Distribution and chemotaxonomy of some members of Lauraceae in Terai and Duars region of West Bengal

A Thesis submitted to the University of North Bengal for the Award of Doctor of Philosophy in Botany

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Dedicated to my Grandfather

Late Manomohan Choudhury

DECLARATION

I declare that the thesis entitled "**Distribution and chemotaxonomy of some members of Lauraceae in Terai and Duars region of West Bengal**" has been prepared by me under the guidance of Dr. A.P. Das, Professor and Dr. Palash Mandal, Assistant Professor, Department of Botany, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

The family Lauraceae comprises a group of flowering plants included in the order Laurales in the Magnoliophyta of the kingdom *Plantae*. The Angiosperm Phylogeny Group classification puts Lauraceae in Laurales under the clade Magnoliids, one of the major clades of angiosperms.

The family Lauraceae is widespread in tropical and subtropical regions throughout the world. The Laurels has a wide range of distribution in India stretching from the coastal plains to the subtropical and temperate regions. Unfortunately, few works were performed on these members particularly, in Terai-Duars belt of West Bengal which is falling under biodiversity hotspot 'Himalaya'. Even complete floristic database of Lauraceae of this region was not obtained through literature survey. As the study on Laurels has not yet completed in Terai-Duars, so there are still some confusions of taxonomic revision of this family, thus, this study will support the taxonomic clarification of these family.

Terai is located between 25° 57" to 26° 36" N, latitude and 89° 54" to 88° 47" E longitude; whereas Duars is situated between 26° 16" