bark of *K. candel*

The leaves of the mangrove plant, *R. apiculata* are found to possess a high flavonol content dominated by quercetin and kaempferol while the bark showed the only the presence of myricetin and quercetin. The presence of apigenin is an important feature of this plant. The accumulation of myricetin only in the bark makes *R. apiculata* distinct from other plants of the study. Warm aqueous extract of the bark of *R. apiculata* is used as astringent for diarrhoea, nausea, and vomiting, and as an antiseptic. The extract is also used to stop bleeding in fresh wounds and for the treatment of chronic typhoid fever. So it can be inferred that the potential bioactive character of the bark of *R. apiculata* may have a positive influence of the two flavonols, myricetin and quercetin. The distribution of flavonoid agylcons in the leaves and bark of *Rhizophora* plants is given in Fig. 4.6.

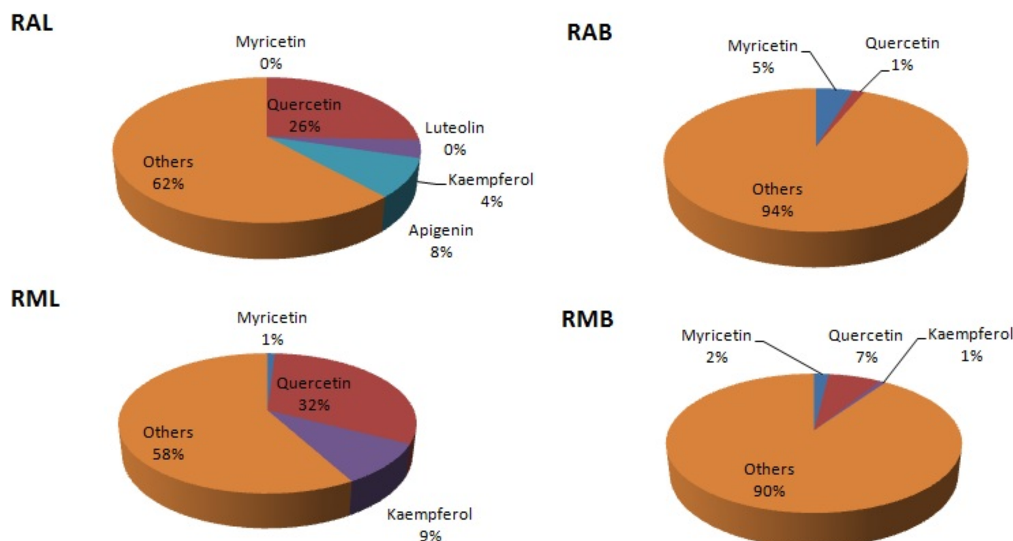

Fig. 4.6 Flavonoid agylcons in the leaves and bark of *Rhizophora* mangroves

R.mucronata is a flavonol rich mangrove plant. It exhibited a qualitatively similar flavonoid profile as that of *B. gymnorrizha* but possess higher concentrations. *R.mucronata* bark was found to be rich in myricetin content than its leaves. When compared with the other plants the bark of this plant has the highest antioxidant flavonoid content. The observation is supported by the result of DPPH radical scavenging assay in which the bark of *R.mucronata* exhibiting a high activity.

Quercetin the most ubiquitous flavonoid found in plants. Quercetin besides being antibacterial is an effective antioxidant. Its glycosidic form should also be equally effective since in biological system glycosides undergo enzymatic hydrolysis to the corresponding aglycone (Murota and Terao, 2003). The present investigation reveals its presence and quantity in the mangrove plants. It is the flavonoid found to be present in all the

mangroves. Among leaf samples, the leaves of *B. cylindrica* contain lowest quercetin content. The bark of *B. gymnorrizha* is bark sample having lowest quercetin content.. In a study conducted by D' souza *et al*., 2010 on phenolics from *L.racemosa*, myricetin was found to be more inhibitory than quercetin, and was effective against six of eight bacterial strains (*Escherichia coli, Klebsiella Pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexineri, Vibrio Cholera* and *Staphylococcus aureus*) tested. *Salmonella typhi* and *Vibrio cholerae* were the two strains that showed resistance to myricetin. Better potency of myricetin as compared to quercetin is attributable to the extra phenolic hydroxyl, since this is the only contrasting feature between the two structures. Evidence obtained with an in vitro oxidation model for heart disease has demonstrated that several plant flavonols, such as quercetin, myricetin, and rutin are more powerful antioxidants than the traditional vitamins (Vinson *et al*., 1995). The flavonol, kaempferol showed its presence in all the *Rhizophoraceae* mangroves under investigation. It showed highest value in *K. candel* while it was absent in the bark of *R. apiculata*. Quercetin and kaempferol are proved to be the most widespread flavonoids in vegetables and herbs (Lugasi *et al*., 2003, Justesen and Knuthsen, 2001). The results of the present study are also in favour of this observation. Apigenin is the major flavonoid found in honey and Urtica sp. (Karakaya and Nehir, 1999). Urtica sp. is one of the herbs used frequently for traditional cancer treatment among Turkish people. It is consumed by boiling of herbs and drinking the boiled juice. The presence of apigenin in mangroves *K. candel* and *R. apiculata* indicate the prospects of these mangroves in development of novel therapeutic agents based this compound.

The health influences of flavonoids have yet to be fully established although they have been shown to function in a way similar to antioxidant vitamins and to protect against lipoprotein oxidation in vitro and to have anti-platelet anti-thrombotic actions (Crozier *et al*., 1997). There are, therefore, grounds for investigating natural sources rich in flavonoids.

4.3.2 Inter-specific variations of flavonoids and their chemotaxonomic significance

The variation of the three flavonols myricetin, quercetin and kaempferol among the plant species is shown in Fig. 4.7. The flavonol concentration dominates the flavones in the mangrove plant parts. Quercetin is the flavonoid found to be present in greater concentration in the plant tissues. In this study, the genus *bruguiera* showed low levels of quercetin when compared with the other two; Kandelia and Rhizophora. Next to *K. candel*, *Rhizophora* mangroves possess higher quercetin concentration. *Bruguiera* plants showed lower quercetin content than the other plants. Previous studies also report quercetin as a major flavonoid present in fruits and vegetables (Hertog *et al*., 1992; Justesen and Knuthsen, 2001). Eventhough the leaves of *R. apiculata* and the bark of *K. candel* lacked myricetin, it showed its presence all the plants of current investigation. From the taxonomic viewpoint, presence and absence of myricetin is very significant. Its presence is considered as a primitive character in dicots, particularly in woody plants (Joshi *et al*., 2004; Harborne, 1966). Thus all the mangroves of the family *Rhizophoraceae* can be regarded as primitive in flavonoid patterns because of the presence of myricetin.

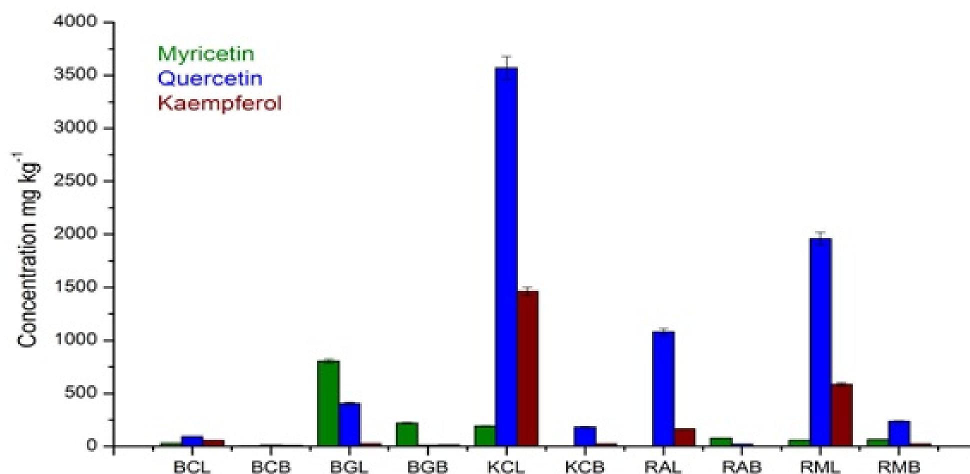

Fig. 4.7 Variation of myricetin, quercetin and kaempferol concentration among *Rhizophoraceae* mangroves.

The flavonol, kaempferol showed its presence in all the *Rhizophoraceae* mangroves under investigation. It showed highest value in *K. candel* while it was absent in the bark of *R. apiculata*. Next to the Kandelia species, the genus Rhizophora was found be the best contributor of kaempferol. The *bruguiera* plants showed very low concentration of kaempferol in their tissues.

Flavones, luteolin and apigenin have restricted distribution in the family *Rhizophoraceae* (Fig. 4.8). Luteolin was found in detectable levels only in *B. cylindrica* which is reveals its chemotaxonomic relevance. In all others species this flavone was not detected. The presence of apigenin was detected only in *K. candel* and *R. apiculata*. It was not detected in the genus Bruguiera pointing towards its chemotaxonomic importance. Comparing the flavonoid composition of the five species reveals greater similarity among *R.mucronata* and B. *gymnorrizha* having only quercetin, myricetin

and kaempferol in their leaves and bark. The plants of the same genus, *R. mucronata* show distinct flavonoid composition with respect to the absence of myricetin and apigenin. Myricetin is not detected in the leaves of *R. apiculata* while apigenin was present only in its leaves but not in *R. mucronata* showing the biochemical distinctiveness of the two plants.

The presence of flavonoids in plant foods is largely influenced by genetic factors and environmental conditions and other factors such as germination,degree of ripeness, variety, processing, and storage also influence the content of plant phenolics (Aherne and O'Brien, 2002; Anderson and Markham, 2010). The aglycone results do not completely agree with the existing classifications. Such disagreement in the aglycon content and classical taxonomy is been previously reported in family *Dipterocarpaceae* in Sri Lanka (Joshi *et al*., 2004) suggesting the need for a revision of the species and sectional levels in the classical taxonomy based on morphological parameters. Eventhough the present findings are useful in chemical characterisation of *Rhizophoraceae* mangroves more comprehensive investigation on other areas, such as molecular, cytological, ecological as well as biogeographical aspects are also needed to draw the specific relationships of these plants as the interaction of a number of factors, including species differences, the light regimes under which the plants were grown, as UV-B irradiation is known to induce the accumulation of flavonoids (Li *et al*., 1993; Lois, 1994; Crozier *et al*., 1997).

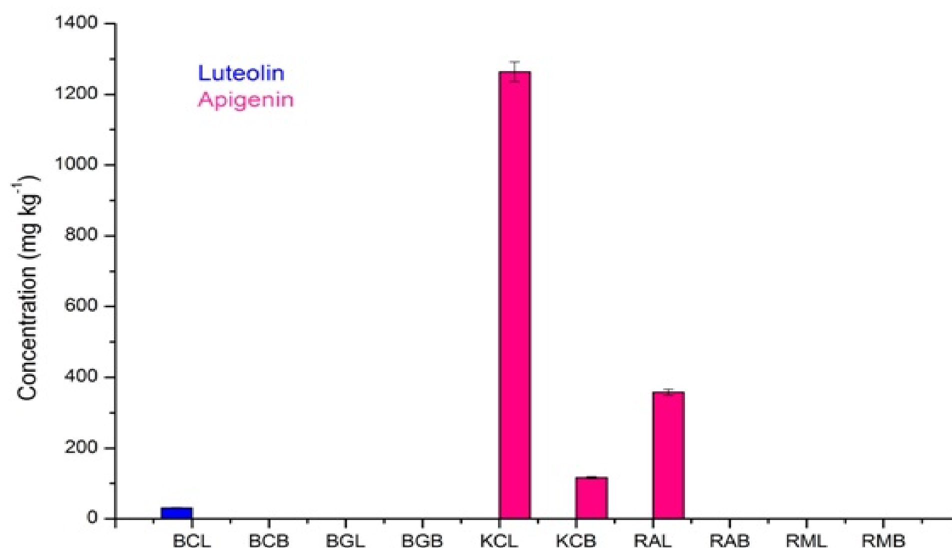

Fig. 4.8 Variation of luteolin and apigenin concentration among *Rhizophoraceae* mangroves

4.3 Flavonoids from *R.mucronata*

R.mucronata can be considered as a high value source of phenolic compound due to the high amount of this group of compounds (Chapter 3, Table 3.3). Comparatively higher amounts of flavonoids (Fig. 4.3) in these plants, its widespread application in ethnopharmaceutical practices and the extensive distribution of these plants along Kerala coast are the main reasons for selecting this plant for extraction of antioxidant metabolites.

DPPH is usually known as a stable free radical and become a stable diamagnetic molecule after assumes electron or hydrogen radical (Soares *et al*., 1997). As it can be inferred from Table 3.3 the leaves and bark of *R.mucronata* exhibits very good antioxidant capacity compared with the other mangroves plants under investigation. The results of free radical scavenging test showed methanol extract of leaves and bark are significantly

comparable with positive controls ascorbic acid in terms of scavenging capacity (Fig. 4.9). After partitioning, the best scavenging activity among the examined extracts was achieved from hexane and ethyl acetate extract of leaves (90.5 and 86.5% respectively) and the aqueous extract showed poor antioxidant activity in terms of scavenging capacity. The ethyl acetate fraction which was reported to contain most antioxidant flavonoids is selected for further separation and analysis.

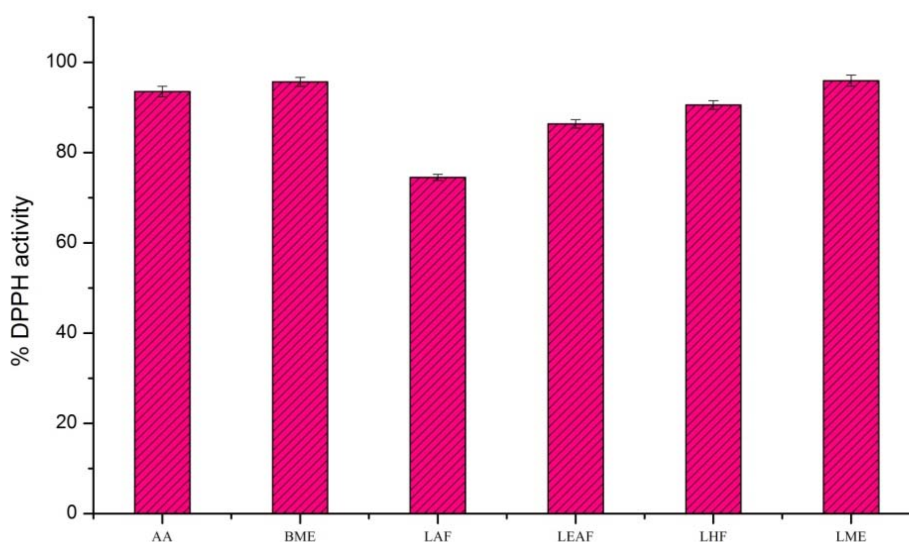

Fig. 4.9 Antioxidant activity of various extracts (LME- Leaf methanol extract, BME- Bark methanol extract, LHF- Leaf hexane fraction, Leaf ethyl acetate fraction, Leaf aqueous fraction, A-Ascorbic acid) of leaves and bark of *R.mucronata* using DPPH activity assay.

Proanthocyanidins are oligomers and polymers of the flavan-3-ol monomer unit that are most commonly built up with repeat units of (epi)catechin, (epi)gallocatechin, or (epi)afzelechin (Fig. 4.10) (Liu *et al.*, 2013). Bioactivities of proanthocyanidins are influenced by the diversity of their structures including the degree of polymerisation, the content of

epicatechin unit in the polymers, the stereochemistry and hydroxylation pattern of the flavan-3-ol starter and extension units, and the position of the linkage between two monomeric units (A type OPCs or B type OPCs) (Wang *et al.*, 2013; Dixon *et al.*, 2005).

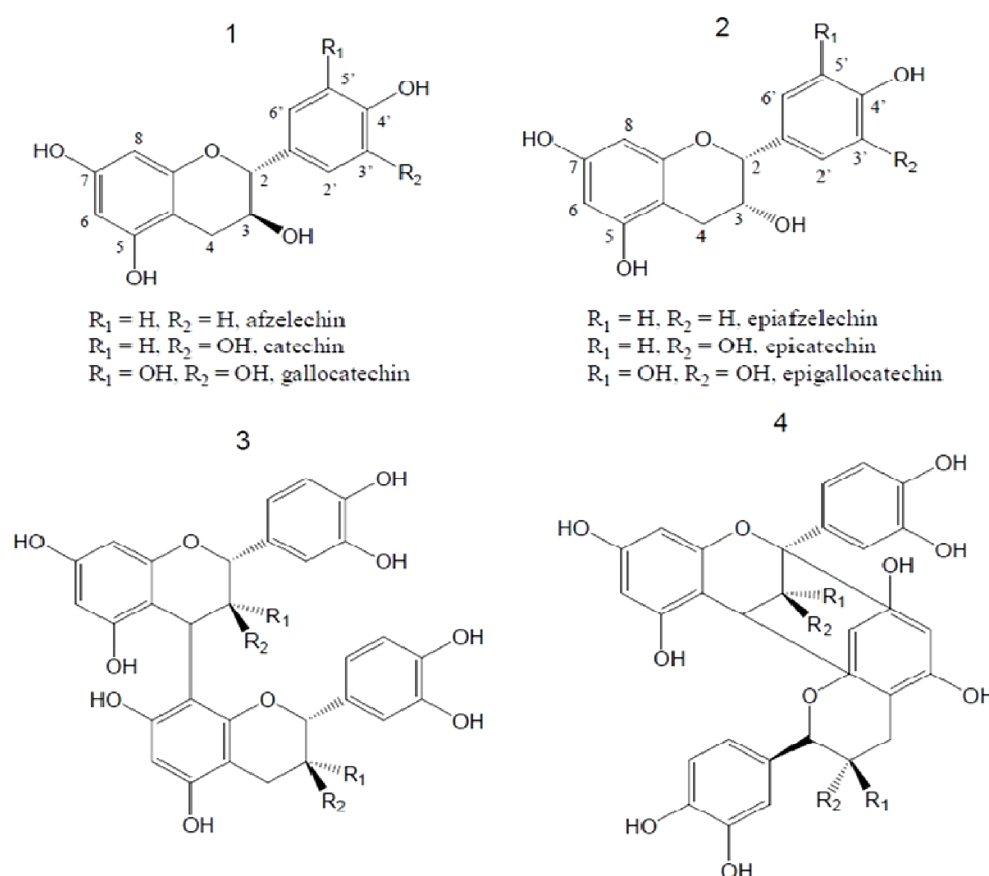

Fig. 4.1 Structures of the flavan-3-ol building blocks (1 and 2) of proanthocyanidins, B-type (3) and A-type (4) dimeric blocks of proanthocyanidins. B-type (dimeric) are characterized by single linked flavonyl units between C-4 and C-8 (3) or C-6 (not shown), while A-type possess an additional ether linkage between C-2 of the upper unit and a 7- and/or 5-OH of the lower unit. Where R1 = H and R2 = OH, catechin; R1 = OH and R2 = H, epicatechin.

On the basis of TLC and HPLC analysis, the fractioned were separated using column chromatography followed by HPLC semipreparative separation to give Fraction 1. Fraction 1 was subjected to LCUV- MS analysis which has given a base peak at 577 $[M–H]^-$ (Fig. 4.10a,b) at 280nm (UV absorption maxima at 278, 234). As literature indicates, this is characteristic to proanthocyanidin dimers (Kajdžanoska *et al*., 2010). Catechin dimers identified in *R.mucronata* leaf extracts include type B proanthocyanidin dimers, detected by molecular ion $[M–H]^-$ *m/z* 577 (Määttä *et al*., 2004).

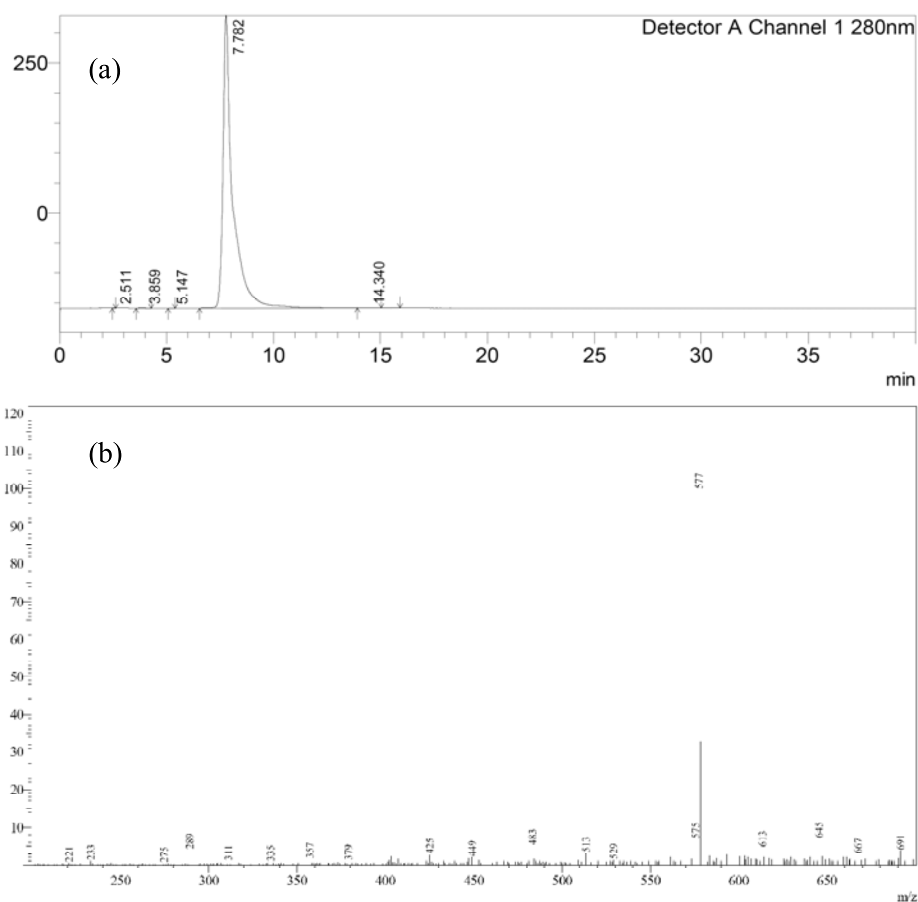

Fig. 4.11 a) LCUV chromatogram and b) LCMS spectrum of fraction 1 isolated from *R. mucronata*

^{1}H NMR spectra (Fig. 4.12) also support the presence of proanthocyanidin, with signals matching published spectra (de Bruyne, 1999). Signals from 6.4 to 7.0 ppm correspond to protons at the 2′-, 5′-, and 6′- positions. Those from 4.8 to 5.2, from 4.2 to 4.8, and from 3.6 to 4.1 ppm correspond to protons at the 2-, 3-, and 4-carbon positions, respectively. Aromatic hydroxyl group signals were apparent from 8.5 to 9.0 ppm. The 6-position proton signals are found at 5.8 ppm with very minimal 8-proton signals at 6.0-6.2 ppm. This, along with the location of the 4-position proton signal, shows the proanthocyanidins are linked with linear 4→8 bonds. If the molecules were composed of 4→6 bonds, the protons at the 8-position would be present and the 4-position proton signals would be shifted downfield.

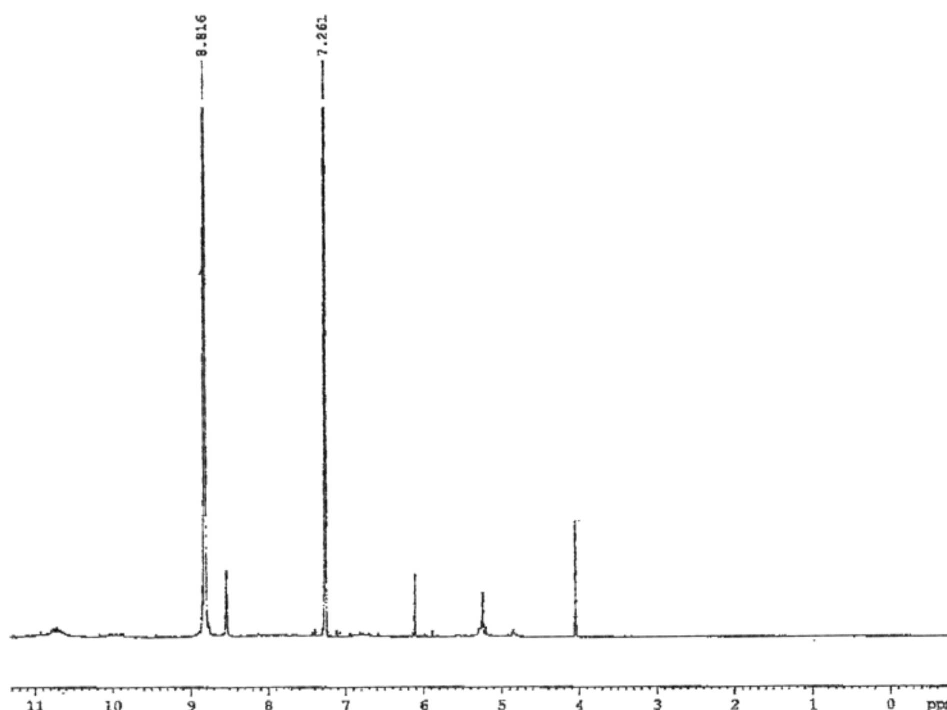

Fig. 4.12 ^{1}HNMR spectrum of proanthocyanidin type B from *R. mucronata* leaves.

This present investigation thus confirms the presence of the bioactive molecule, proanthocyanidin B in the leaves of R. mucronata. Among these proanthocyanidins, proanthocyanidin B has been shown to be the most effective compound in trapping oxygen free radicals (Lopez da Silva *et al*., 2007). Further investigation is needed for the detailed structural determination and confirmation as well as the abundance of this molecule to evaluate its beneficial properties which are outside the scope of this study.

4.4 Conclusion

This study suggests B. *gymnorrizha* can be a very good source of myricetin. Quercetin was found to be the ubiquitous flavonoid and found to be present in high concentration. It is the flavonoid aglycon which is present in highest concentration in *Rhizophora* plants and *K. candel*. The leaves of *K. candel* were found to be a rich source of quercetin than the other species under investigation followed by *R.mucronata* leaves. The observed levels of flavonoid contents confirm the importance of these mangrove plants as excellent sources of plant antioxidants. The HPLC results showed that the five *Rhizophoraceae* mangroves studied are rich sources of the important biologically active food flavonoids. The quantitative analysis of these flavonoids from the plants under investigation is being for the first time. The presence of luteolin in B. *cylindrica* alone as well as the variations in different flavonols and flavones in these plants can be helpful for providing chemotaxonomic relationships between different genus of this family. *R. mucronata* contains the flavonoid, Proanthocyanidin Type B which is isolated for the first time from this plant.

If it is accepted that higher intakes of flavonoids are associated with long-term health benefits, then the data presented in this chapter offer possible avenues for horticultural approaches toward health promotion, by identifying and selecting varieties rich in flavonoids. However, further investigations involving more detailed studies are required to ascertain inclusive phenolics anti-oxidant system of these plant materials as well as to establish factors effecting the variations in flavonoid content in order to develop their application for particular chemotaxonomic, food and nutraceutical purposes.

References

Abdul-Jalil, T. Z., Saour, K., and Nasser, A. M. (2010). Phytochemical Study of some Flavonoids Present in the Fruits of Two Ammi L. Species Wildly Grown in Iraq. *Iraqi J. Pharm. Sci*, *19*, 48-57.

Agati, G., and Tattini, M. (2010). Multiple functional roles of flavonoids in photoprotection. *New Phytologist*, *186*(4), 786-793.

Agoramoorthy, G., Chen, F. A., Venkatesalu, V., Kuo, D. H., and Shea, P. C. (2008). Evaluation of antioxidant polyphenols from selected mangrove plants of India. *Asian Journal of Chemistry*, *20*(2), 1311-1322.

Aherne, S. A., and O'Brien, N. M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition*, *18*(1), 75-81.

Amoros, M., Simõs, C. M. O., Girre, L., Sauvager, F., and Cormier, M. (1992). Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. *Journal of Natural Products*, *55*(12), 1732-1740.

Andersen, O. M., and Markham, K. R. (Eds.). (2010). *Flavonoids: chemistry, biochemistry and applications*. CRC Press.

Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R., and Kinae, N. (2000). Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *The Journal of nutrition*, *130*(9), 2243-2250.

Arivuselvan, N., Silambarasan, D., Govindan, T., and Kathiresan, K. (2011). Antibacterial activity of mangrove leaf and bark extracts against human pathogens. *Advances in Biological Research*, *5*(5), 251-254.

Bandaranayake, W. M. (1998). Traditional and medicinal uses of mangroves.*Mangroves and salt marshes*, *2*(3), 133-148.

Basu, B. D., Kirtikar, K. R.. (1999). *Indian medicinal plants* . 2nd ed. International Book Distributors, Dehradun, p. 1012.

Cao, G., Sofic, E., and Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine*,*22*(5), 749-760.

Critchfield, J. W., Butera, S. T., and Folks, T. M. (1996). Inhibition of HIV activation in latently infected cells by flavonoid compounds. *AIDS research and human retroviruses*, *12*(1), 39-46.

Crozier, A., Lean, M. E., McDonald, M. S., and Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry*, *45*(3), 590-595.

de Bruyne, T., Pieters, L., Dommisse, R., Kolodziej, H., Wray, V., Berghe, D. V., Vlietinck, A., (1999). NMR characterization and biological evaluation of proanthocyanidins: a systematic approach. *Plant Polyphenols 2 Basic Life Sciences, 66*, 193-209

Dinelli, G., Bonetti, A., Minelli, M., Marotti, I., Catizone, P., and Mazzanti, A. (2006). Content of flavonols in Italian bean (< i> Phaseolus vulgaris</i> L.) ecotypes. *Food chemistry*, *99*(1), 105-114.

Dixon, R. A., Xie, D. Y., and Sharma, S. B. (2005). Proanthocyanidins–a final frontier in flavonoid research?. *New phytologist*, *165*(1), 9-28.

D'Souza, L., Wahidulla, S., and Devi, P. (2010). Antibacterial phenolics from the mangrove Lumnitzera racemosa. *Indian Journal of Marine Sciences*, *39*(2), 294.

Fischer-Iglesias, C., Sundberg, B., Neuhaus, G., and Jones, A. M. (2001). Auxin distribution and transport during embryonic pattern formation in wheat. *The Plant Journal*, *26*(2), 115-129.

Gordon, M. H., and Roedig-Penman, A. (1998). Antioxidant activity of quercetin and myricetin in liposomes. *Chemistry and physics of lipids*, *97*(1), 79-85.

Harborne, J. B., Ed. The Flavonoids, Advances in Research Since 1986; Chapman and Hall: London, 1994.

Heim, K. E., Tagliaferro, A. R., and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of nutritional biochemistry*, *13*(10), 572-584.

Hertog, M. G., Hollman, P. C., and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of agricultural and food chemistry*, *40*(12), 2379-2383.

Hertog, M. G., Hollman, P. C., and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of agricultural and food chemistry*, *40*(12), 2379-2383.

Hertog, M. G., Hollman, P. C., and Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of agricultural and food chemistry*, *41*(8), 1242-1246.

Hertog, M. G., Hollman, P. C., and Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of agricultural and food chemistry*, *41*(8), 1242-1246.

Jaakola, L., and Hohtola, A. (2010). Effect of latitude on flavonoid biosynthesis in plants. *Plant, cell* and *environment*, *33*(8), 1239-1247.

Jaakola, L., Määttä-Riihinen, K., Kärenlampi, S., and Hohtola, A. (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (Vaccinium myrtillus L.) leaves. *Planta*, *218*(5), 721-728.

Joshi, K., Seneviratne, G. I., and Senanayake, S. P. (2004). Leaf flavonoid aglycone patterns in the species of Dipterocarpaceae in Sri Lanka. *Biochemical systematics and ecology*, *32*(3), 329-336.

Justesen, U., and Knuthsen, P. (2001). Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes.*Food Chemistry*, *73*(2), 245-250.

Justesen, U., and Knuthsen, P. (2001). Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes.*Food Chemistry*, *73*(2), 245-250.

Kajdžanoska,M., Gjamovski,V., Stefova1, M. (2010). HPLC-DAD-ESI-MS^n identification of phenolic compounds in cultivated strawberries from Macedonia. *Macedonian Journal of Chemistry and Chemical Engineering*. *29*(2), 181–194.

Karakaya, S., and Nehir EL, S. (1999). Quercetin, luteolin, apigenin and kaempferol contents of some foods. *Food chemistry*, *66*(3), 289-292.

Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., ... and Aromaa, A. (2002). Flavonoid intake and risk of chronic diseases. *The American journal of clinical nutrition*, *76*(3), 560-568.

Kong, L., Luo, C., Li, X., Zhou, Y., and He, H. (2013). The anti-inflammatory effect of kaempferol on early atherosclerosis in high cholesterol fed rabbits. *Lipids in health and disease*, *12*(1), 112-115.

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, *2013*.

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, *2013*.

Li, J., Ou-Lee, T. M., Raba, R., Amundson, R. G., and Last, R. L. (1993). Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *The Plant Cell Online*, *5*(2), 171-179.

Liu, Y., Feng, S., Song, L., He, G., Chen, M., and Huang, D. (2013). Secondary Metabolites in Durian Seeds: Oligomeric Proanthocyanidins. *Molecules*, *18*(11), 14172-14185.

Lois, R., and Buchanan, B. B. (1994). Severe sensitivity to ultraviolet radiation in anArabidopsis mutant deficient in flavonoid accumulation. *Planta*, *194*(4), 504-509.

Lopes-da-Silva, F., Escribano-Bailon, M. T., Perez Alonso, J. J., Rivas-Gonzalo, J. C., Santos-Buelga, C. (2007). Anthocyanin pigments in strawberry, *Lebensm. Wiss. Technol.*, 40, 374–382.

Lopez-Lazaro, M. (2009). Distribution and biological activities of the flavonoid luteolin. *Mini reviews in medicinal chemistry*, *9*(1), 31-59.

Lugasi, A., Hóvári, J., Sági, K. V., and Bíró, L. (2003). The role of antioxidant phytonutrients in the prevention of diseases. *Acta Biologica Szegediensis*, *47*(1-4), 119-125.

M Calderon-Montano, J., Burgos-Morón, E., Pérez-Guerrero, C., and López-Lázaro, M. (2011). A review on the dietary flavonoid kaempferol. *Mini reviews in medicinal chemistry*, *11*(4), 298-344.

Määttä-Riihinen, K. R., Kamal-Eldin, A., and Törrönen, A. R. (2004). Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (family Rosaceae). *Journal of Agricultural and Food Chemistry*, *52*(20), 6178-6187.

Maggiolini, M., Recchia, A. G., Bonofiglio, D., Catalano, S., Vivacqua, A., Carpino, A., ... and Ando, S. (2005). The red wine phenolics piceatannol and myricetin act as agonists for estrogen receptor α in human breast cancer cells.*Journal of molecular endocrinology*, *35*(2), 269-281.

Maggiolini, M., Recchia, A. G., Bonofiglio, D., Catalano, S., Vivacqua, A., Carpino, A., ... and Ando, S. (2005). The red wine phenolics piceatannol and myricetin act as agonists for estrogen receptor α in human breast cancer cells.*Journal of molecular endocrinology*, *35*(2), 269-281.

Manach, C., Mazur, A., and Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. *Current opinion in lipidology*, *16*(1), 77-84.

Manach, C., Mazur, A., and Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. *Current opinion in lipidology*, *16*(1), 77-84.

Marinova, D., Ribarova, F., and Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, *40*(3), 255-260.

Miean, K. H., and Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, *49*(6), 3106-3112.

Miean, K. H., and Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, *49*(6), 3106-3112.

Murota, K., and Terao, J. (2003). Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Archives of Biochemistry and Biophysics*, *417*(1), 12-17.

Natake, M., Kanazawa, K., Mizuno, M., Ueno, N., Kobayashi, T., Danno, G. I., and Minamoto, S. (1989). Herb water-extracts markedly suppress the mutagenicity of Trp-P-2. *Agricultural and biological chemistry*, *53*(5), 1423-1425.

Nöthlings, U., Murphy, S. P., Wilkens, L. R., Henderson, B. E., and Kolonel, L. N. (2007). Flavonols and Pancreatic Cancer Risk The Multiethnic Cohort Study.*American journal of epidemiology*, *166*(8), 924-931.

Nurulhuda, M. N., Chew, L. T., Mohd, N. M. Y., and Abdul, R. M. A. (1990). Tannin properties of Rhizophora mucronata barks of different ages. *Holz als Roh-und Werkstoff*, *48*(10), 381-383.

Pace-Asciak, C. R., Hahn, S., Diamandis, E. P., Soleas, G., and Goldberg, D. M. (1995). The red wine phenolics< i> trans</i>-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease. *Clinica Chimica Acta*, *235*(2), 207-219.

Pandey, A. K., and Kumar, S. (2012). Antioxidant, lipo-protective and antibacterial activities of phytoconstituents present in Solanum xanthocarpum root.*International Review of Biophysical Chemistry (IREBIC)*, *3*(3), 42-47.

Patel, D., Shukla, S., and Gupta, S. (2007). Apigenin and cancer chemoprevention: progress, potential and promise (review). *International journal of oncology*, *30*(1), 233-246.

Premanathan, M., Kathiresan, K., and Nakashima, H. (1999). Mangrove halophytes: A source of antiviral substances. *South Pacific Study*, *19*(1-2), 49-57.

Sabitha, V., and Panneerselvam, K. (2103). Docking studies on polyphenols derivatives with insulin receptor tyrosine kinase - an insilico approach. *International Journal of Pharmaceutical Research and Bio-Science*, 2(5), 350-358.

Sadhu, S. K., Ahmed, F., Ohtsuki, T., and Ishibashi, M. (2006). Flavonoids from Sonneratia caseolaris. *Journal of Natural Medicines*, *60*(3), 264-265.

Samejima, K., Kanazawa, K., Ashida, H., and Danno, G. I. (1995). Luteolin: a strong antimutagen against dietary carcinogen, Trp-P-2, in peppermint, sage, and thyme. *Journal of Agricultural and Food Chemistry*, *43*(2), 410-414.

Samejima, K., Kanazawa, K., Ashida, H., and Danno, G. I. (1995). Luteolin: a strong antimutagen against dietary carcinogen, Trp-P-2, in peppermint, sage, and thyme. *Journal of Agricultural and Food Chemistry*, *43*(2), 410-414.

Shahidi, F., Janitha, P. K., and Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science* and *Nutrition*, *32*(1), 67-103.

Smith, C., Lombard, K. A., Peffley, E. B., and Liu, W. (2003). Genetic analysis of quercetin in onion (Allium cepa L.)'Lady Raider'. *Texas Journal of Agriculture* and *Natural Resources*, *16*.

Soare, J. R., Dinis, T. C., Cunha, A. P., and Almeida, L. (1997). Antioxidant activities of some extracts of Thymus zygis. *Free Radical Research*, *26*(5), 469-478.

Sokół-Łętowska, A., Oszmiański, J., and Wojdyło, A. (2007). Antioxidant activity of the phenolic compounds of hawthorn, pine and skullcap. *Food chemistry*,*103*(3), 853-859.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., and Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*, *43*(11), 2800-2802.

Wang, X. H., Huang, L. L., Yu, T. T., Zhu, J. H., Shen, B., Zhang, Y., ... and Gao, S. (2013). Effects of Oligomeric Grape Seed Proanthocyanidins on Heart, Aorta, Kidney in DOCA-Salt Mice: Role of Oxidative Stress. *Phytotherapy Research*, *27*(6), 869-876.

Yu, M. S., Lee, J., Lee, J. M., Kim, Y., Chin, Y. W., Jee, J. G., ... and Jeong, Y. J. (2012). Identification of myricetin and scutellarein as novel chemical inhibitors of the SARS coronavirus helicase, nsP13. *Bioorganic* and *medicinal chemistry letters*, *22*(12), 4049-4054.

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Chapter 5

FATTY ACIDS

Contents

5.1 Introduction
5.2 Results
5.3 Discussion
5.4 Conclusion

5.1 Introduction

A high genetic diversity within and among populations of a large number of mangrove species are demonstrated by a number of studies (Duke *et al*., 1998, 2002; Melville *et al*., 2004; Tan *et al*., 2005), which have led to contrasting conclusions in term of evolution and distribution of these plants (Dodd *et al*., 1998; Duke *et al*., 2002). This diversity may be due to inadequate species identification of mangroves (Dodd *et al*., 1998) but it nevertheless reflects a high degree of adaptability to the extreme conditions that characterise their environments (Hogarth, 1999). This adaptability can lead to a greater inter-specific similarity of biochemical compounds than that of geographically isolated populations of the same species (Dodd *et al*., 1998).

Mangroves contribute to complex food webs and important energy transfers. They create unique ecological environments that host rich

assemblages of species, from microfungi (Schmit and Shearer, 2004) to brachyuran crabs (Lee, 1997) and fishes (Sheaves and Molony, 2000). Fatty acids are ubiquitous in living organisms, and due to their biological specificity can act as biomarkers for prokaryotes, fungi, diatoms, dinoflagellates or vascular plants (Kristeinsen *et al*., 2008). They are therefore useful tracers of the origin and flow of mangrove-derived organic carbon through estuarine food webs (Gireeshkumar, 2013). Chemical tracers are increasingly used for investigating the dietary source of organisms (Meziane and Tsuchiya, 2000; Dalsgaard *et al*., 2003; Howell *et al*., 2003) and for differentiating taxa and even species of plants and animals (Graeve *et al*., 2002; Meziane *et al*., 2006). This application is based on the assumption that populations of the same species exhibit smaller degrees of variation compared to inter-specific differences in chemical signatures. Even though chemical tracers are used for trophodynamic analysis, few attempts have been made to evaluate this assumption underlying the techniques. The chemical character of the environment as well as geographic location may influence the universal nature of chemical tracer signatures.

The Fatty acids (FAs) identified as vascular plant markers in coastal environment are mainly the long chain fatty acids (LCFAs≥24:0) (Meziane and Tsuchiya 2000; Dalsgaard *et al*. 2003) and certain polyunsaturated fatty acids (PUFAs), especially 18:2ω6 and 18:3ω3 (Napolitano *et al*. 1997; Meziane *et al*. 2006). However, the PUFAs, 18:2ω6 and 18:3ω3, in contrary to LCFAs, are also common in other organisms than vascular plants, such as fungi and macroalgae (Chen *et al*. 2001; Graeve *et al*. 2002). Moreover, with respect to nutritional capacity, PUFAs are of much relevance. Therefore, in studies concerned with the export of the mangrove leaf

organic matter to the sediment through litter production and with the role of the mangrove as a primary food source for animals, preliminary checking must be done to ensure the importance of these plants in the environment and the composition of FAs in their tissues.

In this study, the variations in fatty acid profiles of common *Rhizophoraceae* mangrove species found in Kochi, southwest coast of India has been investigated with the aim of determining if differences occur in the fatty acid profiles of mangrove tissues (leaves and bark) and examining the prospect of using individual or groups of FAs as taxonomic and biomarkers. Also, effort has been done to analyse the nutritional aspects with respect to FA composition.

5.2 Results

5.2.1 Variation among different species

Detailed analysis of the FAs present in the leaves and bark of mangroves; *B. cylindrica*, *B. gymnorrizha, K. candel*, *R. apiculata* and *R. mucronata* are reported in Table 5.1. Twenty three fatty acids among the FAs from 7:0 to 30:0 were found to be present in the mangrove plants of this study. The FAs found ranged from FAs 12:0 to 30:0 with major FAs present (present in >90% of plant parts) being FAs 12:0, 14:0, 16:0,17:0, 18:0, *cis*-18:2ω6, 18: 3ω3 and *cis*-18: 1 ω9. The total concentration of the FAs; 16:0, *cis* -18:2ω6, 18: 3ω3 and 18: 1 ω9 formed 2.65 to 28.54% of the total lipid content in the plant parts. The total FA concentration ranged from 6210.2±12.5 µg g^{-1} to 12215.9±87.2 µg g^{-1} in the leaves of mangroves with the maximum value observed in *B. cylindrica* and minimum concentration in *R. mucronata*. Among the bark samples it ranged from 1395.8 ± 9.21µg g^{-1}

to 4140.06±10.09 µg g^{-1} with the maximum in *K. candel* and minimum *in R. mucronata.*

5.2.2 Variation within the plants

B. cylindrica

21 individual FAs were detected in *B.cylindrica* leaves while in its bark, only 14 FAs were detected with a total FA concentration of 12228.9±187.2 µg g^{-1} and 3318.7 ±57.19 µg g^{-1} in the leaves and bark respectively. In this plant, *cis*-18:2ω6 dominated other FAs by possessing a relative abundance of 58.86% in the leaves (18.30% of LPD) and 58.94% (6.26% of LPD) in the bark. This was followed by 16:0 showing a relative abundance of 16.12% (5.01% of LPD) in the leaves and a higher abundance (1.5% of LPD) of 18: 1ω9 which is in the bark. FAs 20:5ω3, 20:3ω6 and 20:2 were found only in the leaves of *B. cylindrica*. In this species, the PUFAs dominated among the FAs detected having relative abundances of 68% in the leaves and 62% in the bark. The SAFAs (saturated fatty acids) and MUFAs (monounsaturated fatty acids) exhibited relative abundances 23% and 9% respectively in the leaves while in the bark they showed relative abundances 23% and 15% respectively. In the leaves, PUFAs formed 21.07% of the total lipid content while SAFAs and MUFAs constitute 7.16 and 2.87% of the total lipid content whereas its bark have 6.58, 2.46 and 1.58% of PUFAs, SAFAs and MUFAs respectively.

B. gymnorrizha

In *B.gymnorrizha* leaves only ten individual FAs showed their presence with a total FA concentration of 5991.4±101.99 µg g^{-1} while in the bark 14 individual FAs were detected with a FA concentration of

2619.32±41.37 μg g^{-1}. In the leaves and bark, *cis*-18:2ω6 showed highest abundance 33.37% and 58.43% respectively. FAs 15:0, 21:0, 22:1ω9 and 26:0 detected in the bark were not detected in the leaves of this plant. The percentage of SAFAs in total lipid content (6.48%) dominates MUFAs (1.34%) and PUFAs (4.67%) in *B. gymnorrizha* leaves while a higher percentage of PUFAs (7.42%) was noticed in the LPD of its bark. The bark of this plant showed 3.25% SAFAs and 1.71% MUFAs in the total lipid content. The observed relative abundances of SAFAs, MUFAs and PUFAs in the leaves are 51.91, 10.70 and 37.39% respectively whereas in the bark they showed relative abundances 26.26, 13.82 and 59.90% respectively.

K. candel

In *K. candel* leaves a total of 12 individual FAs were detected with *cis*- 18:2ω6 having highest abundance, 12.11% of the LPD and a relative abundance of 57.27%. In the bark also , *cis*-18:2ω6 is the most abundant FA which possessed a relative abundance of 70.38% and forms 4.94% of the LPD. The total FA concentration found in the leaves of *K. candel* is 11860.21± 253.32μg g^{-1} whereas the total FA concentration in its bark is found to be 4140.06±53.32 μg g^{-1}. Both the leaves and bark showed higher percentage of PUFAs (13.78 and 4.99% of the LPD respectively) followed by 5.78% and 1.38% SAFAs in the LPD of the leaves and bark respectively. The relative abundances clearly expresses these variations with a higher percentage of PUFAs 65.16 and 71.15% in the leaves and bark respectively followed by relative abundances of SAFAs in the leaves (27.35%) and bark (19.65%). Eventhough the individual concentrations of FAs were found to be lower in the bark than in the leaves, the bark of KC shows higher relative abundance of PUFAs.

Table 5.1 Fatty acid distribution (in µg g^{-1} of dry weight) in the leaves and bark of *Rhizophoraceae* mangroves

Fatty acids	BCL	BCB	BGL	BGB	KCL	KCB	RAL	RAB	RML	RMB
12:0	38.80±1.79	38.40±1.56	570±12.12	23.10±1.45	25.30±0.75	20.70±1.76	11.30±0.54	19.01±0.91	ND	13.00±0.50
14:0	474.10±15.31	208.20±7.53	1240.70±19.01	168.80±4.91	961±4.31	293.80±9.71	593.60±7.9	658.34±8.76	571.7±7.54	348.9±5.61
15:0	6.8±0.32	ND	ND	3.12±0.14	ND	ND	ND	ND	ND	ND
16:1	90.70±5.43	ND	ND	ND	ND	ND	101.90±5.42	ND	ND	ND
16:0	1968.80±50.00	415.2±15.41	1592.3±13.72	404.2±9.87	2062.9±19.87	388.7±12.21	1367.6±23.76	242.31±5.43	870.2±4.32	187.7±3.81
17:0	40.30±2.00	20.90±7.97	27.30±6.54	17.80±0.98	24.2±1.86	9.96±0.54	27.6±1.23	7.57±0.34	ND	5.9±0.24
***cis*-18:2ω6**	7190.6±312.00	1956.2±29.07	1999.4± 19.03	1530.4±5.71	6791.8±197.82	2913.9±16.54	4156.1±45.12	647.12± 9.71	2074.2±13.32	594.4±41.00
18:3ω3	1034.2±32.00	99.1±5.32	240.7±10.01	38.7±2.31	935.8±3.97	31.9±1.76	1004.4±20.17	13.11±0.54	217.9±9.32	12.4±0.57
***cis*-18:1ω9**	1016.7±23.00	478.5±12.11	641±15.52	357.8±9.14	867.4±3.99	373.7±7.65	938.1±15.67	142.15±1.23	451.1±13.2	196.6±2.65
***Trans*-18:1ω9**	15.4±0.56	10.6±0.54	ND	ND	21.4±0.87	7.12±0.34	18.7±2.31	14.91±0.74	5.9±0.23	4.01±0.23
18:0	208.5±10.00	50.2±2.56	175.9±4.65	51.2±2.31	139.6±3.2	37.7±1.32	125.1±3.71	42.73±1.99	65.9±1.98	23.31±1.99
20:5ω3	40.6±2.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
20:3ω6	6.4±0.30	ND	ND	ND	ND	ND	ND	ND	ND	ND
20:2	3.3±0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND
20:0	17.8±0.56	4.8±0.23	8±0.45	3.9±0.15	8.8±0.43	ND	9.7±0.56	2.37±0.13	ND	ND
21:0	9.6±0.43	ND	ND	3.3±0.23	ND	ND	ND	ND	ND	ND
22:1ω9	4.8±0.32	6±0.32	ND	4.4±0.14	ND	ND	ND	ND	ND	ND
22:0	ND	ND	ND	ND	6.51±0.32	4.5±0.23	10.9±0.65	6.00±0.32	1953.3±15.43	ND
23:0	18.2±0.60	8.2±0.43	ND	ND	ND	ND	17.6±0.55	ND	ND	ND
24:0	17.4±0.71	13±0.61	9.1±0.65	7.4±0.45	15.5±0.45	6.6±0.32	12.1±0.71	9.5±0.54	ND	5.12±0.19
26:0	7.5±0.32	9.4±0.49	ND	5.2±0.25	ND	7.6±0.34	0	11.5±0.12	ND	4.53±0.38
28:0	5.4±0.19	ND	ND	ND	ND	20.1±1.2	0	13.1±0.23	ND	ND
30:0	ND	ND	ND	ND	ND	23.9±1.76	0	ND	ND	ND

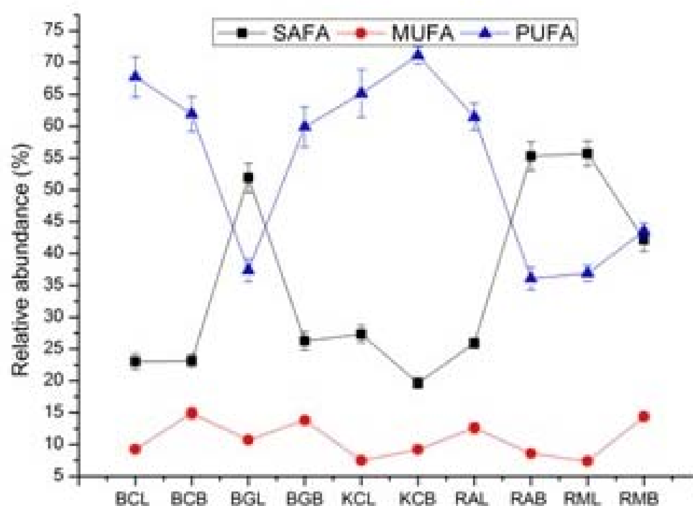

Fig. 5.1 Distribution of polyunsaturated FAs (PUFA), monounsaturated FAs (MUFA), saturated FAs (SAFA) in leaves and bark of *Rhizophoraceae* mangroves

R. apiculata

14 individual FAs were obtained both in the leaves and bark of *R. apiculata* with a higher relative abundance of *cis*-18:2ω6; 49.51% and 35.38% in the leaves and bark respectively. *cis*-18:2ω6 thus constitute 9.11% of the LPD in the leaves and 3.70% of the LPD in bark of *R. apiculata.* The total FA concentration observed for the leaves is 8394.7±124.32μg g^{-1} whereas in the bark it is 1829.17±57.23μg g^{-1}. PUFAs (11.31% of the LPD of leaves) dominated the leaf FAs with a relative abundance of 61.47% while SAFAs (5.79% of the LPD of bark) showed dominance in the bark with a relative abundance of 55.32%. The FAs 16:1 and 23:0 found to be present in the leaves were not detected in the bark of *R. apiculata* whereas the FAs 26:0 and 28:0 showed their presence only in its bark but not in its leaves.

R. mucronata

Only 8 FAs were obtained from the leaves of *R. mucronata* whereas its bark showed the presence of 11 FAs. A total FA concentration of 6210.2 ±152 µg g^{-1} in the leaves and 1395.8 ±42.23 µg g^{-1} in the bark was obtained for *R. mucronata*. SAFAs formed 7.66% of the LPD in the leaves followed by PUFAs and MUFAs which made up 5.07 and 1.62% of the LPD in the leaves. The dominance of SAFAs over PUFAs and MUFAs in the leaves of this plant is clear from their relative abundances among the FAs detected. The relative abundance of SAFAs obtained for the leaves was 55.73% while PUFAs showed a relative abundance of 36.90%. The remaining 7.35% of the total FA (analysed in the present study) concentration in its leaves was MUFAs. In the bark of *R. mucronata*, the total FA (analysed in the present study) concentration constitute 42.15% SAFAs, 14.37% MUFAs and 43.47% PUFAs which is 1.57, 0.54 and 1.62% of the LPD in the bark respectively.

The FA, *cis*-18:2ω6 showed an abundance of 4.32% of the LPD and the highest relative abundance (33.40%) followed by C_{22} which formed 4.32% of the LPD and a relative abundance of 31.45% in the leaves of this plant. The most abundant FA in the bark of *R. mucronata* is found to be *cis*-18:2ω6 with a relative abundance of 42.58% and constituting 1.59% of the LPD. C_{22} which exhibited a high relative abundance (31.45%) next to *cis*-18:2ω6 (33.40%) in the leaves was not detected in its bark.

5.3 Discussion

5.3.1 Nutritional role

In general, PUFAs dominated the FA compounds, followed by SAFAs. There was significantly higher concentration of PUFAs in *B. cylindrica*, *K. candel* and *R. apiculata* than *B. gymnorrhiza* and *R. mucronata* and significantly higher concentration of SAFAs in *B. gymnorrhiza* and *R. mucronata* leaves (Fig. 5.1). This could be related to differences in leaf chemistry between the these species, because *B. cylindrica*, *K. candel* and *R. apiculata* leaves contain higher N and lower C:N ratios than those of *B. gymnorrhiza* and *R. mucronata* leaves (Chapter 3) which also indicates that *B. cylindrica*, *K. candel* and *R. apiculata* leaves are of significantly higher nutritional quality than *B.gymnorrhiza* and *R. mucronata* leaves (Mfilinge *et al*., 2005). The nutritional quality in these leaves was also indicated by their significantly higher concentrations of essential fatty acids (EFAs) ω3 and ω6 (Sargent *et al*., 1990) than those of *B. gymnorrhiza* and *R. mucronata* , which indicates that *B. cylindrica*, *K. candel* and *R. apiculata* leaves are more nutritious than *B.gymnorrhiza* and *R. mucronata* leaves and that they are likely to contribute more EFAs to the estuarine ecosystem and the ocean than those of *B.gymnorrhiza* and *R. mucronata* leaves (Table 5.1). On the other hand, the higher concentration of PUFAs, in particular the EFAs, in the leaves possibly suggests an adaptation to the cold temperatures during monsoon (sampling was done in August), by biosynthesising large quantities of PUFAs from SAFAs through desaturation reactions in order to maintain membrane fluidity and other cell functions such as photosynthesis at low environmental temperatures (Sargent *et al*., 1990). The detection of 20:5ω3

(a microalgae marker) in the leaves of *B. cylindrica* may also be attributed to colonisation of epiphytic diatoms and protozoans, since these organisms colonise fresh plant materials rich in N (Mfilinge *et al*., 2003; Tenore *et al*., 1988). These differences in nutritional qualities between species and state of degradation influence feeding preferences by macrozoobenthos, in particular crabs.

Polyunsaturated FAs (PUFAs) contribute <7% of total energy intake by human beings. Among which the essential FAs (EFA), Linoleic acid (LA, $C_{18:2\omega6}$) is the major PUFA, comprising 84–89% of the total PUFA energy, whereas α-linolenic acid (ALA; $C_{18:3\omega3}$) contributes 9–11% of the total PUFA energy in the diets of the adult population. Omega 3 FAs are becoming of increasing interest as medical research has shown that the increased intake of these FAs can reduce the risk of coronary heart disease, improve inflammatory condition and lower blood pressure (Pereira *et al*., 2001). The elevated concentrations in comparison with other food sources (Kris-Etherton *et al*., 2000, Pereira *et al*., 2001, Simopoulos 2004) observed for both LA and ALA in the leaves and bark of mangroves (12.4-7190.6μg g^{-1}) of the present investigation points towards the use of these plant parts as sources of these molecules.

FAs play an important role in many functions of the skin (Elias, 1981). The role of FAs in trans-epidermal water loss via the skin indicates that a number of FAs have a specific function in restoring the permeability barrier (Elias, 1981; Puniras, 1989). Some polyunsaturated FAs such as linoleic acid, linolenic acid and arachidonic acid are necessary for growth and protection of the skin. Also, lauric acid is a potential antimicrobial agent, suitable for

external application and it is an inexpensive material useful for infection control in hospitals (Kitahara *et al.*, 2006). The present investigation reveals the presence of these compounds in the leaves and bark of mangrove plants in considerably higher concentration. So, the present investigation justifies the use of mangroves in traditional medicine. Higher percentages of EFAs were observed in the mangroves of the present study when compared with mangroves from other parts of the world (Hogg and Gillan 1984, Mfilinge *et al.*, 2003, 2005, Mezaine *et al.*, 2007, Chandrasekaran *et al.*, 2010) proclaims the high nutritional character of these mangroves. As the results indicate, in *K. candel*, 70% of the total FA content in its bark has linoleic acid (18:2ω6). So it can be assessed that *K. candel* can be used as a reliable source of this particular essential FA with less contamination.

5.3.2 Fatty acid composition of *Rhizophoraceae* mangroves

Data on the FAs composition of mangrove tissues provide insight into the nature of FAs present in *Rhizophoraceae* mangroves. The FA distribution of mangrove tissues proclaims the terrestrial character of these plants in that carbon number ranges from 12:0 to atleast 30:0 (Tulloch, 1976). The presence of 14:0, 16:0, 17:0, 18:0, *cis*-18:2ω6, 18:3ω3 and 18:1ω9*cis* as major FAs in the *Rhizophoraceae* mangroves under investigation (Fig 5.2) is supported by earlier results for other mangroves (Hogg and Gillan, 1984; Wannigama *et al.*, 1981). Each of the major plant FAs as defined by Hitchcock and Nicholas (1971) was detected. Of these 18:2ω6*cis*, 18: 1ω9*cis*, 18: 3ω3, 16:0 and 18:0 were present in highest percentages. The higher contribution of the FAs 16:0 , 18:2ω6*cis*, 18: 3ω3, 18:1ω9 to the total FA content is in

accordance with the previously recorded data (Meziane *et al*., 2007). It is apparent from the present data that short chain saturated and unsaturated FAs (10:0 to 19:0) are dominant in the mangrove leaves. This observation is supported by the reports of Sassen 1977 and Mezaine *et al*., 2007. Concentrations of FAs are higher in mangrove leaves than in bark.

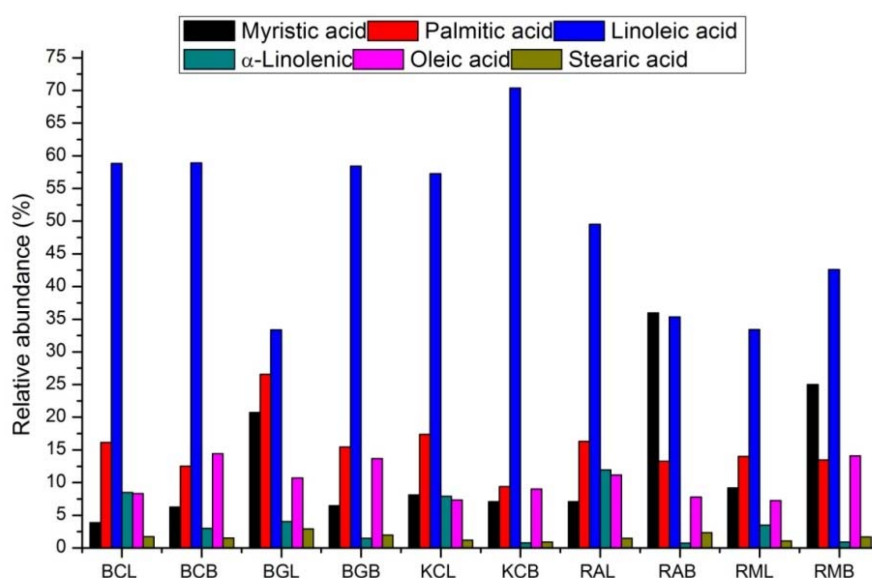

Fig. 5.2 The relative abundances of dominating FAs in the leaves and bark of *Rhizophoraceae* mangrove

Table 5.2 gives a comparison of the dominating FAs in *Rhizophoraceae* mangroves of different locations. The most abundant FA in the *Rhizophoraceae* mangroves was found to be *cis*- 18:2ω6. Most of the previous works report the most abundant FA in mangroves as 16:0 (Sassen *et al*., 1977, Hall et. al., 2006, Mfilinge et. al., 2003, 2005). But it can be noticed in the study by Mezaine *et al*., 2007 that 18:3ω3 is the FA having higher relative abundance of than the other FAs detected in several

mangrove leaves including *B. gymnorrhizha* and *R. stylosa* collected from certain locations in Australia while at some locations the same species exhibited the highest relative abundance for 16:0. His work proves that in some species, with the use of FAs it is able to differentiate mangrove populations spatially. The prevalence of 18:2 as the FA major component has been reported in mangroves from Sunderbans (Misra *et al.*, 1987). The current observations thus indicate that the mangroves of this particular location, ie; Kochi, southwest coast of India, hold their identity by the highest *cis*-18:2ω6abundance. In this study, saturated FAs dominate the fatty composition of mangrove leaves in terms of their presence but polyunsaturated FAs dominate the FA composition in terms of concentration.

The FA composition of bark of mangroves is very seldom found in literature. In the present investigation, the FA profiling of bark of five plants has been carried out. In general it has been observed that the FA contents of the leaves are higher than that of bark. This observation is implies the major function of lipids in conferring resistance to water shortage or herbivory attack in mangroves and hence accumulation in the leaves as cuticular waxes.

Table 5.2 Dominating FAs in *Rhizophoraceae* mangroves of different regions

Mangrove plants	Location	Dominating FAs	References
B. cylindrica, Ceriops decandra, R. apiculata, R. mucronata	Pichavaram, Tamil Nadu, India	Palmitic acid (16:0)	Chandrasekharan *et al*., 2010
K. candel, R. stylosa, B. gymnorrizha	Okinawa, Japan	Palmitic acid (16:0)	Meziane *et al*., 2007, Mfilinge *et al*., 2005, 2003
K. candel	Hong Kong, China	α- Linolenic acid (18:3ω3)	Meziane *et al*., 2007
R. stylosa *Ceriops australis*	Moreton Bay, Australia	Oleic acid (18:1ω9) Palmitic acid (16:0)	Meziane *et al*., 2007
B. gymnorrizha *R. mucronata*	Sunderbans, India	Linoleic acid (18:2ω6) Palmitic acid (16:0)	Misra *et al*., 1984
R. mangle	Triton Bay	Palmitic acid (16:0)	Sassen, 1977
R. stylosa, R. apiculata, R. mucronata, Ceriops tagal, R. mucronata, B. sexangula, B. gymnorrizha	Shade house grown	α- Linolenic acid (18:3ω3)	Hog and Gillan, 1984
B. cylindrica, B. gymnorrizha, K. candel, R. apiculata, R. mucronata	Kochi, India	Linoleic acid (18: 2ω6)	Present study

5.3.3 Chemotaxonomic relevance and implications for their use as biomarker

FA profile study is a promising taxonomic tool that enables the characterisation of mangrove populations according to specific identity and intra-specifically, their geographical location. Meziane *et al*. (2007) showed that leaves of different mangrove species can be differentiated using their FA profiles, and that geographically-separated populations of the same species can be identified. It has been argued that the FA profile in microalgae is a result of both genotypic and phenotypic influences (Dalsgaard *et al*. 2003). Intra-specific variations in FA profiles have also been recorded in animal populations exposed to different environmental conditions, e.g., the mussel Mytilus galloprovincialis (Freites *et al*. 2002) and fish species (Budge *et al*. 2002). The present study also implies that albeit broad patterns of similarity existing amongst different mangrove species (i.e., the high proportion of 18:2ω6 and 18:3ω3) there are substantial differences in the FA profile to differentiate species.

The result of Hierarchical cluster analysis of the FAs from leaves and bark was carried out. It can be seen from the dendogram (Fig. 5.3) of the leaves that *K. candel* and *B. cylindrica* shows similar behaviour with respect to their FA content whereas the other three plants forms another cluster with *B. gymnorrizha*, *R. mucronata* and *R. apiculata*, in which the leaves of *B, gymnorrizha* and *R. mucronata* showed much similarity in their behaviour. Thus, it can be said that the morphological classification and chemotaxonomy with respect to FA profiles of the leaves do not go in line with each other. The HCA analysis of the FAs of the bark samples shows that the plants of same genera are grouped together. This implies that the FA

profile supports the morphological classification of these plants. From the dendograms, it can also be noted that *K. candel* is more close to *Bruguiera* plants than with *Rhizophora* plants.

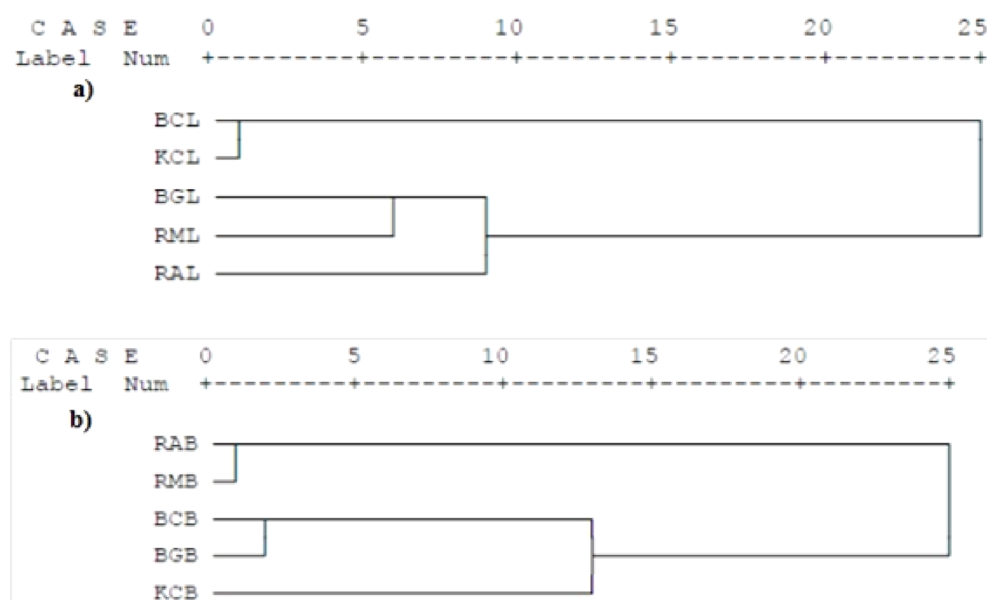

Fig. 5.3 Dendogram obtained from Hierachical Cluster Analysis of FA profiles of the a) leaves and b) bark of the five mangrove plants

The differences in FAs profiles n the plants of the present study can be attributed to the genetic differences as they all belong to same climatic regime. *B. cylindrica* dominates the other mangrove plants of the current study in diversity of FAs with the presence of 21 FAs. The presence of 20:5ω3 and 20:3ω6 was observed only in the leaves of *B. cylindrica* which proclaims the chemotaxonomic significance of these two FAs. The absence of *trans*-18:1ω9 in *B. gymnorrhizha* and its presence in all other mangrove plants of this study suggest the importance of this FA in chemotaxonomy. The absence of FA 22:0 in the genus *Bruguiera* while its presence in the

other two genera; *Kandelia* and *Rhizophora* in the study area reveals their chemotaxonomic distinction. In contrast, the genus *Bruguiera* is distinguished by the presence of 22:1ω9 while the other two genera; *Kandelia* and *Rhizophora,* lack its presence.

Table 5.3 and 5.4 compare the FA profiles of *Rhizophoraceae* mangroves of the present study with the FA profiles of previous studies from different geographical locations. Table 5.3 compares the FA distribution in the leaves of *B. cylindrica* and *K. candel* of Kochi, China and Japan in an effort to clarify the geographical variations within the species and in between species. It can be inferred that the variations in the FA distribution are mainly due the high abundance of the FAs 14:0 and *cis*-18:2ω6 in the leaves of *B. cylindrica* from Kochi. Higher abundance of FA 16:0 in the *B. cylindrica* from Japan can also be noticed. The observed difference between *K. candel* from Japan, China and Kochi is due to the relatively higher contribution of *cis*-18:2ω6 in Kochi mangroves as well as the higher abundance of 18:3ω3 in *K. candel* from China. Long chain FAs which showed lower abundances in the leaves of *K. candel* of Japan were not detected in samples from China and Kochi. The FA variations in *K. candel* from different locations indicate that FA abundance can be used as geographical index.

Table 5.3 Fatty acid distribution in the leaves of *B. gymnorrizha and K. candel* from different geographical locations

FAs	*B. gymnorrizha*		*K. candel*		
	Okinawa, Japan*	**Kochi, India****	**Okinawa, Japan***	**Hongkong, China***	**Kochi, India****
12:0	1.00	0.95	0.56	0.80	0.21
14:1	0.45	ND	0.65	0.68	ND
14:0	4.09	20.71	3.48	3.99	8.10
15:0	0.05	0.00	0.24	0.18	ND
16:1	11.57	0.00	8.40	0.76	ND
16:0	39.88	26.58	38.05	20.22	17.39
17:1	ND	ND	0.15	ND	ND
17:0	1.84	0.46	1.99	1.16	0.20
***cis*-18:2ω6**	6.99	33.37	11.12	17.34	57.27
18:3ω3	10.15	4.02	13.01	39.61	7.89
***cis*-18:1ω9**	9.17	10.70	9.13	9.12	7.31
18:0	8.33	2.94	5.20	3.27	1.18
20:2	0.21	ND	ND	0.16	ND
20:0	0.57	0.13	0.38	0.48	0.07
21:0	ND	ND	ND	ND	ND
22:2	0.18	ND	0.33	ND	ND
22:0	0.46	ND	0.56	0.42	0.05
24:0	0.86	0.15	0.95	0.30	0.13
26:0	0.40	ND	0.51	0.00	ND
28:0	0.98	ND	0.43	0.00	ND
30:0	0.79	ND	1.68	0.00	ND

ND- Not detected, *Meziane *et al.*, 2007, **Present study

Table 5.4 Fatty acid distribution in the leaves of *Rhizophoraceae* mangroves from Pichavaram and Kochi.

FAs	*B. cylindrica*		*R. apiculata*		*R. mucronata*	
	Pichavaram, India*	**Kochi, India****	**Pichavaram, India***	**Kochi, India****	**Pichavaram, India***	**Kochi, India****
12:0	12.17	0.32	19.29	0.13	12.50	ND
13:0	0.64	ND	ND	ND	ND	ND
14:0	8.58	3.88	2.40	7.07	3.80	9.21
15:0	1.26	0.06	0.26	ND	0.65	ND
16:0	56.27	16.12	55.02	16.29	59.05	14.01
17:0	1.27	0.33	0.85	0.33	0.91	ND
18:2ω6	2.95	58.86	4.16	49.51	4.31	33.40
18:3ω3	2.34	8.47	3.73	11.96	8.81	3.51
18:1ω9	9.62	8.32	9.51	11.17	17.66	7.26
18:0	0.48	1.71	0.05	1.49	0.60	1.06
20:0	0.25	0.15	0.37	0.12	0.03	ND
21:0	0.01	0.08	0.01	ND	0.01	ND
22:0	0.61	ND	0.42	0.13	0.31	31.45

ND- Not detected, *Chandrasekharan *et al.*, 2010, **Present study

The FA distribution in the leaves of mangroves from Pichavaram, India and Kochi (Table 5.4) indicate that the dissimilarity in the fatty acid distribution is primarily due to the higher abundance of *cis*-18:2ω6 in the Kochi mangroves. The mangroves from Pichavaram show the highest abundance for the FA, 16:0. The *R. mucronata* is the mangrove showing highest variations in the two locations, Pichavaram and Kochi by exhibiting higher FA abundances at Pichavaram and lower FA abundances at Kochi, except a higher abundance of 22:0 in the Kochi sample. So, it can be seen that geographical variations are integral part of the fatty acid abundances.

Wide range of variation in the dominant FAs; 16:0, *cis*-18:2ω6, 18:3ω3 and *cis*-18:1ω9 can be observed within the same species from different locations. The *Rhizophoraceae* mangroves of Kochi show a same pattern of variation of the two most dominant FAs, ie; *cis*-18:2ω6> 16:0 where as *Rhizophoraceae* mangroves of Japan show the dominance in the order 16:0>18:3. Among the FAs; 16:0, *cis*-18:2ω6, 18:3ω3 and *cis*-18:1ω9, the highest abundance of the FA 16:0 is found to be the only common element in the fatty profiles of *Rhizophoraceae* mangroves of Pichavaram. These comparisons indicate that the most dominant fatty remains the same in all mangrove plants for a particular geographical location, but there can be variations in the order of abundances of other fatty acids in them.

It has been demonstrated that genetic differences of populations from the same species can induce biochemical differentiation in the tissues (Kathiresan and Bingham 2001). But the distinct profiles of FA cannot be attributed solely to an expression of genetic differences, as local climatic and physical conditions may modify the composition of FAs (Hogarth 1999; Kunst and Samuels 2003). So the discrepancies observed in this study with various studies of the past (Chandrasekharan *et al*., 2010; Sassen, 1977; Mfilinge *et al*., 2005; Hall *et al*., 2006) can be endorsed to the genetic differences and physical as well as local climatic differences.

The high content of PUFAs, in particular 18:2ω6 and 18:3ω3, has been identified as useful biomarkers of mangrove leaves in estuarine food chains (Sassen, 1977; Hall *et al*., 2006; Meziane *et al*., 2007). Mangrove leaves also include LCFAs 24:0, 26:0 and 28:0, which are typical vascular plant markers (Alfaro *et al*., 2006; Hall *et al*., 2006; Meziane *et al*., 2007).

The PUFA 18:2ω6 which is used a biomarker for mangrove plants dominates in the *Rhizophoraceae* mangroves found in the southwest coast of India thereby supporting the results of various studies on source determination of organic matter (Gireeshkumar *et al*., 2013, Joseph *et al.,* 2012).

The LCFAs are usually cuticular wax materials that are important in conferring resistance to water shortage or herbivory attack in mangroves. These protections are expected to vary according to local growing conditions as well as other chemical and structural adaptations acting in concert. FA distribution in the mangrove tissues contained LCFAs in 20:0 to 30:0 range. Data indicate that the long chain FAs are present in significantly smaller percentages than short chain FAs. The presence of FA 22:0 in the leaves of *R. mucronata* as one of the major component that can be considered as a remarkable observation towards the chemotaxonomic distinction. The low values reported for long chain alkanes in the leaves of *Rhizophoraceae* mangroves from various parts of the world (Mezaine *et al*., 2007; Hog and Gillan, 1984) and the results of the present study points towards the general behaviour of the mangroves of this family. It has been suggested that differences in lipid diversity observed between South American and African mangroves populations including *Avicennia* and *Rhizophora* species, are influenced by climatic diversity (Dodd *et al*., 1998). Some FAs are precursor to hydrocarbon synthesis (Kunst and Samuels, 2003) and both compounds, as membrane constituents, are strongly affected by the physiological condition of the plants.

Hence, with respect to these five mangrove plants of the family *Rhizophoraceae*, and considering the importance of mangrove as source of

organic matter, it is clear that the polyunsaturated 18:2ω6 and 18:3ω3, because of their prevalence in the FA composition of the mangrove leaves and bark, can always be used as tracers of mangrove-derived organic matter. On the other hand, long chain FAs may not be useful as biomarkers of mangroves since their presence is either not found or only little in the plant components of the study location.

5.4 Conclusion

The present study identifies C18:2ω6 (linoleic acid) as the most abundant FA found in the *Rhizophoraceae* mangroves from Kochi, southwest coast of India. The study confirms the use of the PUFAs *cis*-18:2ω6and $C_{18:3\omega3}$ as biomarker for mangrove plants whereas it brings about the limitation of long chain FAs as biomarkers for mangrove plants by their lower or nil concentration in the mangroves plants of the present study. The chemotaxonomic distinction of *B. cylindrica* and *B. gymnorrizha* from other mangrove plants by the presence of $C_{20:5\omega3}$ and $C_{20:3\omega6}$ in the former and by the absence of absence of $C_{18:\,1\omega9trans}$ in the latter was identified. The similarity between the genera *Kandelia* and *Rhizophora* with respect to the presence of $C_{22:0}$ and absence of $C_{22:1\omega9}$ has been traced out. It can be concluded that the variation of FA profiles depends upon geographical location and this variation is not the same in all plants except for the most dominant FA. Also, these variations cannot be related to classical taxonomic variations. From the study it was found that the leaves and barks contribute differently to the FA composition of the plants, thereby to the surrounding sediment environment. The variation in the content of nutrient content of the mangrove leaves with respect to fatty content can be used to study the food

preferences in the mangrove dependent organisms. The high PUFA content of the mangrove plants implies the high nutrient content of these plants which reveals the prospects for using these plants for the extraction of these essential FAs of manifold benefits. In developing new sources of food, the study of the dietary composition of wild plants, like mangroves is essential. Their cultivation should lead to increased production of plants rich in omega-3 FAs and antioxidants, both of which reduce the risk of chronic diseases.

References

Alfaro, A. C., Thomas, F., Sergent, L., and Duxbury, M. (2006). Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science*, *70*(1), 271-286.

Budge, S. M., Iverson, S. J., Bowen, W. D., and Ackman, R. G. (2002). Among-and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(5), 886-898.

Chandrasekaran, M., Kumar, A. S., Kannathasan, K., and Venkatesalu, V. (2010). Fatty-acid composition of some mangroves. *Chemistry of natural compounds*,*46*(1), 92-94.

Chen, J., Ferris, H., Scow, K. M., and Graham, K. J. (2001). Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi.*Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *130*(2), 135-144.

Dalsgaard, J., St John, M., Kattner, G., Müller-Navarra, D., and Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in marine biology*, *46*, 225-340.

Dodd, R. S., Rafii, Z. A., Fromard, F., and Blasco, F. (1998). Evolutionary diversity among Atlantic coast mangroves. *Acta Oecologica*, *19*(3), 323-330.

Duke, N. C., Benzie, J. A., Goodall, J. A., and Ballment, E. R. (1998). Genetic structure and evolution of species in the mangrove genus Avicennia (Avicenniaceae) in the Indo-West Pacific. *Evolution*, 1612-1626.

Duke, N. C., Lo, E., and Sun, M. (2002). Global distribution and genetic discontinuities of mangroves–emerging patterns in the evolution of Rhizophora.*Trees*, *16*(2-3), 65-79.

Elias, P. M., Cooper, E. R., Korc, A., and Brown, B. E. (1981). Percutaneous transport in relation to stratum corneum structure and lipid composition. *Journal of Investigative Dermatology*, *76*(4), 297-301.

Freites, L., Fernández-Reiriz, M. J., and Labarta, U. (2002). Fatty acid profiles of< i> Mytilus galloprovincialis</i>(Lmk) mussel of subtidal and rocky shore origin.*Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *132*(2), 453-461.

Gireesh Kumar, T.R. (2013). Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach, PhD. Thesis, Cochin University of Science and Technology, Kochi, India.

Gireeshkumar, T. R., Deepulal, P. M., and Chandramohanakumar, N. (2013). Distribution and sources of sedimentary organic matter in a tropical estuary, south west coast of India (Cochin estuary): A baseline study. *Marine pollution bulletin*, *66*(1), 239-245.

Graeve, M., Kattner, G., Wiencke, C., and Karsten, U. (2002). Fatty acid composition of Arctic and Antarctic macroalgae: indicators for phylogenetic and trophic relationships. *Marine ecology-progress series*, *231*, 67-74.

Hall, D., Lee, S. Y., and Meziane, T. (2006). Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. *Journal of Experimental Marine Biology and Ecology*, *336*(1), 42-53.

Hitchcock, C., and Nichols, B. W. (1971). *Plant lipid biochemistry. The biochemistry of fatty acids and acyl lipids with particular reference to higher plants and algae*. Academic Press, Inc..

Hogarth, P.J. (1999). *The biology of mangroves*. Oxford University Press, New York pp. 228.

Hogg, W., R., and Gillan, T.,F. (1984). Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. *Phytochemistry*, *23*(1), 93-97.

Howell, K. L., Pond, D. W., Billett, D. S., and Tyler, P. A. (2003). Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. *Marine ecology. Progress series*, *255*, 193-206.

Joseph, M. M., Renjith, K. R., Ratheesh Kumar, C. S., and Chandramohanakumar, N. (2012). Assessment of organic matter sources in the tropical mangrove ecosystems of Cochin, Southwest India. *Environmental Forensics*, *13*(3), 262-271.

Kathiresan, K., and Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in marine biology*, *40*, 81-251.

Kitahara, T., Aoyama, Y., Hirakata, Y., Kamihira, S., Kohno, S., Ichikawa, N., Nakashima, M., Sasaki, H., and Higuchi, S. (2006). In vitro activity of lauric acid or myristylamine in combination with six antimicrobial agents against methicillin-resistant< i> Staphylococcus aureus</i>(MRSA). *International journal of antimicrobial agents*, *27*(1), 51-57.

Kristensen, E., Bouillon, S., Dittmar, T., and Marchand, C. (2008). Organic carbon dynamics in mangrove ecosystems: a review. *Aquatic Botany*, *89*(2), 201-219.

Kris-Etherton, P. M., Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R. L., Zhao,G., and Etherton, T. D. (2000). Polyunsaturated fatty acids in the food chain in the United States. *The American journal of clinical nutrition*, *71*(1), 179S-188S.

Kunst, L., and Samuels, A. L. (2003). Biosynthesis and secretion of plant cuticular wax. *Progress in lipid research*, *42*(1), 51-80.

Lee, S. Y. (1998). Ecological role of grapsid crabs in mangrove ecosystems: a review. *Marine and Freshwater Research*, *49*(4), 335-343.

M. Puniras, *Precis de Cosmetologie Dermatologique*, Masson Eds., Paris, 1989, pp. 220–222.

Melville, F., Burchett, M., and Pulkownik, A. (2004). Genetic variation among age-classes of the mangrove *Avicennia marina* in clean and contaminated sediments. *Marine pollution bulletin*, *49*(9), 695-703.

Meziane, T., and Tsuchiya, M. (2000). Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. *Marine ecology. Progress series*, *200*, 49-57.

Meziane, T., d Agata, F., and Lee, S. Y. (2006). Fate of mangrove organic matter along a subtropical estuary: small-scale exportation and contribution to the food of crab communities. *Marine Ecology Progress Series*, *312*, 15-27.

Méziane, T., Lee, S. Y., Mfilinge, P. L., Shin, P. K. S., Lam, M. H. W., and Tsuchiya, M. (2007). Inter-specific and geographical variations in the fatty acid composition of mangrove leaves: implications for using fatty acids as a taxonomic tool and tracers of organic matter. *Marine Biology*, *150*(6), 1103-1113.

Mfilinge, P. L., Meziane, T., Bachok, Z., and Tsuchiya, M. (2003). Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality.*Marine Ecology Progress Series*, *265*, 97-105.

Mfilinge, P. L., Meziane, T., Bachok, Z., and Tsuchiya, M. (2005). Litter dynamics and particulate organic matter outwelling from a subtropical mangrove in Okinawa Island, South Japan. *Estuarine, Coastal and Shelf Science*, *63*(1), 301-313.

Misra, S., K Datta, A., Chattopadhyay A Choudhury, S., and Ghosh, A. (1987). Hydrocarbons and wax esters from seven species of mangrove leaves.*Phytochemistry*, *26*(12), 3265-3268.

Napolitano, G. E., Pollero, R. J., Gayoso, A. M., Macdonald, B. A., and Thompson, R. J. (1997). Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology*, *25*(8), 739-755.

Pereira, C., Li, D., & Sinclair, A. J. (2001). The alpha-linolenic acid content of green vegetables commonly available in Australia. *International journal for vitamin and nutrition research*, *71*(4), 223-228.

Sargent, J. R., Bell, M. V., Henderson, R. J., and Tocher, D. R. (1990). Polyunsaturated fatty acids in marine and terrestrial food webs. *Animal nutrition and transport processes*, *1*, 11-23.

Sassen, R. (1977). Early diagenesis of fatty acids in mangrove peats, St Croix, US Virgin Island. *Interdisciplinary Studies of Peat and Coal Origins, Geological Society of America Microform Publication* , *8*, 7.

Schmit, J. P., and Shearer, C. A. (2004). Geographic and host distribution of lignicolous mangrove microfungi. *Botanica Marina*, *47*(6), 496-500.

Sheaves, M., and Molony, B. (2000). Short-circuit in the mangrove food chain.*Marine Ecology Progress Series*, *199*, 97-109.

Simopoulos, A. P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International*, *20*(1), 77-90.

Tan, F., Huang, Y., Ge, X., Su, G., Ni, X., and Shi, S. (2005). Population genetic structure and conservation implications of *Ceriops decandra* in Malay Peninsula and North Australia. *Aquatic Botany*, *81*(2), 175-188.

Tenore, K. R. (1988). Nitrogen in benthic food chains. *Nitrogen cycling in coastal marine environments. Wiley, New York*, 191-206.

Tulloch, A. P. (1976). Chemistry of waxes of higher plants. In *Chemistry and biochemistry of natural waxes* (pp. 235-287). Elsevier.

Wannigama, G. P., Volkman, J. K., Gillan, F. T., Nichols, P. D., & Johns, R. B. (1981). A comparison of lipid components of the fresh and dead leaves and pneumatophores of the mangrove *Avicennia marina*. *Phytochemistry*,*20*(4), 659-666.

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Chapter 6

n-ALKANES

Contents

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6.1 Introduction

Wax lipids cover all aerially exposed organs of higher land plants and can constitute of a wide range of chemical components. Among these, *n*-alkanes contribute to the hydrophobic properties of leaf wax and serve as part of the plant's first barrier from the external environment, protecting the leaf from water loss via evaporation (Post-Beittenmiller, 1996; Jetter *et al.*, 2008). After the decay of the plant, alkanes which are relatively resistant against degradation either persist in the area of production or transported by rivers and wind and get incorporated into soil, sediment or water (Vogts *et al.*, 2009; Ratheeshkumar, 2011; Gireeshkumar, 2013). Thus, biomarkers of terrestrial plants can end up in soils as well as in lake or ocean sediments and give an integrated signal of the wax composition of the plant species within the catchment area. If deposition is continuous and there is no

selective degradation of certain compounds, chronological, geological records of the original wax components can develop.

As they are straight-chain hydrocarbons lacking functional groups, *n*-alkanes are especially stable and long-lived molecules that can survive in the fossil record for tens of millions of years (Eglinton *et al*., 1991; Peters *et al*., 1993). *n*- alkanes occur in both modern and fossil leaves, in soils, paleosols, and fluvial sediments; and in both lacustrine and marine sediments (Bush and McInerney, 2013).The stable isotopic compositions ($\delta^{13}C$ and δD) of *n*-alkanes and their applications in paleoecology and paleoclimatology have been studied extensively (Castaneda and Schouten, 2011; Sachse *et al*., 2012). Long chain *n*-alkanes have great potential to inform us on past terrestrial ecosystems and environments, but their interpretation as paleo-proxies requires a strong understanding of variations in n-alkane production both within and between modern plants.

6.2 *n*-Alkanes as biomarkers

Plants typically produce a range of *n*-alkanes, commonly with characteristic odd/even carbon number predominance and one or two dominant chain lengths (Eglinton and Hamilton, 1963, 1967). It has been suggested that plants of warmer tropical and subtropical climates biosynthesise longer chain wax components than plants in habitats of the temperate regions (Gagosian and Peltzer, 1986). This concept has been used as a proxy for climate dependent vegetation changes (Bush and McInerney, 2013). Geochemical studies of sedimentary long- chain *n*-alkanes have focused on the application of ratios of particular chain lengths in an effort to reconstruct past ecosystems. The ratio of longer chain lengths (e.g. C_{29} or

C_{31}) to C_{17} has been used as a proxy for relative inputs of terrestrial plants versus aquatic algae and phytoplankton in lake sediments (Cranwell *et al*., 1987; Meyers and Ishiwa- tari, 1993). *n*-Alkanes with more intermediate chain lengths—C_{23}, and to a lesser extent C_{25} have been utilised to model Sphagnum peat moss (Nott *et al*., 2000; Pancost *et al*., 2002) and aquatic plants (Ficken *et al*., 2000; Mugler *et al*., 2008). Longer chain lengths have also been used as identifiers for different vascular plant groups. For example, in studies of lake sediments it has been postulated that C_{31} represents input from grasses while C_{27} and C_{29} represent input from trees and shrubs (Meyers and Ishiwatari, 1993; Meyers, 2003). This reasoning has been used to interpret ratios constructed from these n-alkanes in lake cores and loss sequences as changes in ecosystem structure over time corresponding with climate or land use change (Brincat *et al*., 2000; Schwark *et al*., 2002; Hanisch *et al*., 2003; Zhang *et al*., 2006). These n-alkane ratios often can be correlated with other evidence of plant community change, such as pollen records, and are compelling in such context. However, the assumption that single n-alkanes represent such large plant groups as grasses and woody plants is based on relatively sparse original data (Wakeham, 1976; Cranwell, 1984; Kawamura and Ishiwatari, 1984; Cranwell *et al*., 1987),.

6.3 *n*- Alkanes as chemotaxonomic markers

A great deal of research has been devoted to identifying, quantifying, and interpreting naturally occurring leaf wax n-alkanes in modern plants, often with the goal of using them as taxon-specific chemical fingerprints. Chemotaxonomic studies often use one or a few plants from a single

location to represent a species (e.g. Maffei *et al*., 1996); however, *n*-alkane distributions can vary within a species across its range (Dodd and Afzal-Rafii, 2000; Dodd and Poveda, 2003). In addition to ratios of individual n-alkane abundances, several other methods have been developed for characterising a given n-alkane distribution. The two most common methods are average chain length (ACL) and carbon preference index (CPI). ACL is the weighted average of the various carbon chain lengths defined as;

$$ACL=\Sigma (C_n x n)/\Sigma C_n$$

where, C_n is the concentration of each n-alkane with n carbon atoms. Land higher plants generally contain *n*-alkanes with 25–31 carbon atoms with a strong odd over even carbon number predominance. CPI measures the relative abundance of odd over even carbon chain lengths where,

$$CPI= [\Sigma odd(C_{25-33})/ \ \Sigma even(C_{24-34})+ \ \Sigma odd(C_{25-35})\Sigma even(C_{24-34})+]/2$$
(Duan and He, 2011)

It captures the degree to which odd carbon number n-alkanes dominate over even carbon numbers (Marzi *et al*., 1993). CPI values greater than 1 mean a predominance of odd over even chain lengths. In sediments, CPI > 1 is used to indicate a terrestrial plant source and thermal immaturity of the source rock (Bray and Evans, 1961; Eglinton and Hamilton, 1967). Other numerical parameters have also been used to describe and distinguish n-alkane distributions, including the location-specific matrix model developed by Jansen and coworkers (Jansen *et al*., 2006, 2010). The robust application of *n*-alkane distributions as paleoecological biomarkers requires the systematic survey of variation among different modern plants (Diefendorf *et al*., 2011).

6.4 Alkanes in mangroves

Several studies have been showed high genetic diversity within and among populations of large number of mangrove species (Duke *et al.*, 1998; 2002; Melville *et al.*, 2004; Tan *et al.*, 2005). Broad geographic studies of mangrove species are needed in order to distinguish possible intraspecific taxa (Tomlinson, 1986). The straight chain alkanes (*n*- alkanes) have already been successfully used in studies of intra-and interspecific variability in mangrove taxa as their composition is generally species-specific, providing useful taxonomic markers of plants (Eglinton *et al.*, 1962; Gülz, 1994; Maffei, 1996; Dodd *et al.*, 1998a) that correlate well with intraspecific ecological diversification, (Dodd *et al.*, 1995, 1998a, b; Rafii *et al.*, 1996). Long-chain n-alkanes (between 25 and 35 carbons), that are characteristic components of epicuticular waxes of mangrove leaf surfaces, can also be used as tracers of higher plant remains (Dodd *et al.*, 1995, 1998; Rafii *et al.*, 1996; Versteegh *et al.*, 2004). The data of alkanes of mangroves of pan-Atlantic region suggest that significant biogeographic variability exists. Also, as mangroves are widely used in ethanomedicinal practices, the quantity and quality of a component like alkane is of much relevance. There is no relevant data available on the alkane composition of mangroves of Kochi, Southwest coast of India. In view of the very wide distribution of the family of *Rhizphoraceae* along this region that might be expected to contribute to significant genetic variation, studies on alkane composition in the plants of this family are of much importance.

6.5 Results

6.5.1 n- alkane distributions across the plant species

Aliphatic hydrocarbons ranging from C_{17} to C_{37} were identified. Their distribution in µg g^{-1} is given in Table 6.1. The total *n*-alkane concentration ranged from 57.24± 2.32µg g^{-1} to 114.85± 4.89µg g^{-1} in the barks of mangroves with the maximum value observed in *R. mucronata* and minimum concentration in *R. apiculata*. High contents of *n*-alkanes distinguished *R. mucronata* (609.34±15.23 µg g^{-1} in the leaves and 114.85± 4.89 µg g^{-1} in the bark) from the other mangrove plants of the present study. In the leaves of *R. mucronata*, the total alkane content formed 1.35% of the total lipid content (LPD). Among the leaf samples, *K.candel* exhibited lowest alkane concentration (9.68± 0.39µg g^{-1}) which is 0.02% of the LPD in its leaves. In the present study, the distribution of n-alkanes of most samples maximises either at n-C_{29} or at n-C_{31}. Other maxima were observed for the barks of *B. gymnorrizha* and *R. apiculata*; nC_{22} and nC_{17} respectively. Except *K.candel*, all the long chain *n*- alkanes in the carbon number range of 25–35 were found to be present in the leaves of all species, atleast in low quantities, with an odd/even predominance.

6.5.2 n- alkane distributions within the plants

B. cylindrica

In the leaves of B. cylindrica, except C_{18} all *n*- alkanes from C_{17} to C_{37} were found to be present while in its bark, the *n*-alkanes, C_{18}, C_{19} and C_{20} were not detected. A total *n*- alkane concentration of 118.60±4.25µg g^{-1} and 96.21±4.08 µg g^{-1} were obtained in the leaves and bark respectively. The total concentration of the alkanes from C_{17-37} is 0.3% of the LPD in leaves and 0.31% of the LPD in the bark of *B. cylindrica*.

Table 6.1 Concentrations of n- alkanes (in µg g^{-1}) in the leaves and barks of *Rhizophoraceae* mangroves

Alkanes	BCL	BCB	BGL	BGB	KCL	KCB	RAL	RAB	RML	RMB
C_{17}	15.09±0.75	13.96±0.49	12.20±0.65	12.16±0.29	0.17±0.01	12.31±0.00	13.91±0.56	10.62±0.75	48.68±3.76	14.92±0.89
C_{18}	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.37±0.56
C_{19}	2.63±0.01	ND	3.77±0.14	ND	ND	3.76±0.16	1.72±0.05	1.54±0.03	16.30±0.65	5.68±0.23
C_{20}	5.38±0.23	ND	ND	2.20±0.17	0.39±0.00	12.76±0.34	ND	5.33±0.23	21.08±1.34	7.93±0.35
C_{21}	0.15±0.00	0.64±0.00	ND	ND	ND	ND	0.53±0.03	ND	12.26±0.56	ND
C_{22}	17.07±0.91	15.13±0.43	ND	14.55±0.	0.27±0.01	11.34±0.49	13.77±0.67	0.02±0.00	8.93±0.46	11.12±0.57
C_{23}	8.38±0.42	7.89±0.36	ND	5.96±0.25	0.03±0.00	5.79±0.50	ND	5.63±0.43	18.90±1.23	6.15±0.34
C_{24}	5.00±0.21	0.01±0.00	6.53±0.31	6.47±0.31	0.32±0.00	7.45±0.37	6.64±0.34	6.16±0.32	31.38±2.56	9.89±0.45
C_{25}	5.20±0.19	0.01±0.00	4.75±0.19	6.00±0.87	0.50±0.00	4.61±0.23	5.21±0.23	3.89±0.13	22.02±1.23	4.97±0.20
C_{26}	2.56±0.10	2.30±0.12	3.09±0.15	1.80±0.02	ND	2.73±0.09	2.31±0.17	2.01±0.16	54.85±2.65	3.36±0.15
C_{27}	1.03±0.04	1.13±0.05	2.28±0.09	ND	ND	1.52±0.07	1.47±0.04	ND	86.24±5.77	1.72±0.05
C_{28}	4.02±0.23	4.13±0.21	5.13±0.21	2.98±0.12	0.72±0.02	4.38±0.23	3.99±0.13	3.19±0.10	85.92±4.21	4.88±0.23
C_{29}	7.26±0.35	7.40±0.36	14.19±0.64	4.59±0.19	0.23±0.02	11.06±0.03	12.07±0.99	1.71±0.08	137.14±5.67	8.16±0.43
C_{30}	4.62±0.23	3.78±0.15	6.55±0.31	5.35±0.19	1.72±0.05	6.60±0.30	5.80±0.19	3.91±0.18	17.37±0.76	5.75±0.25
C_{31}	18.29±0.97	22.83±1.2	22.93±1.87	3.41±0.12	3.55±0.12	22.63±2.76	18.56±1.45	6.27±0.39	15.94±0.67	7.21±0.35
C_{32}	5.81±0.00	3.62±0.12	5.38±0.23	0.98±0.01	0.19±0.00	3.67±0.25	4.94±0.27	1.26±0.03	8.53±0.45	2.00±0.10
C_{33}	10.49±0.52	8.76±0.43	7.71±0.27	2.15±0.00	0.32±0.00	6.37±0.47	5.63±0.31	2.37±0.01	7.38±0.37	3.39±0.16
C_{34}	1.45±0.03	0.85±0.37	0.70±0.19	0.59±0.01	0.06±0.00	0.88±0.46	0.92±0.03	0.55±0.02	2.10±0.10	0.75±0.00
C_{35}	1.62±0.05	1.49±0.00	0.93±0.43	1.75±0.04	0.77±0.00	1.57±0.04	1.12±0.00	1.28±0.03	4.62±0.23	1.66±0.03
C_{36}	1.47±0.00	2.02±0.00	1.43±0.01	1.48±0.01	0.24±0.00	0.68±0.00	1.74±0.00	0.48±0.00	6.68±0.34	1.18±0.02
C_{37}	1.09±0.02	0.86±0.00	1.25±0.00	2.11±0.07	0.19±0.00	1.16±0.06	0.73±0.00	1.04±0.00	3.01±0.15	1.76±0.01

Value expressed as Concentration±Standrard deviation

In this plant, C_{31} dominated other *n*- alkanes by possessing a relative abundance of 15.42% (0.05% of the LPD of the leaves) in the leaves and 23.58% (0.07% of the LPD of the bark) in the bark. This was followed by C_{22} showing a relative abundance of 14.39% (0.04% of the LPD) and 15.63% (0.05% of the LPD) in the leaves and bark respectively. None of the alkanes showed their presence exclusively in *B. cylindrica.* Among the long chain n- alkanes (C>25) C_{31}, C_{33} and C_{29} was found to the major ones.

B. gymnorrizha

In leaves only 16 *n*- alkanes among the *n*- alkanes from C_{17} to C_{37} showed their presence with a total *n*- alkane concentration of 98.81± 3.29 µg g^{-1}. The bark of this plant showed a total *n*- alkane concentration of 74.55±2.91 µg g^{-1}. In the leaves, C_{31} showed highest abundance (0.05% of LPD) while in the bark, C_{22} (0.07% of the LPD) was found to be most abundant *n*- alkane. C_{20}, C_{22} and C_{23} detected in the bark were not detected in the leaves of this plant whereas the absence of long chain alkane C_{27} can be noticed in the bark *n*- alkane distribution. The total concentration of the detected alkanes is 0.21% of the LPD in leaves and 0.35% of the LPD in the bark of *B.gymnorrizha*.

K. candel

In the leaves and the bark of *K.candel*, C_{31} showed the highest abundance; 0.006% and 0.04% of the LPD respectively. The total *n*-alkane concentration found in the leaves of *K. cande*l is 9.68± 0.39µg g^{-1} which is 0.02% of the LPD of the leaves whereas the total *n*-alkane concentration in its bark is found to be 121.27±3.23µg g^{-1} which is 0.21% of the LPD in the bark. The leaves of this plant were found to be inferior in *n*- alkane content

than the bark. The long chain alkanes show a greater abundance than the short chain *n*-alkanes in this plant.

R. apiculata

Among the straight chain *n*-alkanes from C_{17} to C_{37}, the *n*-alkanes; C_{18}, C_{20} and C_{23} were found to be absent **i**n the leaves of *R. apiculata* while its bark was found to be deficient in C_{18}, C_{21} and C_{27}. The total *n*-alkane concentration obtained for the leaves is 101.07±4.79 µg g^{-1} and that for the bark is 57.24±2.23 µg g^{-1}. In the leaves of this plant, the long chain *n*-alkane, C_{31} (0.04% of the LPD) showed the highest abundance. In the bark, eventhough C_{31} (0.04% of the LPD) was found to be dominant among the long chain *n*-alkanes, the most abundant alkane was found to be C_{17} (0.06% of the LPD). The *n*- alkanes form 0.22% of the LPD in leaves and 0.33% of LPD in bark.

R. mucronata

Except C_{18} all the other *n*- alkanes from C_{17} to C_{37} were detected in the leaves of *R. mucronata*. The *n*- alkanes studied form 1.35% of the LPD in leaves and 0.31% of the LPD in bark. The bark of this plant is the only plant component which showed the presence of the alkane, C_{18} in this study. C_{21} is the only *n*-alkane, in the range C_{17}-C_{37} which was found to be absent in the bark of this plant. C_{29} (0.3% of the LPD) was found to the most dominant *n*-alkane in the leaves of R. mucronata whereas C_{17} (0.04%) is the *n*-alkane showing highest abundance in its bark. Among the long chain alkanes (C>25), the alkane C_{29}, was found to be dominant in both leaves and bark. The total concentration of *n*-alkanes obtained was 609.34 ±15.23 µg g^{1} and 101.30 ±5.23 µg g^{-1} in the leaves and bark respectively.

6.6 Discussion

6.6.1 *n*- Alkane distributions in *Rhizophoraceae* mangroves

Analyses of variance revealed 21 alkanes vary significantly among the leaves of different species (Table 6.2) with a P value less than 0.01. No significant variation was observed for different alkanes of the leaves. The bark samples exhibited significant variation of alkanes among different species with highly significant variation between different alkanes. Thus the ANOVA results not only suggest significant variation in alkane distribution among species both in bark and leaves but also it indicate that the plant components differently contribute to the nature of alkanes in the neighbouring ecosystems. The quantitative variation in the hydrocarbons was related to the environmental conditions while the qualitative variation is genetic in nature (Rafii *et al.*, 1996; Dodd *et al.*, 1999; Said, 2011). So, while using alkane as a biomarker in source identification studies, the above observation point towards the significance of considering the alkane composition of the dominant mangrove species of that particular geographical area.

Table 6.2 Results of ANOVA of alkanes in the leaves and bark of five *Rhizophora*ceae mangroves

Plant part	Source of Variation	Df	F	P- Value
Leaves	n- alkanes	20	1.28	ns
	Species	4	9.6	0.000**
Bark	n- alkanes	20	9.06	0.000**
	Species	4	3.17	0.018*

**Significant at 0.01 level, *Significant at 0.05 level, ns- not significant

The results of the present study on *n*-alkane composition of mangrove tissues bring about the nature of *n*-alkanes present in *Rhizophoraceae* mangroves. The hydrocarbon profile for *Rhizophoraceae* mangroves obtained in the current investigation was found to be similar with that was reported by Dodd *et al*., 1995 but with a high concentration for Kochi mangroves than African mangroves. C_{31} can be termed as the most abundant foliar *n*-alkane due to its dominating presence in all the leaves, except *R. mucronata* leaves. In *R. mucronata*, C_{29} was found to be the most abundant *n*-alkane. This observation is supported by the results of C_{31} dominance in a few mangroves in West Africa, Arabian Gulf and north Queensland (Hog and Gillan, 1984; Dodd *et al*., 1995, 1998, 1999). Dodd *et al*. (1999) suggested that the *n*-alkane composition of mangroves can be linked to environmental conditions, and attributed the dominance of longer chained C_{31} and C_{33} n-alkanes of *Avicennia marina* in the United Arab Emirates to its evolution under arid conditions. Versteegh *et al*. (2004) and Mead *et al*. (2005) found that the most abundant lipid at the *Rhizophora* leaf surface, C_{29} n-alkane, accounts for 0.22% of the dry leaf material. However, it seems that the n-alkanes composition in mangrove plants is susceptible to biogeographic variations. Rafii *et al*. (1996) and Dodd *et al*. (1998) reported unusually high concentration of C_{28} n-alkane in *Avicennia* and *Rhizophora* from French Guiana, whereas C_{31} is also important in plants of these genera in West Africa. Chemical genetic studies have revealed the biosynthetic steps necessary for the production of different hydrocarbon homologues and the presence of mutants in which certain homologues are absent due to lack of the appropriate enzyme systems (Dodd *et al*., 1999). So this may be the reason for the distinct behaviour of

R. mucronata from other mangroves. Concentrations of *n*-alkanes are found to be higher in mangrove leaves than in bark. The *n*- alkane compositions of the bark thus indicate the tendency of the plants to accumulate lipids in their leaves as cuticular wax which provides stress protection to this delicate part of the plants.

The *n*- alkane distributions in the leaves and bark of *Bruguiera* mangroves (Fig. 6.1) show that the short chain alkanes predominate in the leaves of *B. cylindrica* while the longer chain length alkanes show a larger distribution in the bark. *n*- Alkanes with carbon number greater than 25 is more abundant than the short chain alkanes in the leaves of *B. gymnorrhiza*. The radicals of *B. gymnorrhiza* are universally used as vegetables. The alkane content of the leaves reveals the non toxicity of its leaves with respect to toxic hydrocarbons towards using it as an edible source. The lower n- alkane absolute concentrations as well as the greater alkane content of the bark than the leaves (Fig. 6.2) makes K. candel different from other plants of the study. The lower concentration of alkanes in *K. candel* implies its nature as a non vascular plant rather than its existence as a tree (Silva and Madureira, 2012). *R. apiculata* and *R. mucronata* possess higher abundance of long chains than short chain alkanes in the leaves but no such preferences were observed in their barks (Fig. 6.3).

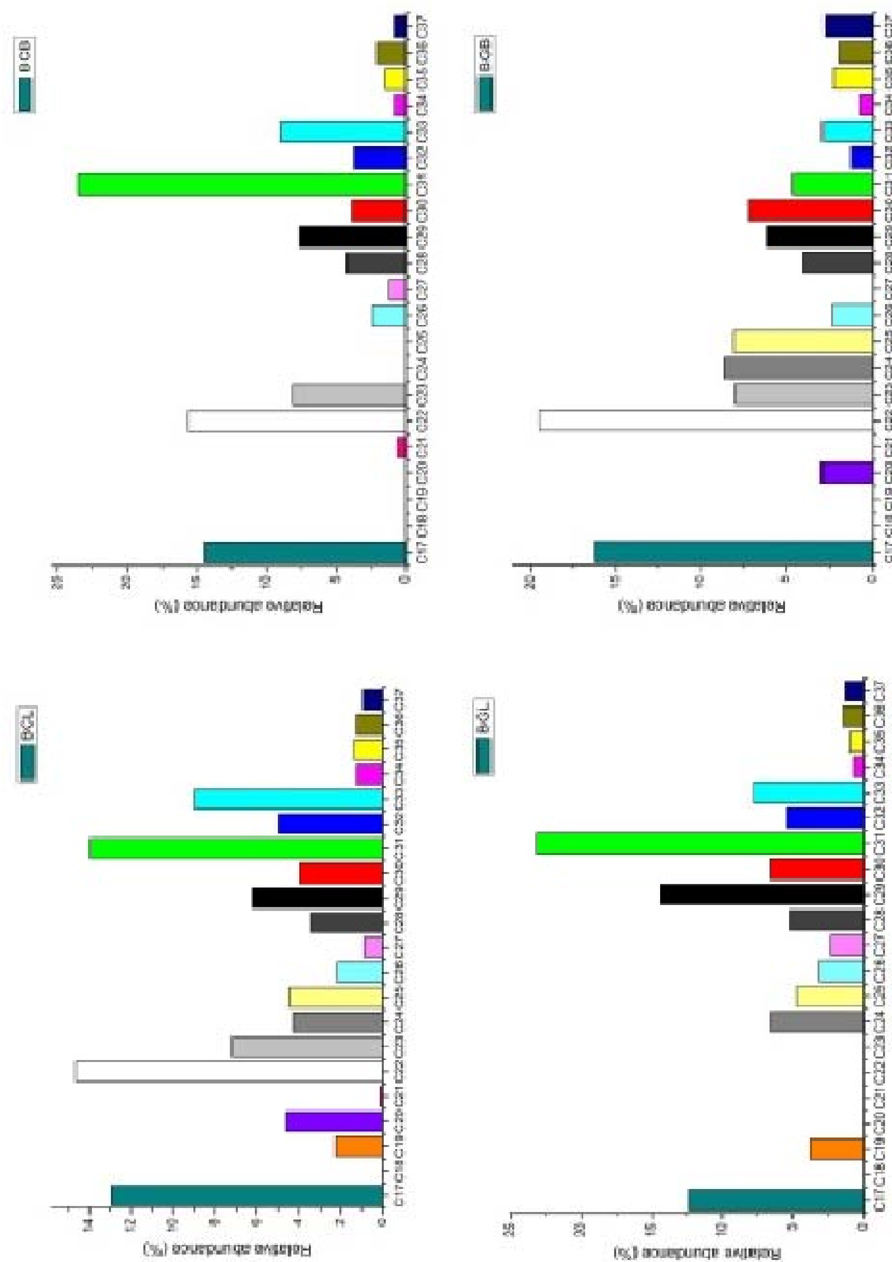

Fig. 6.1 n- alkane profiles of *Bruguiera* mangroves

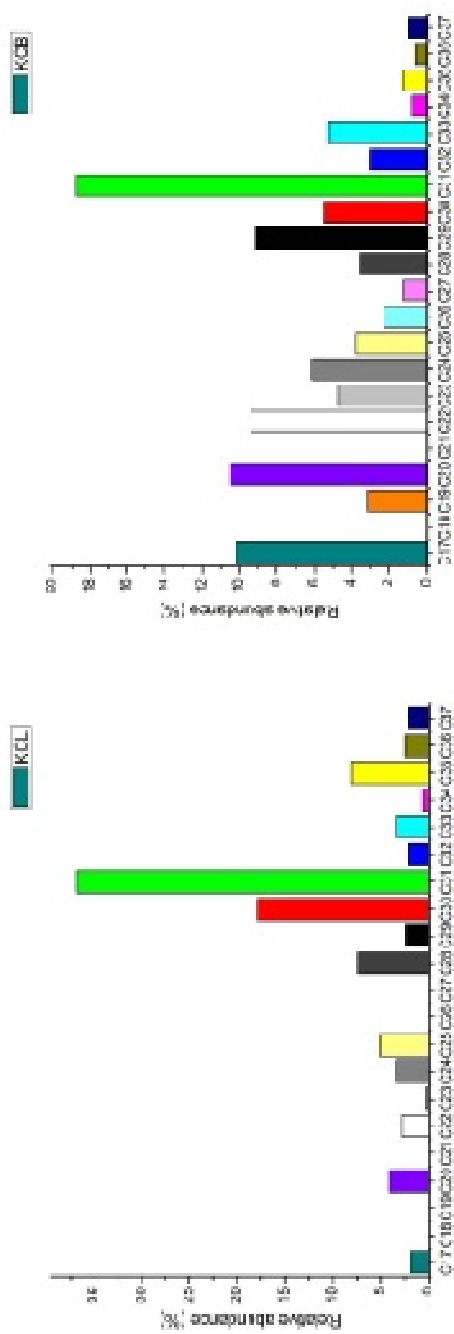

Fig. 6.2 n- alkane profiles of *Kandelia* mangroves

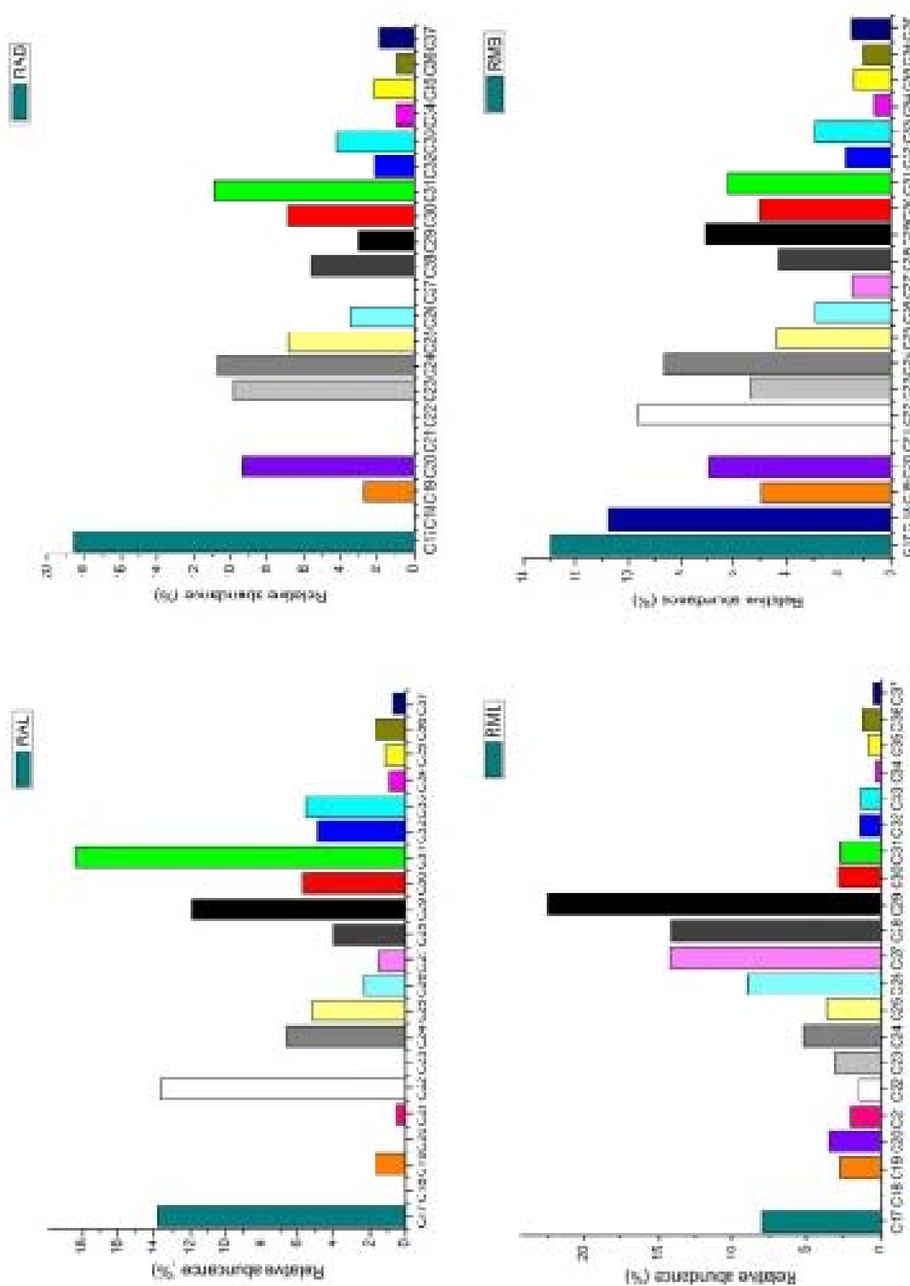

Fig 6.3 n- alkane profiles of *Rhizophora* mangroves

The short carbon chain length *n*- alkane predominance observed in this study can be attributed to the incorporation of hydrocarbons inside the cells as these hydrocarbons has the flat distribution of the chain lengths and the lack of alternation between odd and even numbers of the homologous series that is characteristic of higher plants in which the leaf wax has a very low hydrocarbon content (Herbin and Robins, 1969). While determining the hydrocarbons in the leaves from eleven mangroves, Hogg and Gillan, 1984 reported the presence long chain alkanes from C_{25} to C_{35} present in *B.gymnorrizha*, *R. apiculata* and *R. mucronata* from Queensland, Australia. Their study reports higher values of alkanes in µg g^{-1} of fresh weight than the values obtained in the present study in µg g^{-1} dry weight. Moreover while the present study reports the presence of all the hydrocarbons from 25 to 30 in the leaves of these plants (with an exception of *K. candel*) atleast in small amounts, C_{28} was found to be absent in the study by Hogg and Gillan, 1984. These differences in the hydrocarbon compositions of these three mangroves of Queens land and Kochi may be due to geographic as well as seasonal and developmental variations (Prasad and Gulz, 1990).

6.6.2 *n*- Alkanes as a wax source

The alkane content of the plants can be used to assess its importance as a source of novel products. Most abundant alkanes found in the *Rhizophora*ceae mangroves are C_{31} followed by C_{33}. These are constituents found ubiquitously in the cuticles of fruits and vegetables. Each of the alkanes (C_{23} – C_{27}) reported to be present in more than trace amounts in these mangroves have been found in significant concentration in other plant waxes as well: apple peel, brussels, sprouts, cocksfoot grass, rye grass and

the leaves of turnip, runner beans, white mustard, green tobacco and cactus. The most abundant alkane found in *Rhizophora*ceae mangroves, C_{31} has been detected in the cuticles of pears, apples, cherries, grapes, oranges and grapefruit as well as in the leaves of various vegetables. C_{29} another of the hydrocarbon in these set of mangroves comprises 99%of the paraffins in the apple cuticle and is one of the most abundant hydrocarbons of all plant waxes thus far analysed. In all these aspects the distribution of *n*- alkanes in these mangroves coincides with that of Candelilla wax, which is a purified wax obtained from the leaves of Candelilla plant, *Euphorbia antisyphillitica.* It is used as a constituent of various polishes, candels, varnishes, sealing waxes, electrical insulators, carbon papers, cosmetic and other wax containing preparations. In Unites states of America it is used as a component of chewing gum base and as a glaze for hard candles. It has also been successfully used as a coating agent to extend the storage life of citrus fruits (Burdock, 1997). So the wax derived from mangroves has the potential to use it as a lubricant or surface polishing agent for fruits and vegetables.

The leaves of the trees B.cylindrica, R. apiculata, and R. mucronata were proved to be an alternate source of tea (Kathiresan, 1995). Mineral oil which contains C_{18} - C_{24} *n*-alkanes act as a laxative (Stoker, 2012). So when these mangrove extracts containing alkanes with chain length between C_{18}-C_{24}, which when taken by mouth it passes through the gastrointestinal tract unchanged and is excreted chemically intact thereby can act as a laxative. The high concentration of higher solid alkanes ($C>25$) points towards the use of mangroves as a source of alkanes for skin treatments. The antioxidant, anticarcenogenic flavonoid rich leaves of mangroves (Chapter 4) containing

appreciable amount of solid alkanes can be a very good source of UV protecting substances and other remedies for skin irritations.

6.6.3 *n*- Alkane composition in biomarker studies

Land higher plants generally contain *n*-alkanes with 25–31 carbon atoms with a strong odd over even carbon number predominance (expressed as the CPI) (Rieley *et al*., 1991a; Collister *et al*., 1994, Duan and Ma, 2001). Therefore, the *n*-alkane CPI in sediment is an indicator of the sources of organic matter (Volkman *et al*., 1983; Simoneit *et al*., 1991; Duan, 2000).The average CPI obtained for the mangroves of the present study is of 2 indicating the predominance of odd chain lengths over even chain lengths. The observed CPI lower than reported CPI for other terrestrial plants (Duan and He, 2011; Bush and McInerney, 2013) indicates the contribution of hydrocarbons other than cuticular hydrocarbons of the mangrove plant parts to the lipid content of the surrounding ecosystems.

In biomarker studies, the predominance of C_{17} is considered as the contribution from algae (Sikes *et al*., 2009), while the mid chain alkanes C_{21}, C_{23} and C_{25} represent aquatic macrophytes. The present study reveals the presence of C_{17} in appreciably higher concentration as well as the alkanes C_{21}, C_{23} and C_{25} in lower concentration in the mangroves. This indicates the similarity in biosynthetic pathway of mangroves with that of algae and aquatic macrophytes. Submerged and floating macrophytes have n-alkane biomarker compositions intermediate between algae and terrestrial vascular plants and are characterised by the dominance of odd mid chain length n-alkanes (C_{21},C_{23} or C_{25}) (Viso *et al*., 1993; Ficken *et al*., 2000).

The proxy P_{aq} is often used for expressing the relative contributions of submerged macrophytes/floating plants.

$$P_{aq} = \frac{C_{23} + C_{25}}{C_{23} + C_{25} + C_{20} + C_{31}}$$

So, it is suggested to account for the contribution of the mangrove plants in these proxies if the study area is close to these ecosystems. Thus it can be assessed that the population and species diversity of a specific mangrove area can bring about alternations in the alkane ratios as well as proxies to differentiate the probable sources of organic matter in sediments. So it is recommendable to adopt or develop methods and proxies in which the quantity and distribution of n- alkanes of the dominant mangrove species of neighbouring mangrove area while using it as a biomarker.

6.6.4 n- alkane chain length and CPI as chemotaxonomic tool

The abundance of n-C_{25} to n-C_{29} alkanes in the leaves decreases from *R. mucronata* over *R. apiculta* and *B. cylindrica* to *B. gymnorrizha* and *K. candel*, whereas the abundance of the n-C_{31} and n-C_{33} alkanes increases. This is numerically expressed by $ACL_{23–35}$- the overall chain length distribution, best expressed by the average chain length parameter in the odd carbon number range from 23 to 35 ($ACL_{23–35}$; Poynter *et al.*, 1989)- increasing from 27.74 to 31.01in the leaves (Fig. 6.4). It ranges from 27.58 to 29.86 in the bark specimens. The ACL values obtained are almost similar that can be attributed to the fact that all the species belongs to a single family. The results of the present study implies the shrubby nature of *K. candel* compared to *R. mucronata* as studies on n-alkane distribution in various

plants and trees report that the abundance of n-C_{25} to n-C_{29} alkanes decreases from trees over shrubs to herbs, whereas the abundance of the n-C_{31} (for shrubs) and n-C_{33} alkanes (for herbs) increases (Dove and Mayes, 1991; Maffei, 1996; Schwark *et al*., 2002; Ali *et al*., 2005; Bi *et al*., 2005; Jansen *et al*., 2006). This is numerically expressed by $ACL_{25–35}$ values increasing from 29.54 to 30.47 (Vogts *et al*., 2009). It is also reported earlier that the chemical differences between New World and African populations of mangroves were confounded by variations, possibly associated with plant form, in African populations. Thus, foliar waxes of a shrubby form were relatively rich in triterpenoids (23%) compared with those from trees (10%) and the shrubby form was richer in the lower carbon number alkanes (Rafii *et al*., 1996).

The interpretation of n-alkane chain-length ratios in terms of plant community rests on a foundational assumption of equivalent n-alkane production by different plant groups. Some species exhibit a high degree of genetic control over their n-alkane distributions, such that different plants growing in different locations can have relatively similar patterns of n-alkane distribution, e.g. the almost exclusive production of C_{27} by Fagus sylvatica leaves (Gu ̈lz *et al*., 1989; Lockheart *et al*., 1997; Nguyen-Tu *et al*., 2007; Sachse *et al*., 2009). However, other species have been shown to have variable n- alkane distributions across different environments, limiting the usefulness of n-alkane profiles in chemotaxonomy (Dodd and Poveda, 2003; Vogts *et al*., 2009).

The CPI shows an average value of 2 with a maximum at 2.7 and minimum at 1.2 indicating that odd chain lengths are more abundant than even

chain lengths. This is in accordance with the observation that the vast majority (but not all) of modern plants have values greater than 1 (Bush and McInerney, 2013). In the case of mangrove plants of this study, although the CPI value indicates the odd over even predominance but to a lesser extent as expected for woody angiosperms. This variation may be because mangroves are vascular plants growing in different environmental conditions than other terrestrial plants. This may be due to the incorporation of internal hydrocarbons (hydrocarbons inside the cells) as a result of prolonged solvent extraction of the dried grounded samples. The internal hydrocarbon pattern has the flat distribution of the chain lengths and the lack of alternation between odd and even numbers of the homologous series that is characteristic of higher plants in which the leaf wax has very low hydrocarbon content (Herbin and Robins, 1969). Therefore it can be assessed that the leaves with lower CPI has higher internal hydrocarbon contribution than the cuticular hydrocarbons to the total lipid content. So in this study, as it is evident from the results *R. mucronata* is the preeminent hydrocarbon contributor to the mangrove ecosystem. It can also be observed that the barks contribute less to the lipid content than the leaves.

The abundance of minor amounts of even carbon number n-alkanes in plant waxes originates from variations in the beginning of the biosynthesis. Instead of utilising a building block with two carbon atoms a molecule with three carbon atoms is used for the synthesis of fatty acids, which are the precursor molecules of n-alkanes and n-alkan-1-ols (Shepherd, 2003). It is unknown why different building blocks are being used at the beginning of the synthesis reactions (or why the extent differs in plants) and thus lead to differences in CPI values. Guyanan populations were found to much richer

in the C_{28} alkane (38.6-66.7% of total extract) compared with 10.9-23.1% for Gabon populations of different mangrove species when alkanes were expressed as a proportion of the hydrocarbon fraction (Rafii *et al.*, 1996). In the present study *Rhizophoraceae* mangroves were found to be richer in the abundance of C_{22} and C_{24}.

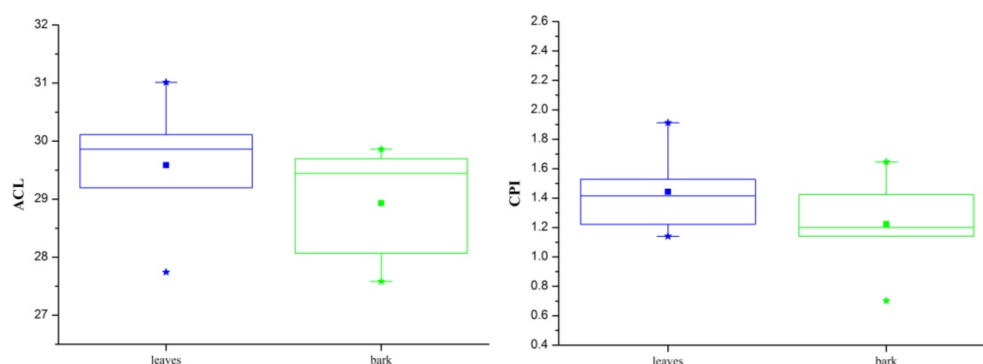

Fig. 6.4 Box plots divided by plant parts for Average Chain Length (ACL) and Carbon preference Index (CPI).

The value of alkanes as genetic markers was indicated by the close correlation between classification from biochemical variation and classical taxonomy. The partition of species were carried out based on Hierarchical cluster analysis, ternary diagrams using the most abundant alkanes C_{29}, C_{31} and C_{33} as vertices as well as using PCA analysis.

6.6.5 Hierarchical Cluster analysis and Ternary diagram

The result of Hierarchical cluster analysis of the *n*- alkanes from leaves and bark (Fig. 6.5) was carried out. It can be seen from the dendogram of the leaves that *R.mucronata* remains separated from all other plants. This can be attributed to the high alkane content of its leaves

and the dominance of short chain length alkane (C_{29}). *B. gymnorrizha* and *R. apiculata* are found to be closer and show similarities with *K. candel*. *B. cylindrica* forms part of the cluster containing all plant leaves other than *R. mucronata* leaves. The HCA analysis of the n- alkanes of the bark samples shows that *B. cylindrica* bark is more similar to the bark of *R. mucronata* than with *B. gymnorrizha* of the same genus. *B.gymnorrizha* is more related to *R. apiculata* in terms of *n*-alkane content. This extreme behaviour observed in *n*-alkane content within the plants of the same genus supports the formation of the *n*- alkanes and depends more on external factors than internal factors.

Higher concentrations of *n*- alkanes are obtained for *R. mucronata* than all other plants. The leaves of *R. mucronata* are distinguished by the presence of higher C_{29}, while all other plants are distinguished by higher C_{31} abundance. This distinction can be clearly observed from the ternary diagram which evaluates the coincidence of the most abundant three *n*- alkanes; C_{29}, C_{31} and C_{33} (Fig. 6.6). This may be due to the difference in distribution of leaf wax in the leaves of different mangroves. The lower content of C_{29} and C_{33} and a corresponding higher content of C_{31} in the leaves of *K.candel* distinguishes this plant leaves from others. With respect to their bark content of these three dominating alkanes, all mangroves can be grouped into one. In general, *K. candel* exhibited very low alkane content compared with others albeit their distribution showed greater similarities with other plants.

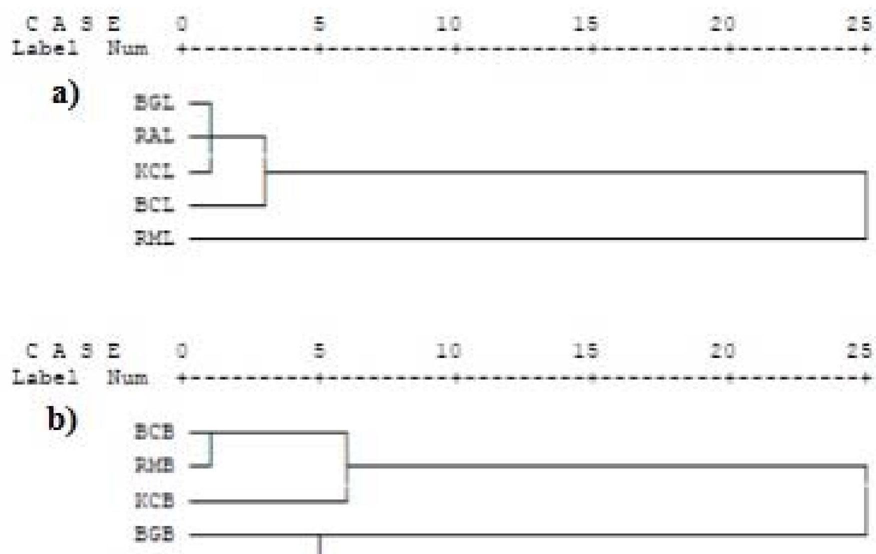

Fig. 6.5 Dendogram obtained from Hierachical Cluster Analysis of n profiles of the a) leaves and b) bark of the five mangrove plants

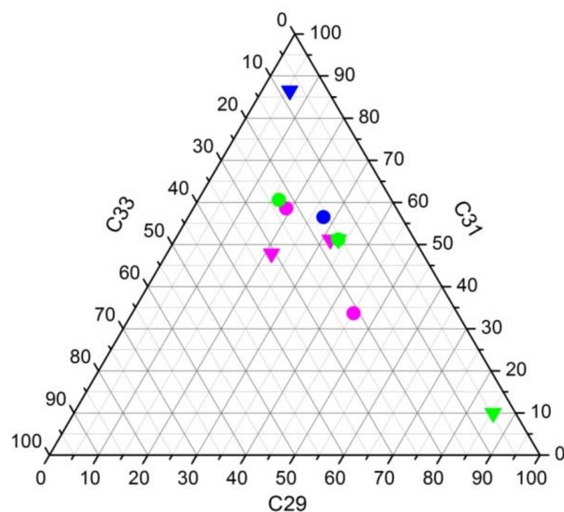

Fig. 6.6 Ternary diagram of n- alkane chain- length abundances by plant species (▼- *Bruguiera* leaves, ▼- *Kandelia* leaves, ▼- *Rhizophora* leaves, ●- *Bruguiera* bark, ●-*Kandelia* bark and ●- *Rhizophora* bark).

6.6.6 Principle component analysis

PCA was employed on two type of data sets; one using the absolute concentration and the other one using relative abundance of *n*-alkanes. In order to classify the mangroves on the basis of their difference in *n*-alkane production PCA was carried out using the absolute concentration. To identify the preferential alkane synthesis the PCA of relative abundance was taken. Extraction was done using Principal Component Analysis while the Rotation Method employed was Varimax with Kaiser Normalization. Principle components were considered significant if the Eigen values were greater than 1.

(i) PCA based on absolute abundance

The results of PCA of absolute abundance of *n*-alkanes components of the leaves of the five mangroves (Fig. 6.7) are focussed on thet three components which explained 97.88% of total variance. The PC1, PC2 and PC3 accounted for 51.96, 31.61, and 14.32 % of the total variance respectively. The components 1, 2 and 3 possessed Eigen values 16.05, 2.29 and 1.23 respectively. All the alkanes except C_{22}, C_{31-33} exhibit significant positive loadings in PC1 with highest loadings for C_{21} and C_{27}. PC2 is affected by the high positive loadings of *n*- alkanes C_{31-33} while PC3 has high positive loading for $C_{22.}$ The PCA results of the foliar alkane profiles thus show significant positive loadings for all alkanes in different components suggesting the role of these alkanes in differentiating the leaves among species with respect to the amount of alkane produced and the greater diversity in the alkane content of the leaves is due to the variation in the biosynthesis and accumulation of these alkanes in the leaves. PC1 represents the biosynthesis process in which all the n-alkanes are produced

whereas the other two factors represent the deviations from the common route of synthesis or accumulation according to the persisting environmental conditions. The uniqueness in the production of the *n*-alkane, C_{31} is clear from its highest positive value. Also, it can be inferred that the *n*-alkanes C_{31-33} formed in the leaves through processes different from other *n*-alkanes or they possess properties different from others. C_{22} can be expected to behave or function in an entirely different manner from other n- alkanes.

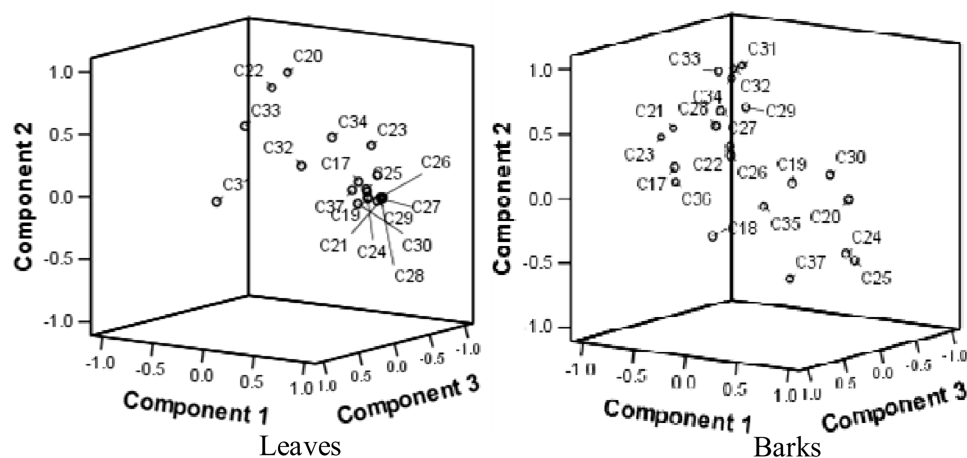

Fig. 6.7 Principle component plot in rotated space for n- alkanes (absolute abundance) in the leaves and barks of *Rhizophoraceae* mangroves (Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization)

PCA was used on data set of absolute abundance of *n*-alkanes of barks of *Rhizophoraceae* mangrove plants. PCA resulted in four components accounting for 31.33 (PC1), 30.974 (PC2), 19.118(PC3) and 18.578 (PC4)% of the total variance (100%) (Fig. 6.7). The Eigen values of these components PC1, PC2, PC3 and PC4 are 9.104, 6.77, 3.50 and 1.62 respectively. Similar

PCA contributions show the different process contributing in the concentration of alkanes in the bark. Significant positive loadings were observed for the *n*- alkanes, C_{19}, C_{20}, C_{24}, C_{25} and C_{30} indicating that the differences in production/ accumulation of these alkanes among the mangroves cause alterations in *n*- alkane profiles among the plants. High negative loadings were observed for the *n*-alkanes C_{21}, $C_{23}$and C_{36} indicating the possibility of removal of these alkanes in the biosynthetic pathway followed by an increased accumulation or generation of other alkanes. In the next level, the *n*- alkanes found to influence the alkane profiles were C_{27}, C_{29}, C_{31-34} with a significant positive loading in PC2 depicting the dominance of these alkanes in all the plants and their variation in the production pathway from other alkanes. PC3 was affected by significant positive loadings of C_{17}, C_{18}, C_{19}, C_{26}, C_{27} and C_{28} while PC4 showed high positive loadings for C_{22} and C_{35}. The PCA results points towards the importance of these *n*- alkanes in distinguishing the mangrove plants on the basis of quantity of these alkanes produced or transported in the bark of the plants.

A usual transportation or biosynthetic path as other plants cannot be expected in mangrove plants. Either preferential transportation or retention of some alkanes in the leaves or in the barks is likely to occur. *n*-Alkanes stable in the leaves are found to be unstable in bark and those *n*- alkanes which are important for the barks are not significant in the leaves. So the processes involved in the distribution of *n*-alkanes in the mangrove plants are so complex to explain using the PCA of absolute concentration. Hence the PCA using relative abundance of *n*- alkanes has been employed to derive better conceptions on the processes involved in their distribution.

(ii) PCA based on relative abundance

PCA was employed on data set of relative abundance of *n*-alkanes of leaves *Rhizophoraceae* mangrove plants. PCA results are focussed on the first three principal components (Fig. 6.8), which explained 92.46% of total cumulative variance. The PC1, PC2 and PC3 accounted for 48.03, 29.13 and 15.30 % of the total variation respectively. The Eigen values of these components1, 2 and 3 are 10.11, 5.90 and 2.93 respectively. The parameters affecting PC1 were C_{30}, C_{31}, C_{35}, C_{36}, C_{37} with a high negative loading pointing towards their same synthetic path in all the species and their least contribution towards the variations in alkane synthesis between the five mangrove plants whereas a high positive loading for C_{17}, C_{19}, C_{26}, C_{27} and C_{29} in PC1 indicates the role of these molecules in the classification of these plants with respect to alkanes. It can be assumed that in some process where there is enrichment of C_{17}, C_{19}, C_{26}, C_{27} and C_{29} there is depletion of C_{30}, C_{31}, C_{35}, C_{36} and C_{37} in the leaves. PC2 showed high negative loadings for C_{21} and C_{28} while high positive loadings were observed for C_{32-34} explaining the variations in biosynthetic pathways in which generation of C_{21} and C_{28} followed by a depletion of C_{32-34}. PC3 is characterised by the high negative loadings of C_{20} and C_{23} explaining the uniformity in production.

As the PCA can be used to express the data in such a way as to highlight their similarities and differences (Winterova et., 2008), it has been used previously to establish the relationships between chemical traits and other characteristics in various plants (Azondanlou *et al.*, 2003; Ruie and eges, 2008; Emeka and Chimaobi, 2012). The PCA results of the present study shows that the greater diversity in the foliar alkane composition is due

to C_{20}, C_{29}, C_{32}, C_{33} and C_{34} .Thus this observation provides a better understanding towards setting up criteria for studies related to chemical classification and in source identification of organic matter in sediments.

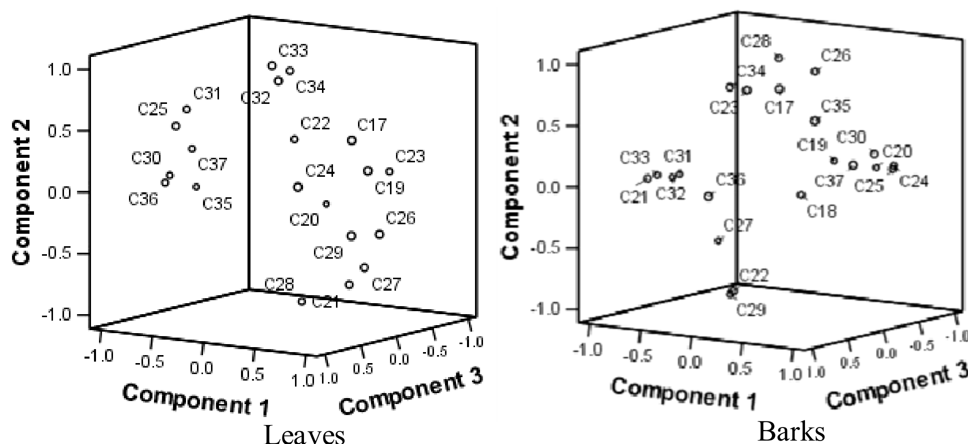

Fig. 6.8 Principle component plot in rotated space for *n*- alkanes (relative abundance) in the leaves and barks of *Rhizophoraceae* mangroves (Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization)

The results of PCA of relative abundance of *n*-alkanes components of the bark of the five mangroves (Fig. 6.8) are focussed on the first three components which explained 87.65% of total variance. The PC1, PC2 and PC3 accounted for 35.48, 29.07 and 23.1 % of the total variance respectively. The components 1, 2 and 3 possessed Eigen values 9.40, 6.23 and 3.66 respectively. The parameters affecting PC1 were C_{24}, C_{25}, C_{27}, C_{30-35}, and C_{37} while C_{17}, C_{22}, C_{23}, C_{26}, C_{28}, C_{29} and C_{34} showed significant loadings in PC2. The PC3 has high loading for C_{19}, C_{20} and C_{36}. The PCA results of the bark alkane profiles showed the *n*-alkanes C_{17}, C_{23}, C_{24}, C_{25}, C_{26}, C_{28}, C_{30}, C_{34} and C_{37} have significant positive loading in the components while C_{19}, C_{20}, C_{22}, C_{27}, C_{29}, C_{31}, C_{32} and C_{33} shows significant

negative loading. These results indicate that C_{17}, C_{23}, C_{24}, C_{25}, C_{26}, C_{28}, C_{30}, C_{34} and C_{37} contribute maximum towards the differences in the barks among species with respect to alkanes and the greater diversity in the alkane composition of the barks is due to the variation in distribution in these alkanes. The components C_{19}, C_{20}, C_{22}, C_{27}, C_{29}, C_{31}, C_{32} and C_{33} show uniform distribution but behaviours opposite to the other alkanes. Thus the current observation of the alkane profiles of the barks of the five mangrove plants extents a thorough base to study the contribution of bark to the organic matter input into the surrounding ecosystems.

Processes that are happening in the plants require a detailed examination of various stages of synthesis. Further studies should lead to identification of the production pathways and transport pathways. Because of the lack of exact knowledge of the steps in the biosynthetic pathways it is difficult to explain the process pathways clearly. The present data on alkanes could explain only the overall plant character as terrestrial, mangrove or aquatic or as tree or shrub. For general characterisation of the *Rhizophoraceae* mangroves alkanes cannot be used. Moreover, no plant specific biomarker component was identified and hence limiting the use of *n*-alkanes as a biomarker.

6.7 Conclusion

The current study reveals that leaves and barks contribute differently towards the chemical classification of the five mangrove plants; *B. cylindrica*, *B. gymnorrizha*, *K. candel*, *R. apiculata* and *R. mucronata* on the basis of their alkane composition. R. mucronata showed distinct alkane distribution in the leaves which separate it from the other plants. The study

identifies C_{31} as the most abundant n- alkane in the *Rhizophoraceae* mangroves with an exception of R. mucronata which showed high abundance for C_{29}. In the present study, the n- alkane profiles of the mangrove plant leaves and bark were characteristic in the sense that, both contained a high proportion of low molecular weight components. The higher proportions of C_{17} and C_{22} detected in the mangroves suggest the need to adopt or develop methods and proxies while using it as a biomarker. *R. apiculata* exhibited similarities with *B. gymnorrizha* while *B. cylindrica* was found to be more related with the alkane composition of *R. mucronata*. *K. candel* showed an intermediate character between the two genera; *Bruguiera* and *Rhizophora*. The current data reveals the potential of *Rhizophoraceae* mangroves, especially *R. mucronata,* towards their use as a source of wax for food use. The abundance of n- alkanes in the leaves and bark supported by the presence of other important health beneficial components such as flavonoids, minerals and metals and other biochemical components are helpful in evaluating their use in ethanopharmaceutical practices thereby opening new access strip for novel drug development. The results of PCA support the limitations of alkanes towards its use in chemotaxonomic a well as biomarker studies.

References

Ali, H. A. M., Mayes, R. W., Hector, B. L., and Orskov, E. R. (2005). Assessment of *n*-alkanes, long-chain fatty alcohols and long-chain fatty acids as diet composition markers: The concentrations of these compounds in rangeland species from Sudan. *Animal Feed Science and Technology*, *121*(3), 257-271.

Bi, X., Sheng, G., Liu, X., Li, C., and Fu, J. (2005). Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. *Organic Geochemistry*, *36*(10), 1405-1417.

Bray, E. E., and Evans, E. D. (1961). Distribution of *n*-paraffins as a clue to recognition of source beds. *Geochimica et Cosmochimica Acta*, *22*(1), 2-15.

Brincat, D., Yamada, K., Ishiwatari, R., Uemura, H., and Naraoka, H. (2000). Molecular-isotopic stratigraphy of long-chain *n*-alkanes in Lake Baikal Holocene and glacial age sediments. *Organic Geochemistry*, *31*(4), 287-294.

Burdock, G. A. (1997). Encyclopedia of food and colour additives, CRC press, USA.

Bush, R. T., and McInerney, F. A. (2013). Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. *Geochimica et Cosmochimica Acta*, *117*, 161-179.

Castañeda, I. S., and Schouten, S. (2011). A review of molecular organic proxies for examining modern and ancient lacustrine environments. *Quaternary Science Reviews*, *30*(21), 2851-2891.

Collister, J. W., Rieley, G., Stern, B., Eglinton, G., and Fry, B. (1994). Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. *Organic Geochemistry*, *21*(6), 619-627.

Cranwell, P. A. (1984). Lipid geochemistry of sediments from Upton Broad, a small productive lake. *Organic Geochemistry*, *7*(1), 25-37.

Cranwell, P. A., Eglinton, G., and Robinson, N. (1987). Lipids of aquatic organisms as potential contributors to lacustrine sediments—II. *Organic Geochemistry*, *11*(6), 513-527.

Diefendorf, A. F., Freeman, K. H., Wing, S. L., and Graham, H. V. (2011). Production of *n*-alkyl lipids in living plants and implications for the geologic past. *Geochimica et Cosmochimica Acta*, *75*(23), 7472-7485.

Dodd, R. S., and Afzal-Rafii, Z. (2000). Habitat-related adaptive properties of plant cuticular lipids. *Evolution*, *54*(4), 1438-1444.

Dodd, R. S., and Poveda, M. M. (2003). Environmental gradients and population divergence contribute to variation in cuticular wax composition in Juniperus communis. *Biochemical systematics and ecology*, *31*(11), 1257-1270.

Dodd, R. S., Blasco, F., Rafii, Z. A., and Torquebiau, E. (1999). Mangroves of the United Arab Emirates: ecotypic diversity in cuticular waxes at the bioclimatic extreme. *Aquatic Botany*, *63*(3), 291-304.

Dodd, R. S., Fromard, F., Rafii, Z. A., and Blasco, F. (1995). Biodiversity among West African *Rhizophora:* Foliar wax chemistry. *Biochemical Systematics and Ecology*, *23*(7), 859-868.

Dodd, R. S., Rafii, Z. A., Fromard, F., and Blasco, F. (1998b). Evolutionary diversity among Atlantic coast mangroves. *Acta Oecologica*, *19*(3), 323-330.

Dodd, R.S., Rafii ,Z.A., and Power, A.B. (1998a). Ecotypic adaptation in Austracedrus Chilensis in cuticular, hydrocarbon composition. *New Phytologist*, *138,* 699-708.

Dove, H., and Mayes, R. W. (1991). The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. *Crop and Pasture Science*, *42*(6), 913-952.

Duan, Y. (2000). Organic geochemistry of recent marine sediments from the Nansha Sea, China. *Organic Geochemistry*, *31*(2), 159-167.

Duan, Y., and He, J. (2011). Distribution and isotopic composition of n-alkanes from grass, reed and tree leaves along a latitudinal gradient in China.*Geochemical Journal*, *45*(3), 199-207.

Duan, Y., and Ma, L. (2001). Lipid geochemistry in a sediment core from Ruoergai Marsh deposit (Eastern Qinghai-Tibet plateau, China). *Organic Geochemistry*,*32*(12), 1429-1442.

Duke, N. C., Benzie, J. A., Goodall, J. A., and Ballment, E. R. (1998). Genetic structure and evolution of species in the mangrove genus Avicennia (Avicenniaceae) in the Indo-West Pacific. *Evolution*, 1612-1626.

Duke, N. C., Lo, E., and Sun, M. (2002). Global distribution and genetic discontinuities of mangroves–emerging patterns in the evolution of *Rhizophora*.*Trees*, *16*(2-3), 65-79.

Eglinton, G., and Hamilton, R. J. (1963). The distribution of alkanes. In: *Chemical plant taxonomy*, Academic Press Inc., London, pp. *187*- 217.

Eglinton, G., and Hamilton, R. J. (1967). Leaf epicuticular waxes. *Science*,*156* (3780), 1322-1335.

Eglinton, G., Gonzalez, A. G., Hamilton, R. J., and Raphael, R. A. (1962). Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. *Phytochemistry*, *1*(2), 89-102.

Eglinton, G., Logan, G. A., Ambler, R. P., Boon, J. J., and Perizonius, W. R. K. (1991). Molecular preservation [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *333*(1268), 315-328.

Ficken, K. J., Li, B., Swain, D. L., and Eglinton, G. (2000). An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry*, *31*(7), 745-749.

Gagosian, R. B., and Peltzer, E. T. (1986). The importance of atmospheric input of terrestrial organic material to deep sea sediments. *Organic Geochemistry*,*10*(4), 661-669.

Gireesh Kumar, T.R. (2013). Source Characterization of Sedimentary Organic Matter in a Tropical Estuary, Southwest Coast of India: A Biomarker Approach, PhD. Thesis, Cochin University of Science and Technology, Kochi, India.

Gülz, P. G. (1994). Epicuticular leaf waxes in the evolution of the plant kingdom.*Journal of Plant Physiology*, *143*(4), 453-464.

Hanisch, S., Ariztegui, D., and Püttmann, W. (2003). The biomarker record of Lake Albano, central Italy—implications for Holocene aquatic system response to environmental change. *Organic geochemistry*, *34*(9), 1223-1235.

Herbin, G. A., and Robins, P. A. (1969). Patterns of variation and development in leaf wax alkanes. *Phytochemistry*, *8*(10), 1985-1998.

Hogg, R. W., and Gillan, T., F. (1984). Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. *Phytochemistry*, *23*(1), 93-97.

Jansen, B., Nierop, K. G., Hageman, J. A., Cleef, A. M., and Verstraten, J. M. (2006). The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. *Organic Geochemistry*, *37*(11), 1514-1536.

Jansen, B., van Loon, E. E., Hooghiemstra, H., and Verstraten, J. M. (2010). Improved reconstruction of palaeo-environments through unravelling of preserved vegetation biomarker patterns. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *285*(1), 119-130.

Jetter, R., Kunst, L., and Samuels, A. L. (2008). 4 Composition of plant cuticular waxes. *Annual Plant Reviews, Biology of the Plant Cuticle*, *23*, 145.

Kathiresan, K.(1995). Studies on tea from mangrove leaves. *Environment and. Ecology*, *13*, 321–323.

Kawamura, K., and Ishiwatari, R. (1984). Fatty acid geochemistry of a 200 m sediment core from Lake Biwa, Japan. Early diagenesis and paleoenvironmental information. *Geochimica et Cosmochimica Acta*, *48*(2), 251-266.

Lockheart, M. J., Van Bergen, P. F., and Evershed, R. P. (1997). Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: implications for the study of higher land plant-derived sedimentary organic matter. *Organic Geochemistry*, *26*(1), 137-153.

Maffei, M. (1996). Chemotaxonomic significance of leaf wax alkanes in the Gramineae. *Biochemical Systematics and Ecology*, *24*(1), 53-64.

Marzi, R., Torkelson, B. E., and Olson, R. K. (1993). A revised carbon preference index. *Organic Geochemistry*, *20*(8), 1303-1306.

Mead, R., Xu, Y., Chong, J., and Jaffé, R. (2005). Sediment and soil organic matter source assessment as revealed by the molecular distribution and carbon isotopic composition of n-alkanes. *Organic Geochemistry*, *36*(3), 363-370.

Melville, F., Burchett, M., and Pulkownik, A. (2004). Genetic variation among age-classes of the mangrove *Avicennia marina* in clean and contaminated sediments. *Marine pollution bulletin*, *49*(9), 695-703.

Meyers, P. A. (2003). Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes.*Organic geochemistry*, *34*(2), 261-289.

Meyers, P. A., and Ishiwatari, R. (1993). Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic geochemistry*, *20*(7), 867-900.

Mohanan, C.N. (1997). Mangroves. In : Ed., Thampi, K.B., Nayar, N.M., and Nair, C.S., *National Resources of Kerala*. WWF- India, Thiruvanathapuram, Kerala. PP 149-158.

Mugler I., Sachse D., Werner M., Xu B., Wu G., Yao T. and Gleixner G. (2008). A multi-proxy approach to reconstruct hydrological changes and Holocene climate development of Nam Co, Central Tibet. *Journal of Paleolimnology*, *43*(4), 625-648.

Nguyen-Tu, T. T., Egasse, C., Zeller, B., and Derenne, S. (2007). Chemotaxonomical investigations of fossil and extant beeches. I. Leaf lipids from the extant *Fagus sylvatica* L. *Comptes Rendus Palevol*, *6*(6), 451-461.

Nott, C. J., Xie, S., Avsejs, L. A., Maddy, D., Chambers, F. M., and Evershed, R. P. (2000). *n*- Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation. *Organic Geochemistry*, *31*(2), 231-235.

Pancost, R. D., Baas, M., van Geel, B., & Sinninghe Damsté, J. S. (2002). Biomarkers as proxies for plant inputs to peats: an example from a sub-boreal ombrotrophic bog. *Organic Geochemistry*, *33*(7), 675-690.

Peters, K. E., and Moldowan, J. M. (1993). *The biomarker guide: interpreting molecular fossils in petroleum and ancient sediments* (Vol. 363). Englewood Cliffs, NJ: Prentice Hall.

Post-Beittenmiller, D. (1996). Biochemistry and molecular biology of wax production in plants. *Annual review of plant biology*, *47*(1), 405-430.

Poynter, J. G., Farrimond, P., Robinson, N., and Eglinton, G. (1989). Aeolian-derived higher plant lipids in the marine sedimentary record: Links with palaeoclimate. In *Paleoclimatology and paleometeorology: modern and past patterns of global atmospheric transport* (pp. 435-462). Springer Netherlands.

Prasad, R. B. N., and Gülz, P. G. (1990). Developmental and seasonal variations in the epicuticular waxes of beech leaves (Fagus sylvatica L.). *Zeitschrift für Naturforschung. Section C, Biosciences*, *45*(7-8), 805-812.

Rafii, Z. A., Dodd, R. S., and Fromard, F. (1996). Biogeographic variation in foliar waxes of mangrove species. *Biochemical systematics and ecology*, *24*(4), 341-345.

Ratheesh Kumar, C.S. (2011). Triterpenoids as biomarkers of mangrove organicmMatter in Cochin estuarine system, PhD Thesis, Cochin University of Science and Technology, Kochi, India.

Rieley, G., Collier, R. J., Jones, D. M., Eglinton, G., Eakin, P. A., and Fallick, A. E. (1991). Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds.

Rommerskirchen, F., Eglinton, G., Dupont, L., and Rullkötter, J. (2006). Glacial/interglacial changes in southern Africa: Compound-specific δ13C land plant biomarker and pollen records from southeast Atlantic continental margin sediments. *Geochemistry, Geophysics, Geosystems*, *7*(8). doi:10.1029/2005GC001223.

Rommerskirchen, F., Plader, A., Eglinton, G., Chikaraishi, Y., and Rullkötter, J. (2006). Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C_4 grass waxes. *Organic Geochemistry*, *37*(10), 1303-1332.

Sachse D., Billault I., Bowen G. J., Chikaraishi Y., Dawson T. E., Feakins S. J., Freeman K. H., Magill C. R., McInerney F. A., van der Meer M. T. J., Polissar P., Robins R. J., Sachs J. P.,Schmidt H.-L., Sessions A. L., White J. W. C., West J. B. and Kahmen A. (2012). Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annual Review of Earth and Planetary Sciences.* 40, 221–249.

Sachse, D., Kahmen, A., and Gleixner, G. (2009). Significant seasonal variation in the hydrogen isotopic composition of leaf-wax lipids for two deciduous tree ecosystems (*Fagus sylvatica* and *Acer pseudoplatanus*). *Organic Geochemistry*,. 40, 732–742.

Said, M., W. (2011.) Variations in the composition of leaf cuticlar hydrocarbons of two varieties in *Avicennia marina (Forssk.) Vierh. African Journal of Biological Science*, *7* (2), 109-117.

Schwark, L., Zink, K., and Lechterbeck, J. (2002). Reconstruction of postglacial to early Holocene vegetation history in terrestrial Central Europe via cuticular lipid biomarkers and pollen records from lake sediments. *Geology*, *30*(5), 463-466.

Shepherd, T., 2003. Wax pathways. In: Thomas, B., Murphy, D.J. (Eds.), Encyclopedia of Applied Plant Sciences. Elsevier, Amsterdam, pp. 1204–1225.

Sikes, E. L., Uhle, M. E., Nodder, S. D., and Howard, M. E. (2009). Sources of organic matter in a coastal marine environment: Evidence from *n*-alkanes and their $\delta^{13}C$ distributions in the Hauraki Gulf, New Zealand. *Marine Chemistry*, *113*(3), 149-163.

Silva, C. A., and Madureira, L. A. (2012). Source correlation of biomarkers in a mangrove ecosystem on Santa Catarina Island in southern Brazil. *Anais da Academia Brasileira de Ciências*, *84*(3), 589-604.

Simoneit, B. R., Sheng, G., Chen, X., Fu, J., Zhang, J., and Xu, Y. (1991). Molecular marker study of extractable organic matter in aerosols from urban areas of China. *Atmospheric Environment. Part A. General Topics*, *25*(10), 2111-2129.

Stoker, H. S. (2012). *General, organic, and biological chemistry*. Cengage Learning.

Tan, F., Huang, Y., Ge, X., Su, G., Ni, X., and Shi, S. (2005). Population genetic structure and conservation implications of *Ceriops decandra* in Malay Peninsula and North Australia. *Aquatic Botany*, *81*(2), 175-188.

Tomlinson, P.B. (1986). The Botany of Mangroves. Cambridge, London, New York: Cambridge University Press. 414 pp.

Versteegh, G. J., Schefuß, E., Dupont, L., Marret, F., Sinninghe Damsté, J. S., and Jansen, J. H. (2004). Taraxerol and *Rhizophora* pollen as proxies for tracking past mangrove ecosystems. *Geochimica et Cosmochimica Acta*, *68*(3), 411-422.

Viso, A. C., Pesando, D., Bernard, P., and Marty, J. C. (1993). Lipid components of the mediterranean seagrass *Posidonia Oceanica*. *Phytochemistry*,*34*(2), 381-387.

Vogts, A., Moossen, H., Rommerskirchen, F., and Rullkötter, J. (2009). Distribution patterns and stable carbon isotopic composition of alkanes and alkan-1-ols from plant waxes of African rain forest and savanna C_3 species. *Organic geochemistry*, *40*(10), 1037-1054.

Volkman, J. K., Barrerr, S. M., Blackburn, S. I., & Sikes, E. L. (1995). Alkenones in *Gephyrocapsa oceanica*: Implications for studies of paleoclimate. *Geochimica et Cosmochimica Acta*, *59*(3), 513-520.

Wakeham, S. G. (1976). A comparative survey of petroleum hydrocarbons in lake sediments. *Marine Pollution Bulletin*, *7*(11), 206-211.

Zhang, Z., Zhao, M., Eglinton, G., Lu, H., and Huang, C. Y. (2006). Leaf wax lipids as paleovegetational and paleoenvironmental proxies for the Chinese Loess Plateau over the last 170kyr. *Quaternary Science Reviews*, *25*(5), 575-594.

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SUMMARY AND CONCLUSIONS

Mangroves acquire a range of features which make them uniquely adaptable to their stressful environment, they are halophytic or salt tolerant, have aerial roots for gathering oxygen and seeds that geminate on the tree. Mangroves like other plants depend on the photosynthetic reduction of carbondioxide to form carbohydrates and other organic constituents necessary for growth and maintenance. They are known to synthesise more polyphenols and tannins in response to salinity and organic acid metabolism which vary with different species (Basak et al., 1996). These salt-tolerant plants have evolved mechanisms to cope with the harmful environmental conditions that include salt and desiccation. Mangroves have enormous economic and ecological value. Extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens. Mangroves may be further developed as sources of high-value commercial products and fishery resources and as sites for a burgeoning ecotourism industry. They protect and stabilise coastlines, enrich coastal waters, yield commercial forest products and support coastal fisheries. Mangrove forests are among the world's most productive ecosystems, producing organic carbon well in excess of the ecosystem requirements and

contributing significantly to the global carbon cycle. Mangroves create typical ecological environments that host rich assemblages of species.

In order to assess the contribution of mangroves towards the environment and their importance as sources for potential chemicals for future use, chemotaxonomy is of much relevance compared with the classical taxonomy based on morphological features. In chemotaxonomy, environment has a specific role. It is linked with environmental compositions as well as the metabolic character of the plants. So, environmental conditions are important. In order to understand the relative importance of biochemical processes and the relevance of chemotaxonomy, it is necessary to characterise and quantify chemical constituents in the plant materials. The *Rhizophoraceae* family of true mangrove plants is the most populated and contains widely distributed species. There is a gap of information towards the chemistry of *Rhizophoraceae* mangroves from Kerala for their use in various applications including extraction of bioactive metabolites and as biomarkers.

The main objective of the study was to identify the major factors contributing to the chemotaxonomy of *Rhizophoraceae* mangroves and to identify the potent species for the extraction of bioactive metabolites. The leaves and bark of five mangrove plants of family *Rhizophoraceae; Bruguiera cylindrica* and *Bruiguiera gymnorrhiza* belonging to the genus *Bruguiera*; *Kandelia candel* of the genus *Kandelia*; *Rhizophora mucronata* and *Rhizophora apiculata* of the genus *Rhizopora* are considered for this study. The basic chemical characterisation of the mangroves have been carried out by determining the elemental, isotopic, mineral and biochemical composition of the leaves and bark. The presence of food flavonoids and

their relevance towards chemotaxonomy has been investigated. The use of n- alkanes and fatty acids in chemotaxonomic studies and biomarker studies has been validated by their quantitative and qualitative analysis.

K.candel was found to exhibit highest carbon content among the five *Rhizophoraceae* mangroves under investigation. The macronutrient elements, H,N, P and S are more concentrated in the leaves and less in the bark of mangroves while C is more concentrated in their bark. No specific trends were observed according to genera. The carbon isotope composition of leaves of the *Rhizophoraceae* mangrove under the present investigation matches well with the all ready established values of $\delta^{13}C$ for C3 plants. The plant components of *K. candel* were enriched in ^{13}C relative to other *Rhizophoraceae* mangroves by 1 to 2.5‰. The most depleted $\delta^{13}C$ was found in *B. gymnorrizha*. The present nitrogen stable isotope results fall within the range of plants that obtain inorganic nitrogen directly from seawater. The lower C/N in the leaves suggests that the leaves have high nutrient quality compared to bark and significantly higher nutritional quality was observed in *B. cylindrica* leaves than the other mangrove plants in this study.

All plants, except *B. gymnorrizha* are found to accumulate sodium in their leaves than in the bark. The genus *Rhizophora* as well as *Kandelia* exhibited higher potassium content than the *Bruguiera* plants. The salt exclusion mechanism is efficiently operative in *K. candel* followed by *Rhizophora* species. Plants of the genus *Bruguiera* were found to be the least salt excluding mangroves among the *Rhizophoraceae* plants under investigation. *R. apiculata* leaves were found to be the richest in magnesium

content while the iron rich mangrove plant was found to be *B. gymnorrizha*. All the mangrove plant parts investigated contained manganese within the recommended level for plants. Zinc and copper content was found to be highest in *K.candel*. Pb in this study was found to be below the recommended levels. In this research study, it was found that the leaves and bark of the mangrove plant *R. mucronata* is free from cadmium.

The present study gives an idea that, except for *Bruguiera* mangroves, the major portion of the total carbohydrates are in the form of low molecular weight carbohydrates. Being high in sugars, proteins and lipids, *K. candel* was found to be species with high calorific value. The leaves of *K. candel*, the bark of *R. apiculata*, the leaves and bark of *R. mucronata* can be used as sources of antioxidant materials. Using PCA, the major processes differentiating the mangroves plants are identified as the transport of photosynthetic products in leaves and accumulation as well as storage of minerals and nutrients. The leaves of the two *Bruguiera* plants exhibited distinct chemical character with respect to the minerals and biochemical parameters, *B. cylindrica* being more similar to the genus, *Rhizophora*. The study also reveals the intermediate chemical character of *K. candel* and the distinct behaviour of *B. gymnorrizha*.

The observed levels of flavonoid contents confirm the importance of these mangrove plants as excellent sources of plant antioxidants. The quantitative and qualitative estimation of the five bioactive food flavonoids- myricetin, quercetin, kaempferol, luteolin and apigenin- in *Rhizophoraceae* mangroves is done for the first time The total flavonoids are found to be more concentrated in the leaves than in the bark. Quercetin was found to be

the ubiquitous flavonoid and found to be present in high concentration in all the mangroves of this study. *B. gymnorrhiza* can be a very good source of myricetin.

The presence of luteolin in *B. cylindrica* alone as well as the variations in different flavonols and flavones in these plants can be helpful for providing chemotaxonomic relationships between different genera of this family. Comparing the flavonoid composition of the five species reveals greater similarity of *R. mucronata* with *B. gymnorrhizha*. The presence of all the flavonoids in their leaves can be regarded as a specific taxonomic character of the plants, *R. mucronata* and *B. gymnorrhizha*. From this study it is found that all the mangroves of the family *Rhizophoraceae* can be regarded as primitive in flavonoid patterns. *R. mucronata* contains the flavonoid, procyanide Type B which is isolated for the first time from this plant.

The leaves and barks contribute differently towards the chemical classification of the five mangrove plants; *B. cylindrica*, *B. gymnorrizha*, *K. candel*, *R. apiculata* and *R. mucronata* on the basis of their alkane composition. The study identifies C_{31} as the most abundant n- alkane in the *Rhizophoraceae* mangroves with an exception of *R. mucronata* which showed high abundance for C_{29}. The n- alkane profiles of the mangrove plant leaves and bark were characteristic by a high proportion of low molecular weight components. The higher proportions of C_{17} and C_{22} detected in the mangroves suggest the need to adopt or develop methods and proxies in which the quantity and distribution of n- alkanes of the dominant mangrove species of neighbouring mangrove area while using it as a

biomarker. Alkane compositions link the two genera, *Bruguiera* and *Rhizophora* closer with *K. candel* showing an intermediate character between the them. *Rhizophoraceae* mangroves, especially *R. mucronata* can be used as a potential source for the production of wax for food use. The results of PCA support the limitations of alkanes towards its use in chemotaxonomic a well as biomarker studies.

Linoleic acid is the most abundant fatty acid found in the *Rhizophoraceae* mangroves from Kochi, southwest coast of India. The study confirms the use of the PUFAs, cis-18:2ω6 and 18:3ω3 as biomarker for mangrove plants. The lower or nil concentration in the mangroves plants use of long chain fatty acids limit their use as biomarkers for mangrove plants. *B. cylindrica* is chemically distinct from other mangrove plants by the presence of fatty acids 20:5ω3 and 20:3ω6 whereas the absence of fatty acid 18: 1ω9trans in *B. gymnorrizha* alone makes it distinct. Similarities exist between the genera *Kandelia* and *Rhizophora* with respect to the presence of FA 22:0 and absence of FA 22:1ω9. The variation of fatty acid profiles depends upon geographical location and this variation is not the same in all plants except for the most dominant fatty acid. Also, these variations cannot be related to classical taxonomic variations. From the study it was found that the leaves and barks contribute differently to the fatty acid composition of the plants, thereby to the surrounding sediment environment. The variation in the foliar nutrient levels with respect to fatty content can be used to study the food preferences in the mangrove dependent organisms. These mangrove plants can be used as a source of essential fatty acids of manifold benefits.

The quantity and quality of the beneficial and toxic components in the mangrove extracts are helpful in evaluating their use in ethanopharmaceutical practices thereby opening new corridors for novel drug development. The chemical parameters of mangrove leaves and bark can assist in an effective application of taxonomic approach towards their use in various economic and ecological services.

Combining the observations of the chemical components-resemblance of C3 plants by the $\delta^{13}C$ values, terrestrial character by fatty acids and intermediate character between terrestrial and aquatic by alkanes-the mangroves can be classified only as modified vascular plants with properties of both terrestrial and aquatic plants. When the beneficial characters are considered chemotaxonomy is more relevant than morphological classification and the former depends on environment settings. So, chemotaxonomy should be defined on environment setting while morphological classification does not have any relation with environment setting. This study provides a significant evidence for this hypothesis. Species diversity is not the same as diversity in chemicals. Plants of same genera show different chemical characters. It can be concluded that alkane profiles cannot end up in specific classification except to define the general character of the plants whereas flavonoids and fatty acids can be used as chemotaxonomic markers for *Rhizophoraceae* mangroves form Kochi.

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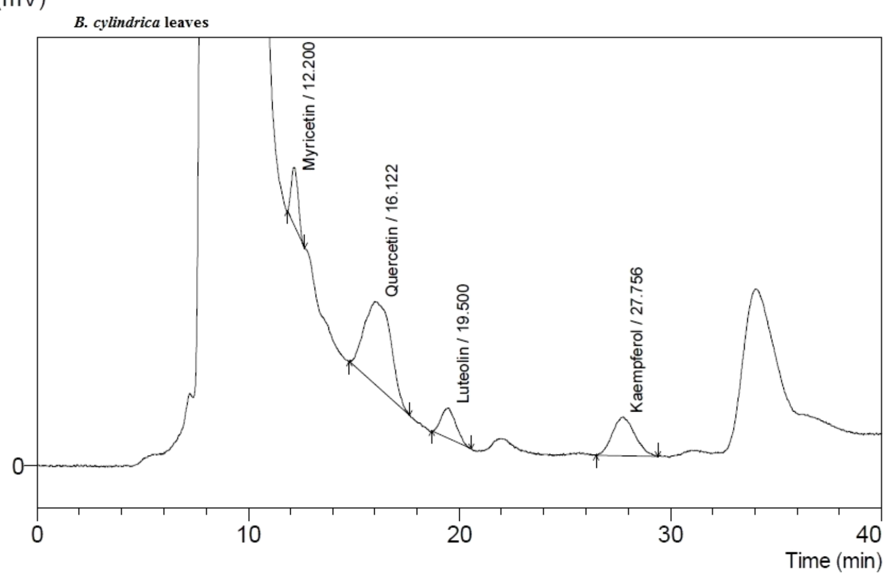
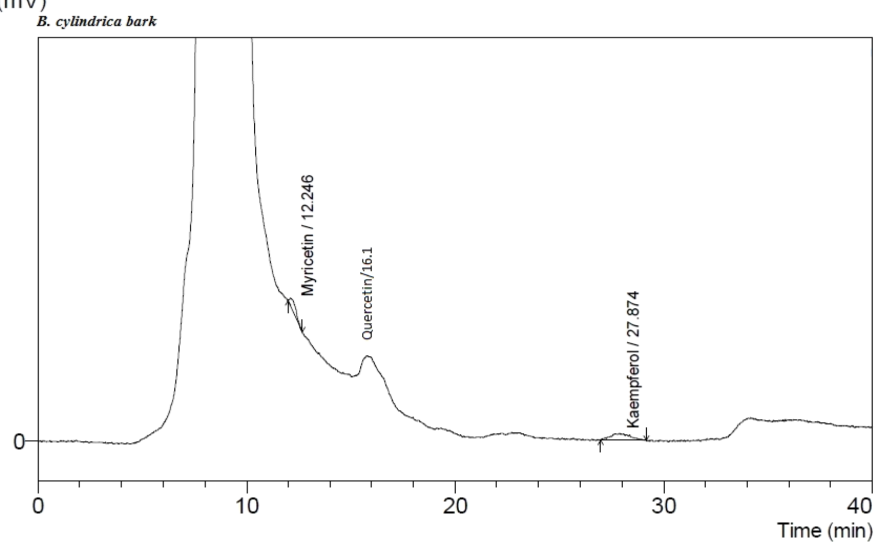
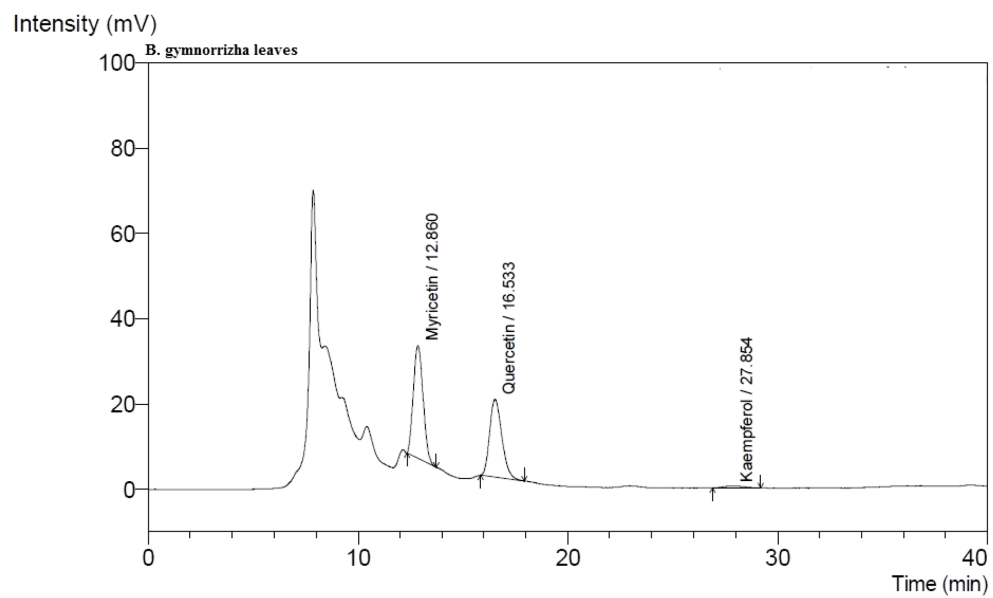
Appendix

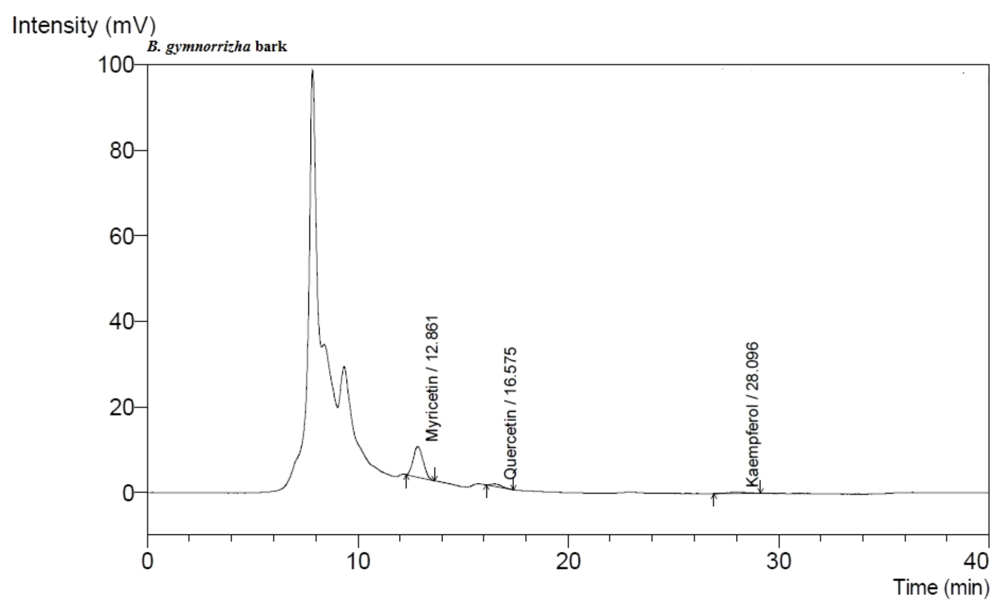
Appendix 1a

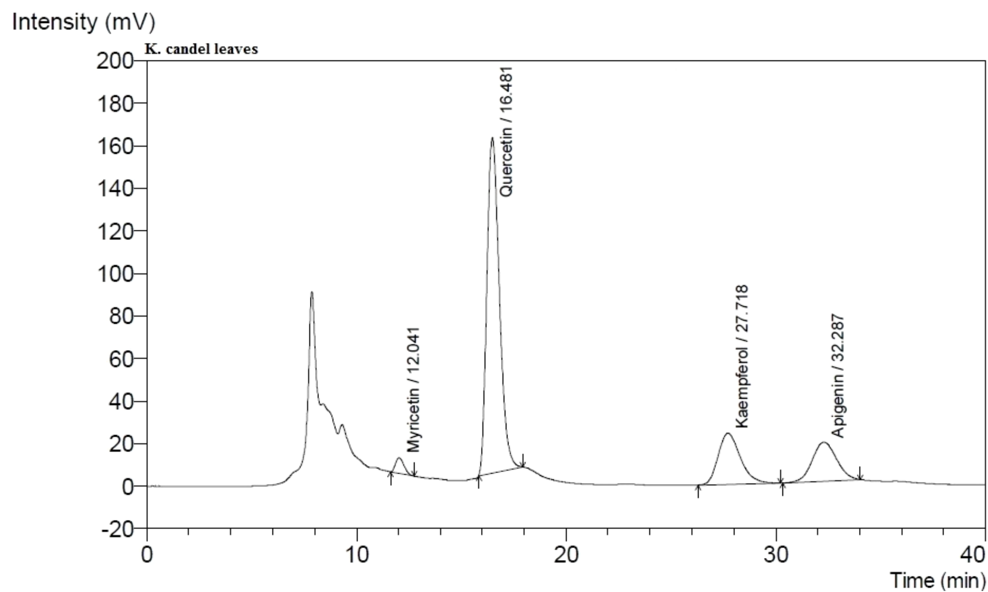
LCUV chromatogram of the flavonoid agylcons in the leaves and bark of *Rhizophoraceae* mangroves

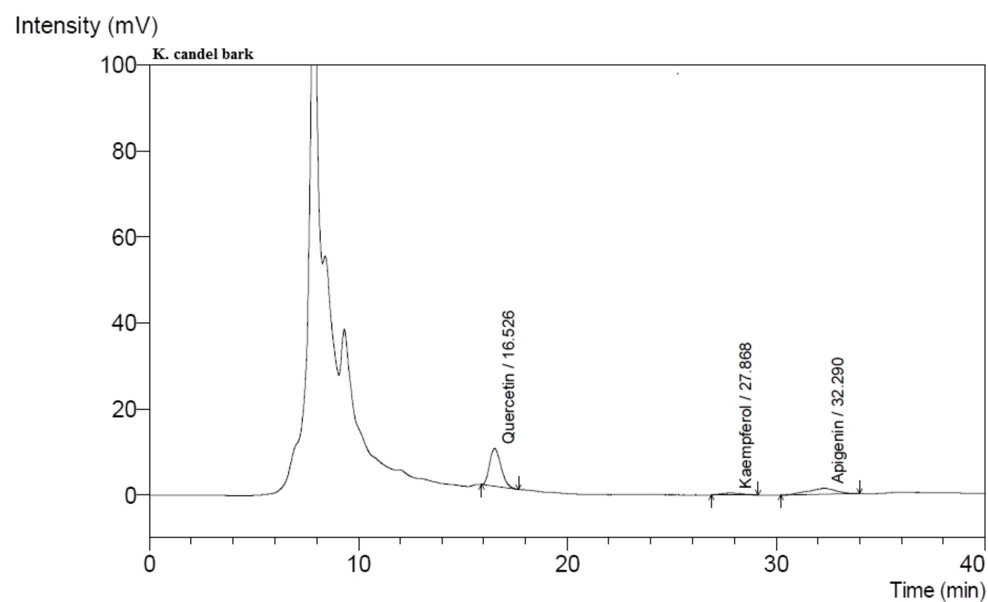


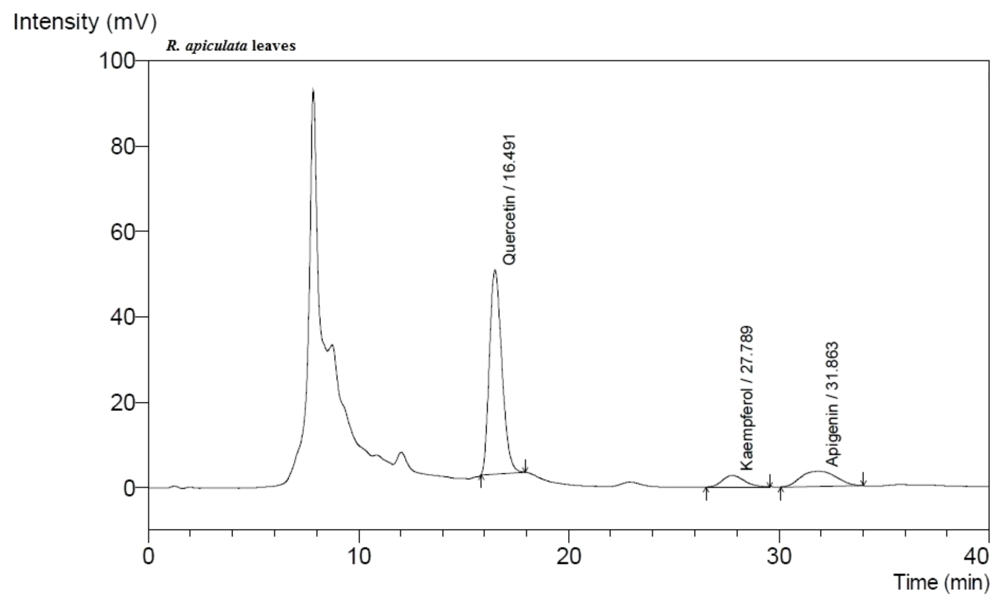


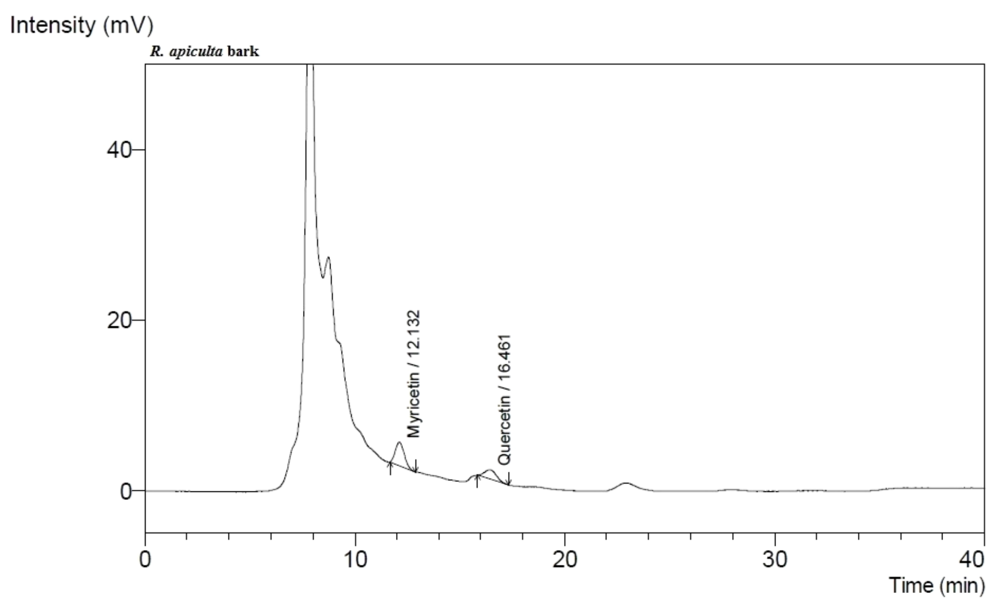

Intensity (mV)
B. gymnorrizha leaves
100
80
60
40
20
0
Myricetin / 12.860
Quercetin / 16.533
Kaempferol / 27.854
0
10
20
30
40
Time (min)

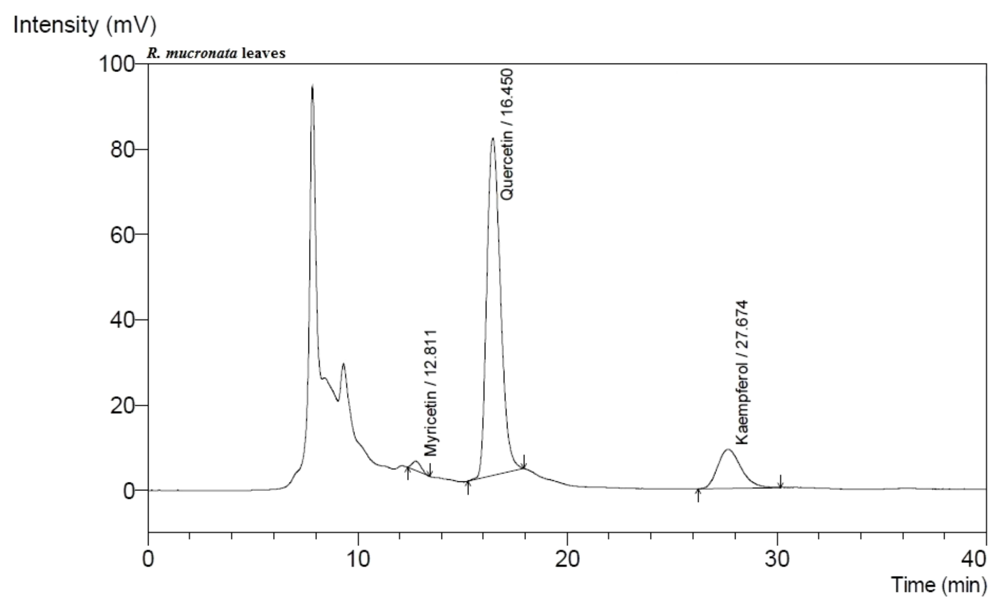

Intensity (mV)
B. gymnorrizha bark
100
80
60
40
20
0
Myricetin / 12.861
Quercetin / 16.575
Kaempferol / 28.096
0
10
20
30
40
Time (min)

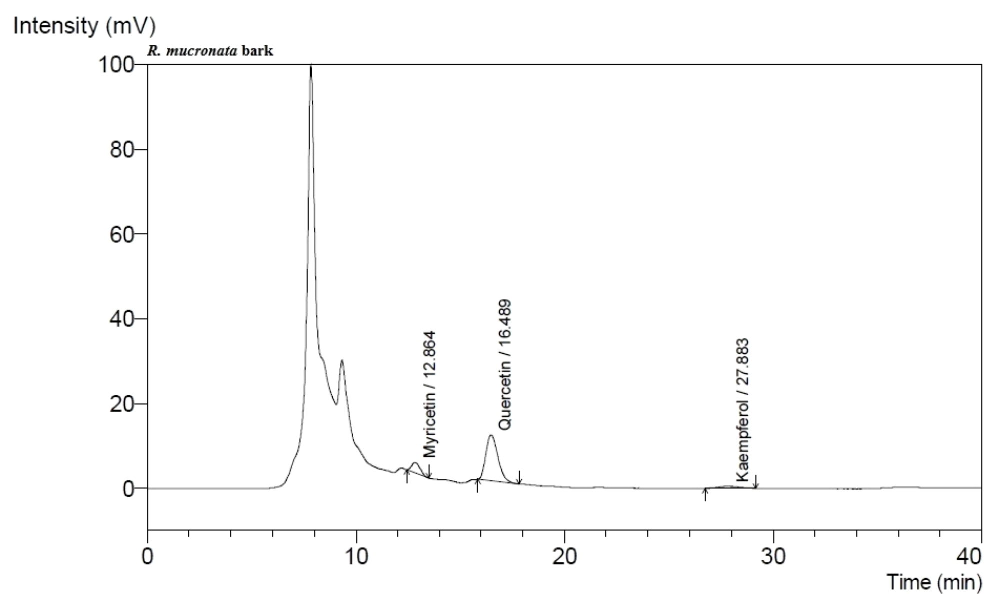

Intensity (mV)
K. candel leaves
200
180
160
140
120
100
80
60
40
20
0
-20
Myricetin / 12.041
Quercetin / 16.481
Kaempferol / 27.718
Apigenin / 32.287
0
10
20
30
40
Time (min)


Intensity (mV)
K. candel bark
100
80
60
40
20
0
Quercetin / 16.526
Kaempferol / 27.868
Apigenin / 32.290
0
10
20
30
40
Time (min)


Intensity (mV)
R. apiculata leaves
100
80
60
40
20
0
Quercetin / 16.491
Kaempferol / 27.789
Apigenin / 31.863
0
10
20
30
40
Time (min)

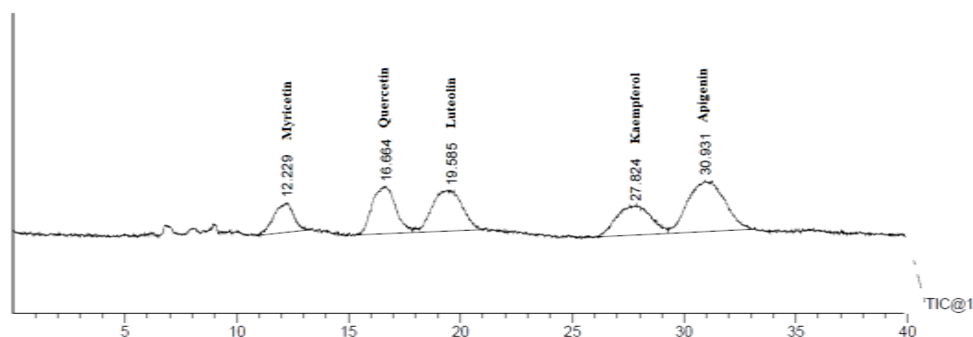

Intensity (mV)
R. apiculta bark
40
20
0
Myricetin / 12.132
Quercetin / 16.461
0
10
20
30
40
Time (min)

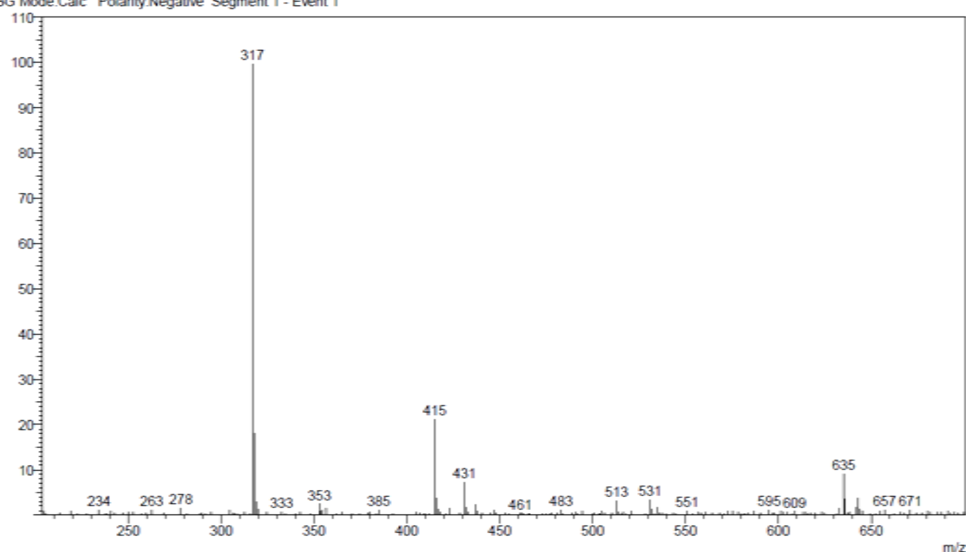

Intensity (mV)
R. mucronata leaves
100
80
60
40
20
0
Myricetin / 12.811
Quercetin / 16.450
Kaempferol / 27.674
0
10
20
30
40
Time (min)

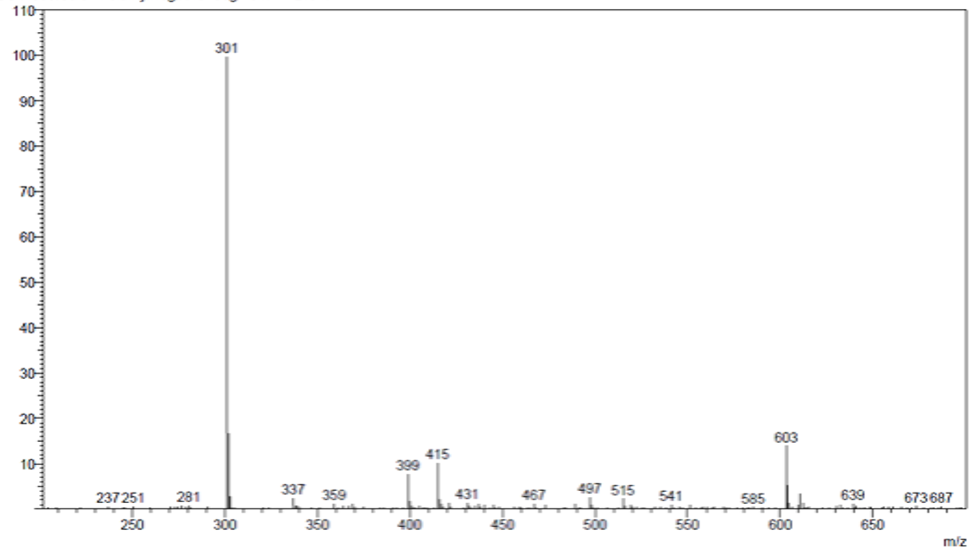

Intensity (mV)
R. mucronata bark
100
80
60
40
20
0
Myricetin / 12.864
Quercetin / 16.489
Kaempferol / 27.883
0
10
20
30
40
Time (min)

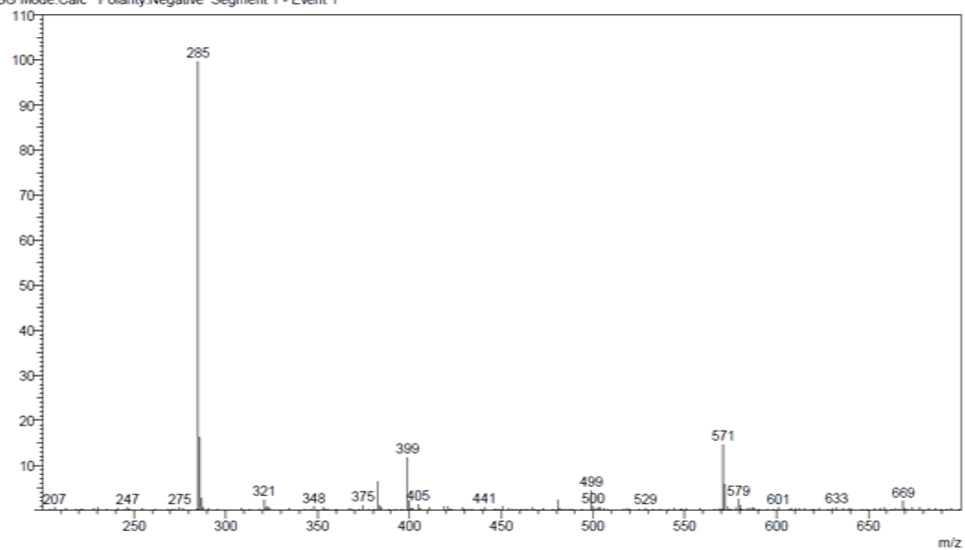
Appendix 1b

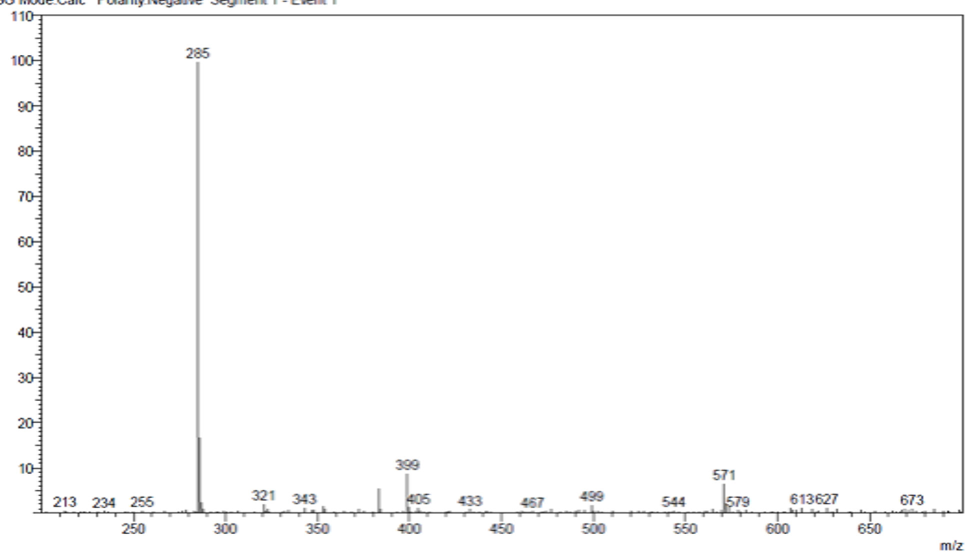
Total intensity chromatogram of flavonoid standard and corresponding mass spectra of flavonoid standards

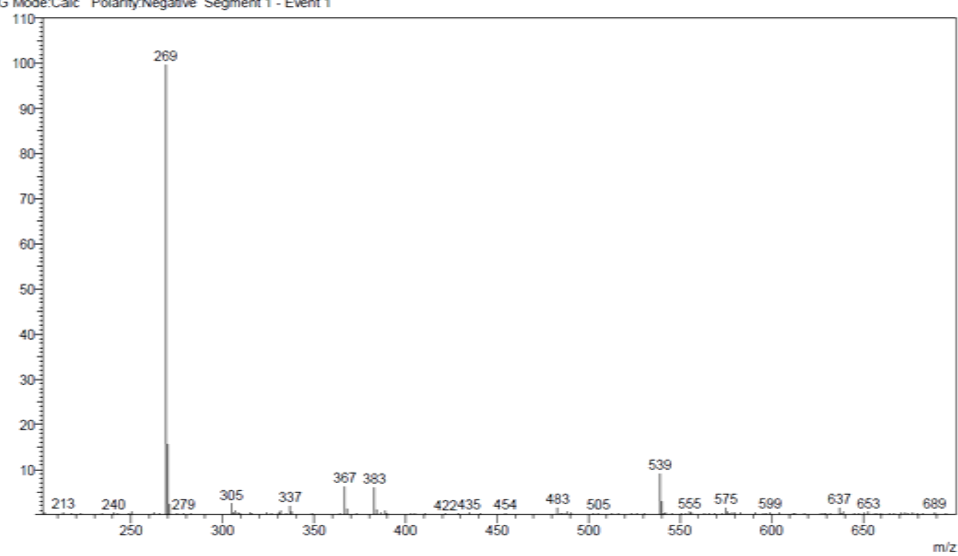





R.Time:16.667(Scan#:1001)
MassPeaks:271 BasePeak:301(77800)
Spectrum Mode:Averaged 16.650-16.683(1000-1002)
BG Mode:Calc Polarity:Negative Segment 1 - Event 1
301
399
415
603
237 251
281
337
359
431
467
497
515
541
585
639
673 687
m/z

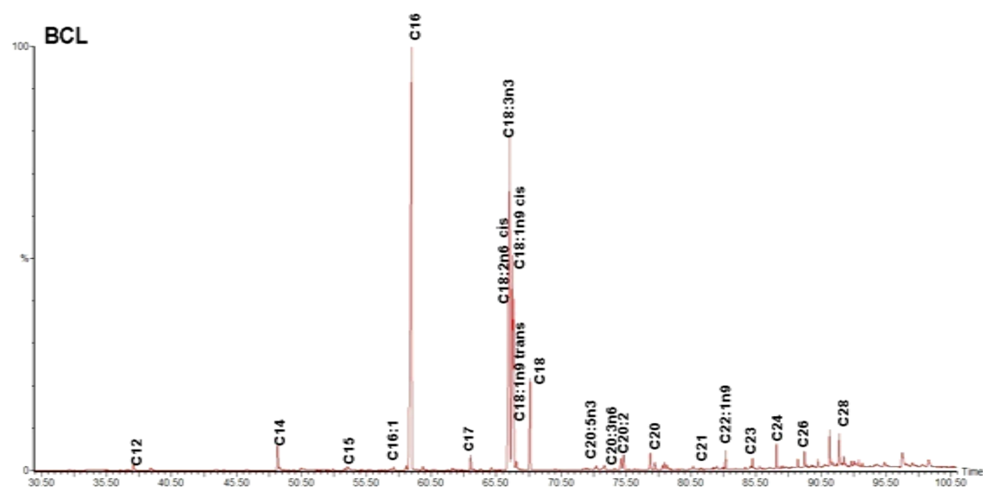

R.Time:19.583(Scan#:1176)
MassPeaks:261 BasePeak:285(72100)
Spectrum Mode:Averaged 19.567-19.600(1175-1177)
BG Mode:Calc Polarity:Negative Segment 1 - Event 1
285
399
571
207
247
275
321
348
375
405
441
499
500
529
579
601
633
669
m/z

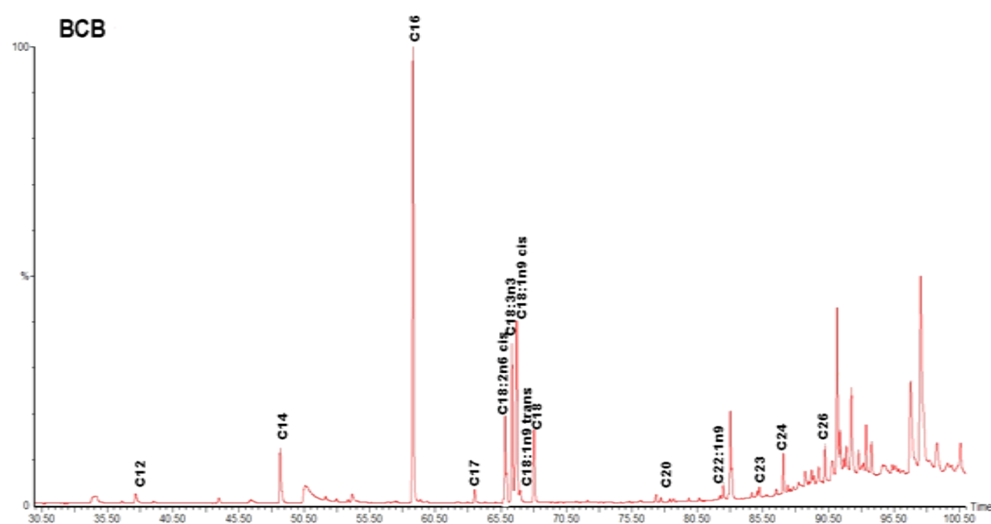

R.Time:27.817(Scan#:1670)
MassPeaks:255 BasePeak:285(56475)
Spectrum Mode:Averaged 27.800-27.833(1669-1671)
BG Mode:Calc Polarity:Negative Segment 1 - Event 1
285
399
571
213 234 255 321 343 405 433 467 499 544 579 613627 673
m/z

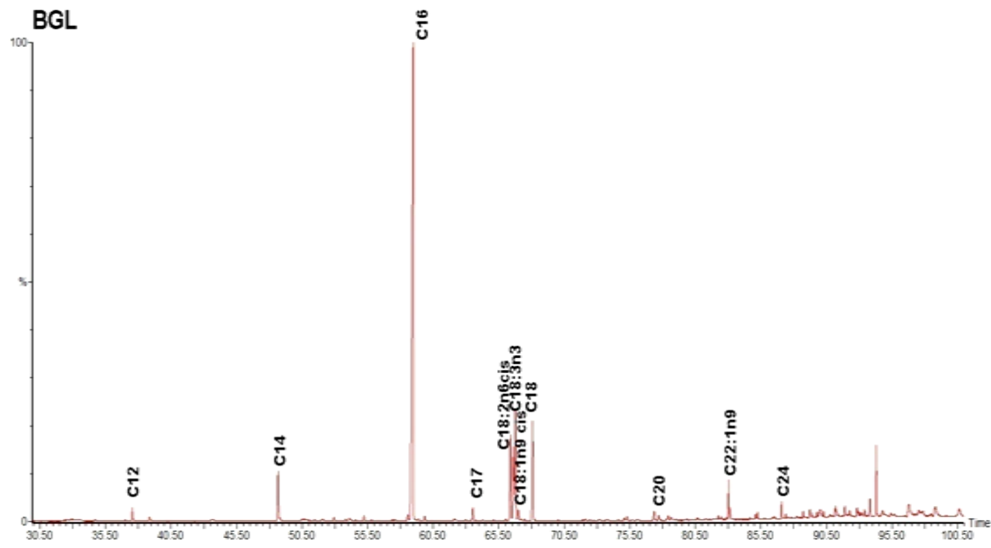

R.Time:30.933(Scan#:1857)
MassPeaks:252 BasePeak:269(105375)
Spectrum Mode:Averaged 30.917-30.950(1856-1858)
BG Mode:Calc Polarity:Negative Segment 1 - Event 1
269
539
367 383
213 240 279 305 337 422435 454 483 505 555 575 599 637 653 689
m/z

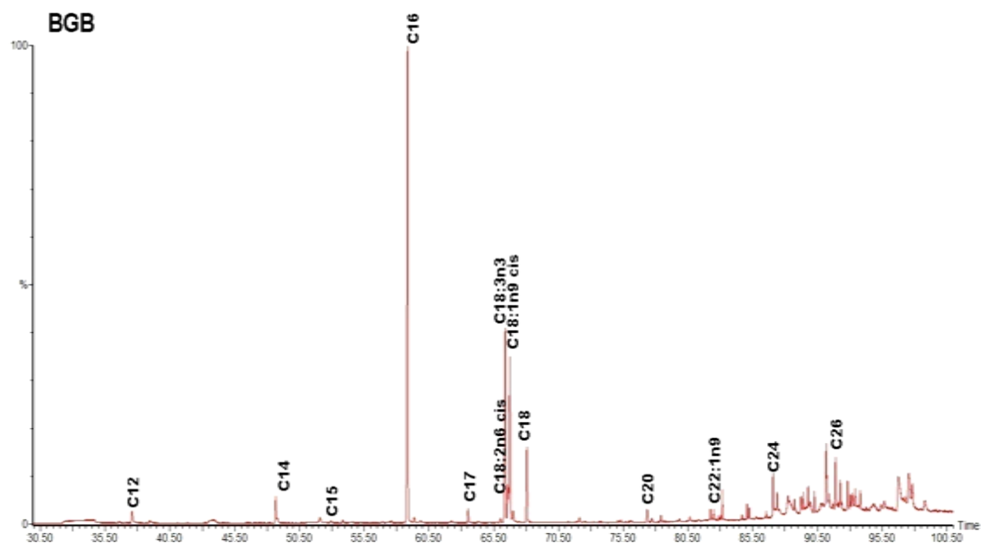
Appendix 2

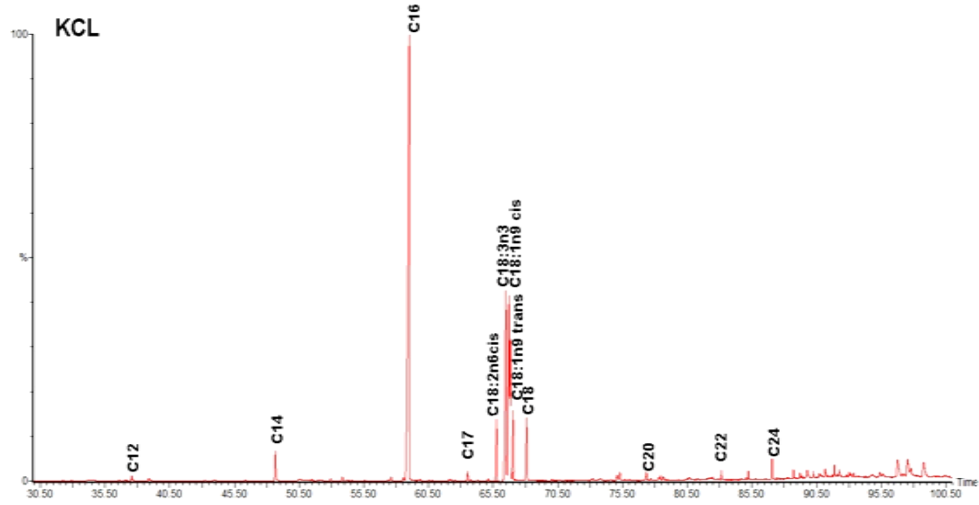
Total ion chromatograms of fatty acids in the leaves and barks of *Rhizophoraceae* mangroves



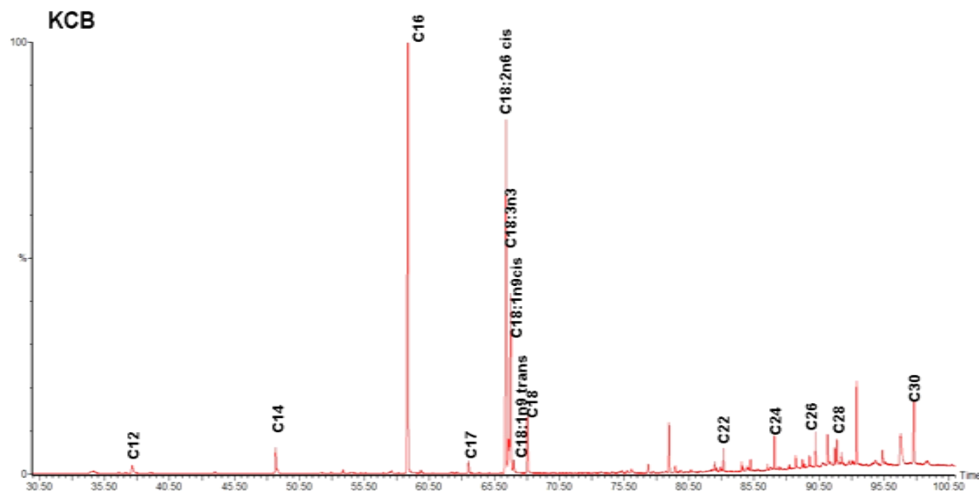



BGL
C16
C18:2n6cis
C18:3n3
C18:1n9 cis
C18
C12
C14
C17
C20
C22:1n9
C24
100
%
0
Time

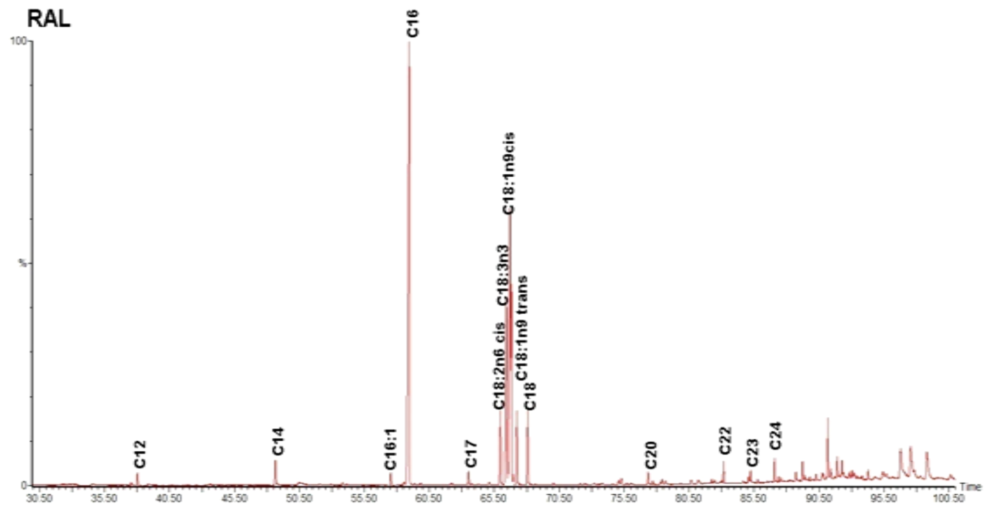

BGB
C16
C18:3n3
C18:1n9 cis
C18:2n6 cis
C18
C12
C14
C15
C17
C20
C22:1n9
C24
C26
100
%
0
Time

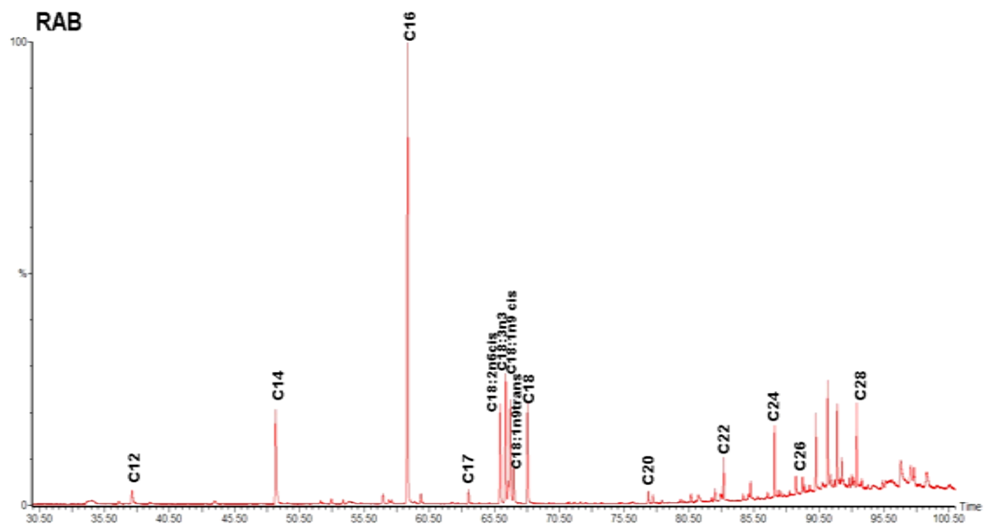


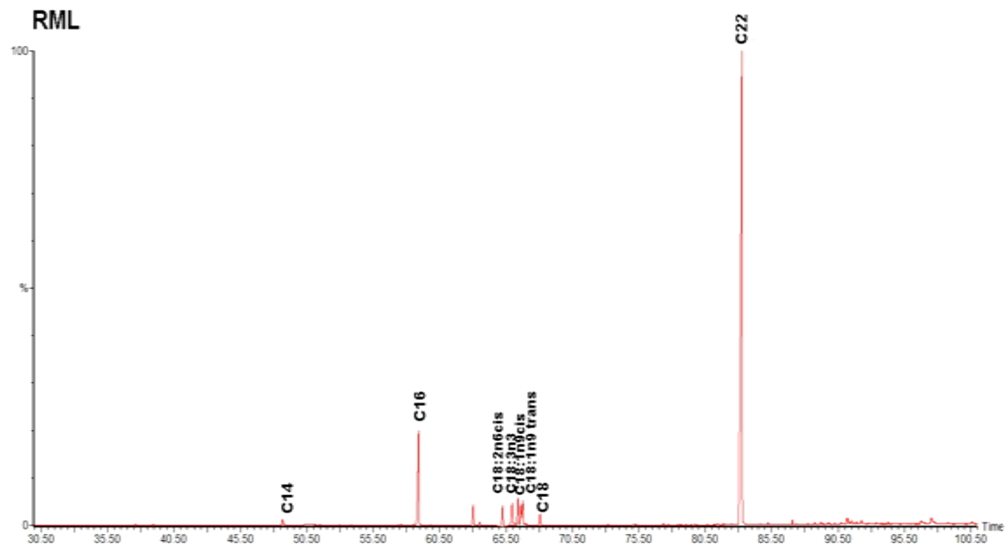
KCL
C12
C14
C16
C17
C18:2n6cis
C18:3n3
C18:1n9 cis
C18:1n9 trans
C18
C20
C22
C24
Time

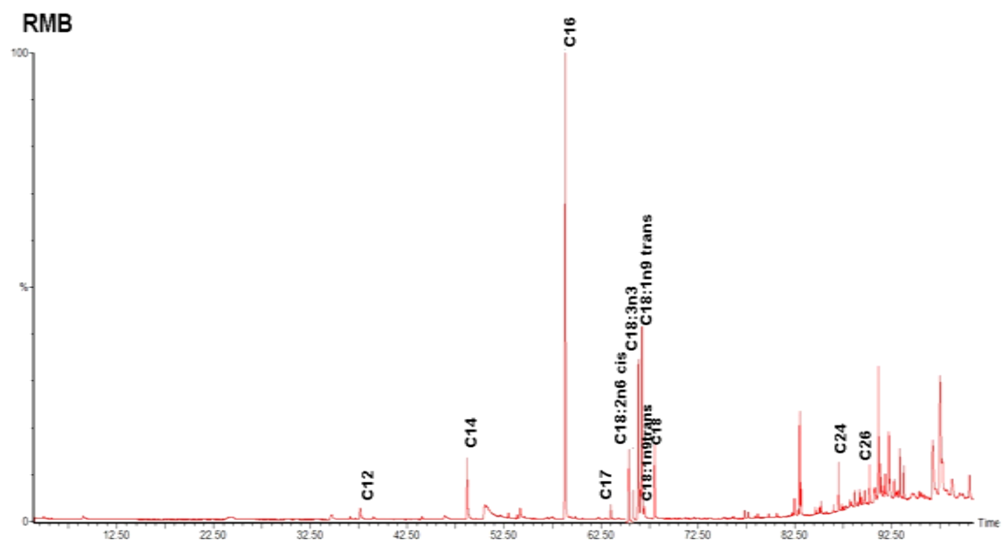


KCB
C12
C14
C16
C17
C18:2n6 cis
C18:3n3
C18:1n9cis
C18:1n9 trans
C18
C22
C24
C26
C28
C30
Time


RAL
C16
C18:1n9cis
C18:3n3
C18:1n9 trans
C18:2n6 cis
C18
C12
C14
C16:1
C17
C20
C22
C23
C24
Time

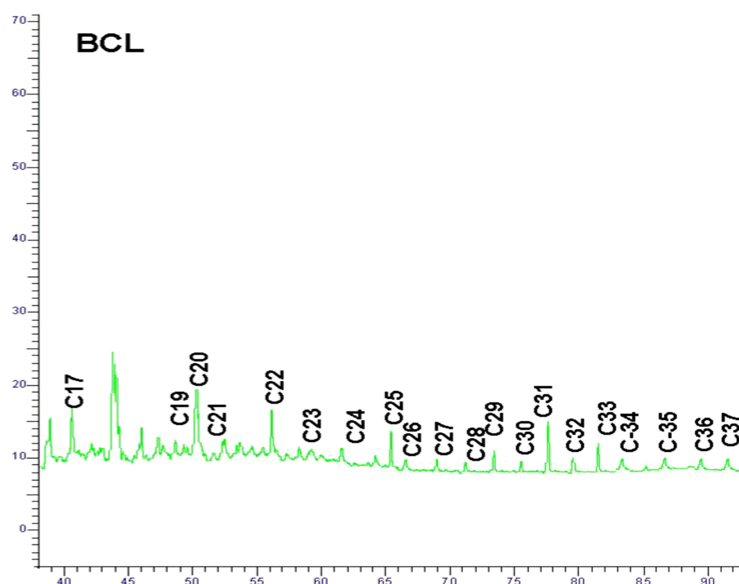

RAB
C16
C18:2n6cis
C18:3n3
C18:1n9 cis
C18:1n9trans
C18
C12
C14
C17
C20
C22
C24
C26
C28
Time

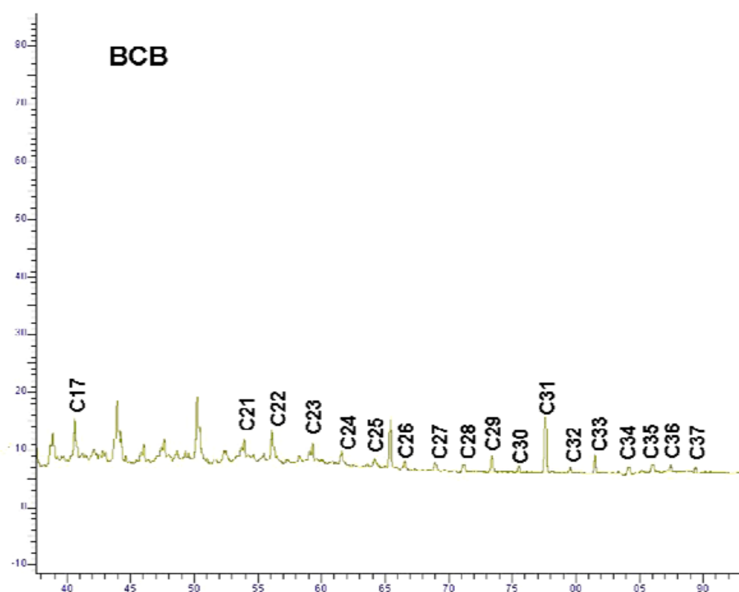

RML
C22
C16
C14
C18:2n6cis
C18:3n3
C18:1n9cis
C18:1n9 trans
C18
Time

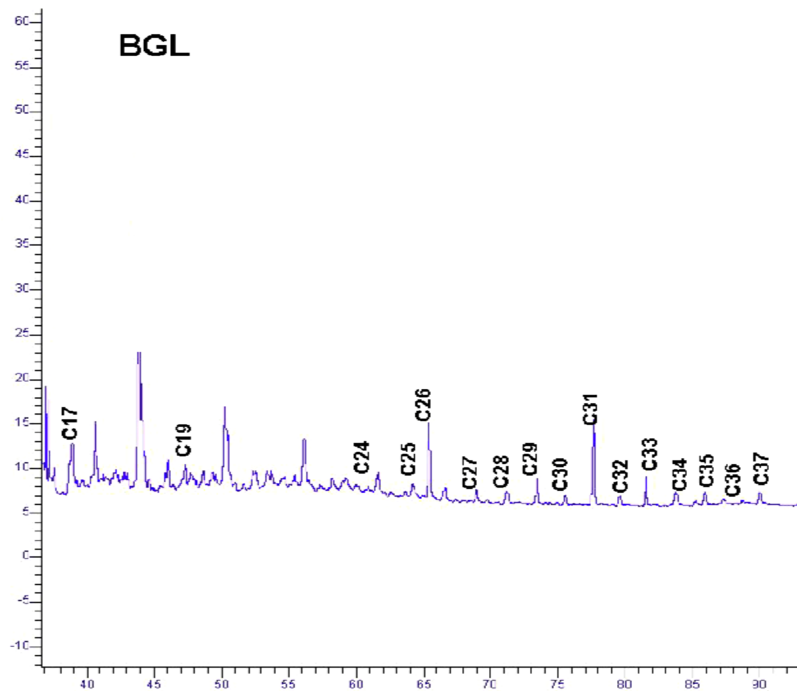

RMB
C16
C18:1n9 trans
C18:3n3
C18:2n6 cis
C18:1n9trans
C18
C14
C12
C17
C24
C26
Time

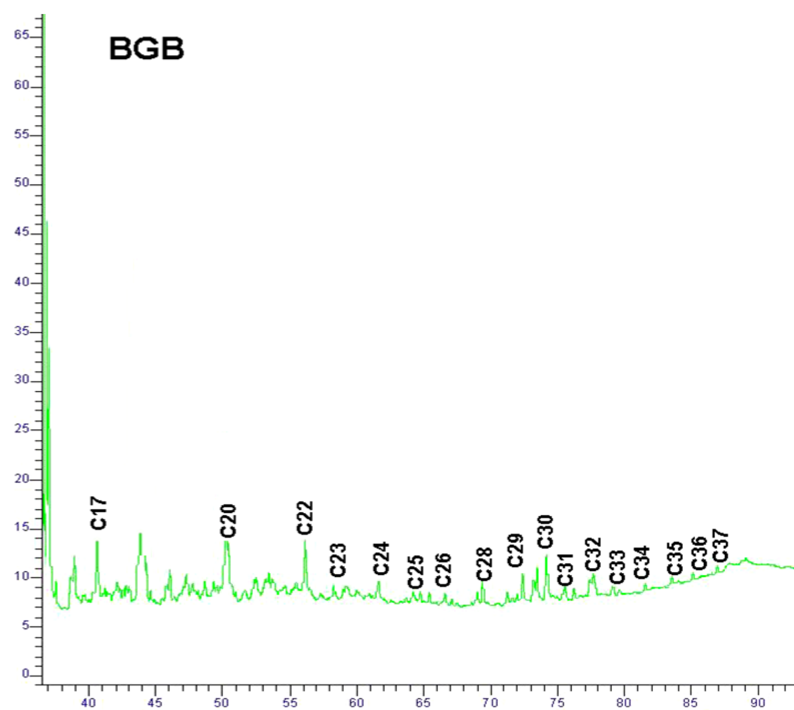
Appendix 3

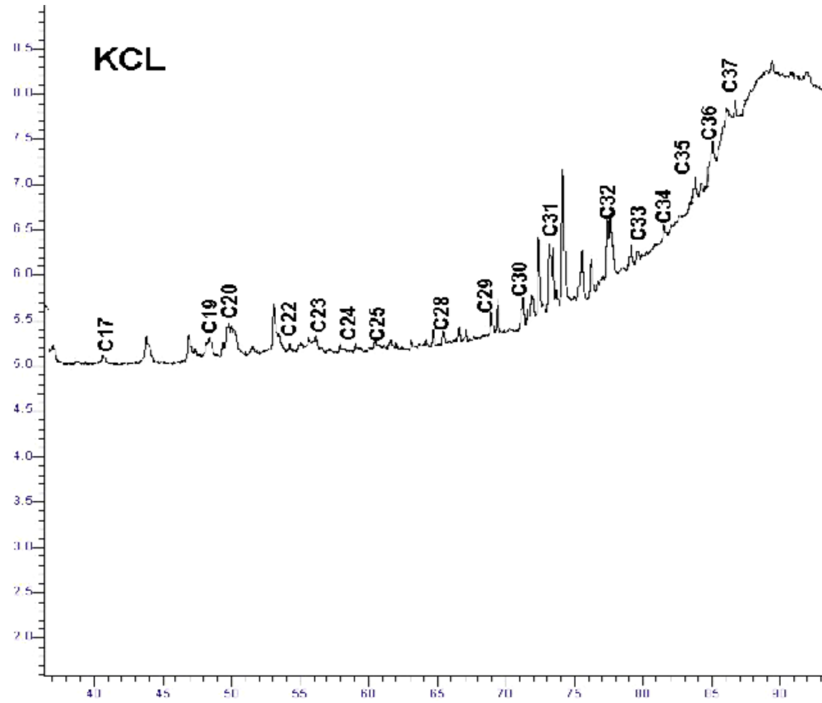
Chromatogram of *n*- alkanes in the leaves and barks of *Rhizophoraceae* mangroves

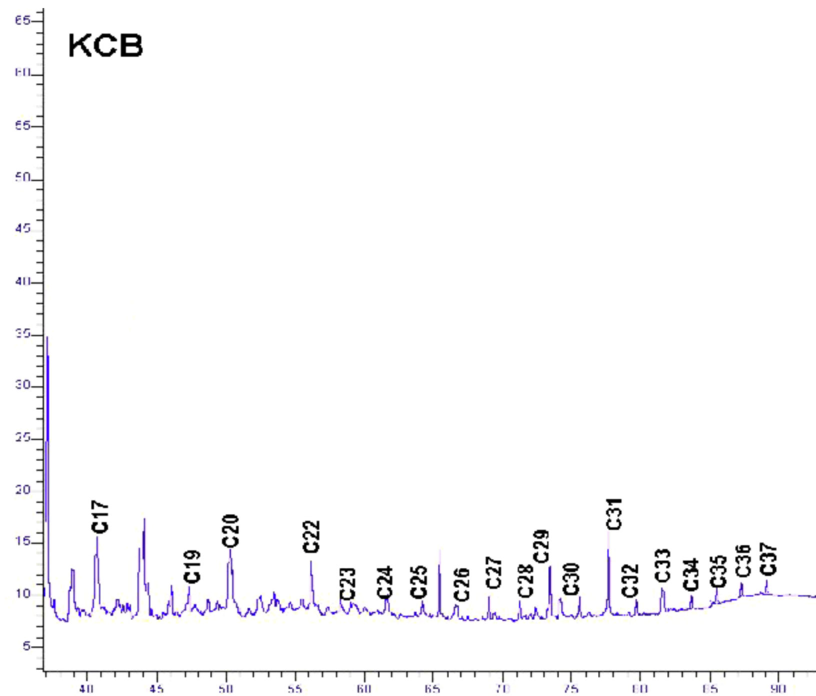


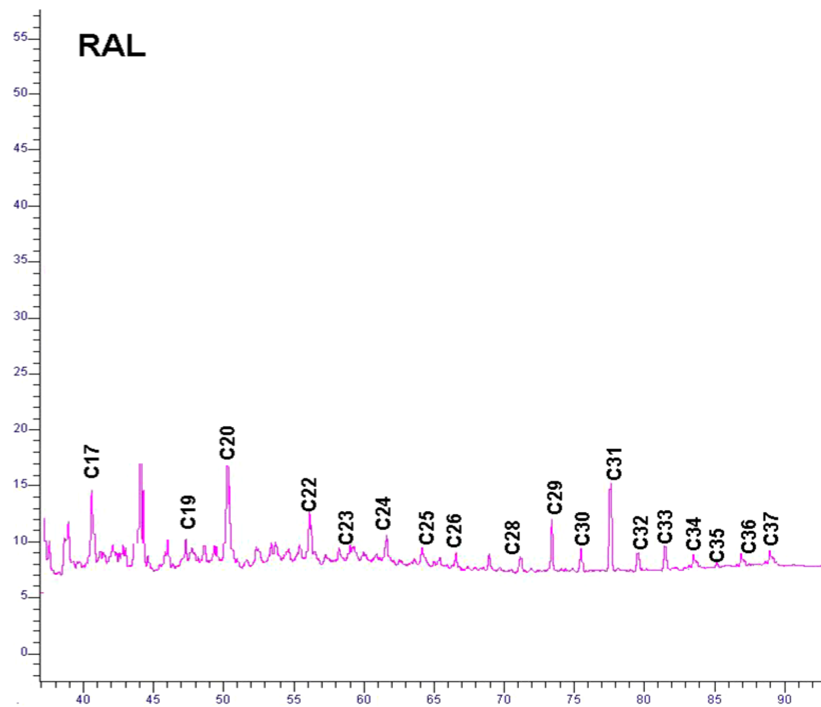


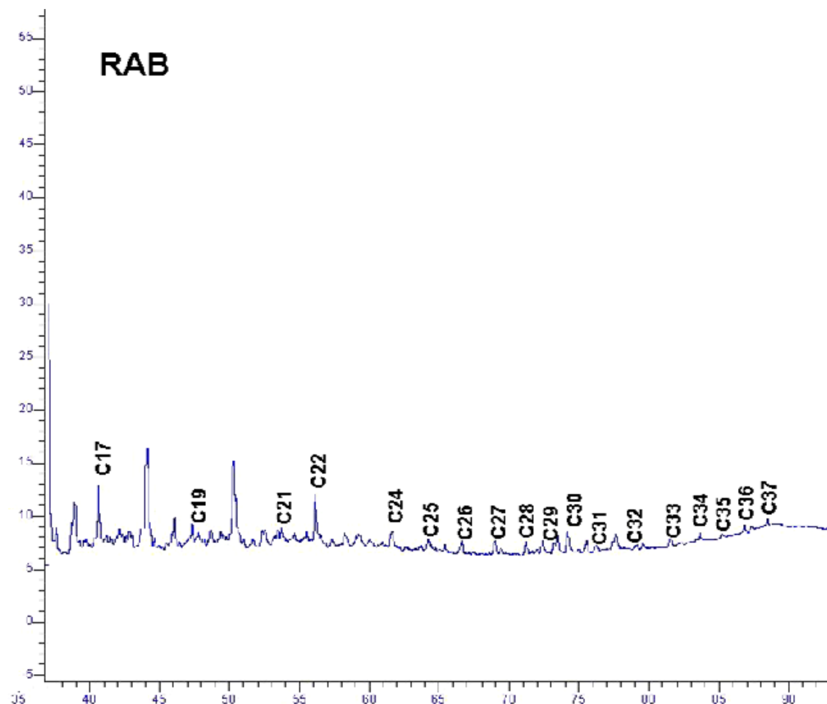

BGL
C17
C19
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37

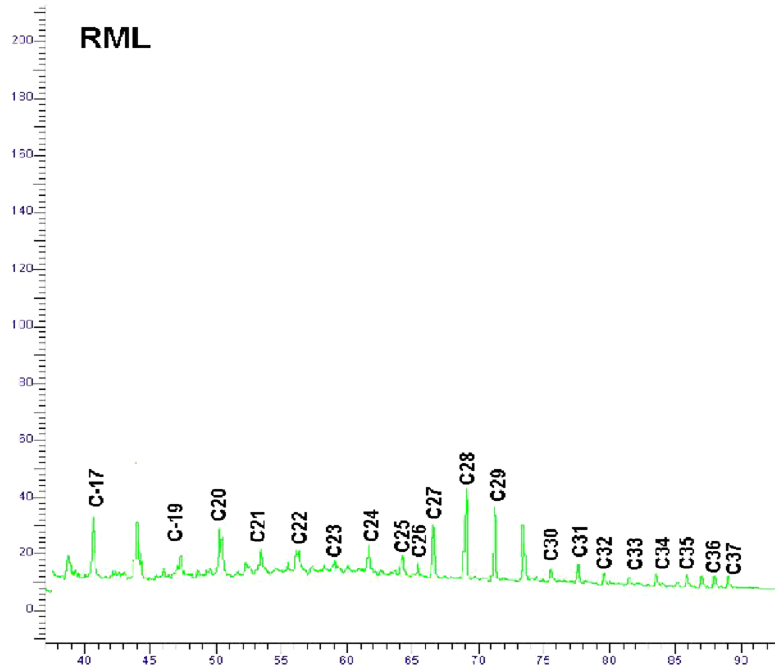

BGB
C17
C20
C22
C23
C24
C25
C26
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37

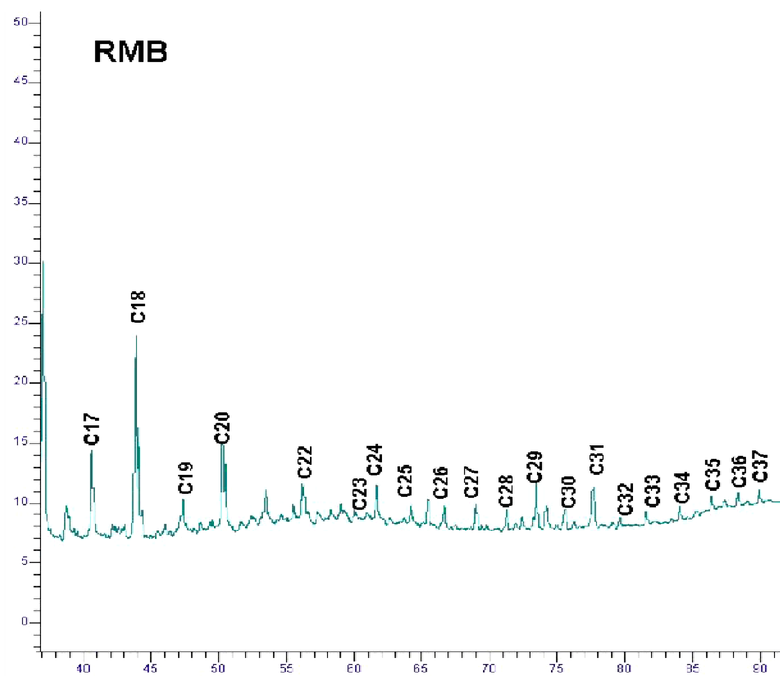

KCL
C17
C19
C20
C22
C23
C24
C25
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37


KCB
C17
C19
C20
C22
C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37


RAL
C17
C19
C20
C22
C23
C24
C25
C26
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37


RAB
C17
C19
C21
C22
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37





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List of Publications

[1]. Nebula, M., Harisankar H.S. and Chandramohanakumar, N. (2013). Metabolites and bioactivities of *Rhizophoraceae* mangroves, *Natural Products Bioprospecting*, 3, 207–232.

[2]. Nebula, M, Harisankar H. S., Chandramohanakumar, N. (2012). Phytochemical constituents and bioactivities of three mangrove plants from Southwest coast of India. In: *Advances in aquatic biotoxins*, Lambert Academic Publishing, ISBN 978-3-8484-1574-8, 2012, 33-45.

[3]. Nebula, M., Harisankar H.S., Gireesh Kumar T.R., Byju P., N. Chandramohanakumar, 2013, Food flavonoids in Rhizophoraceae mangroves from southwest coast of India , Proceedings of Aquasem 2013, Dept. of Chemical Oceanography, CUSAT.

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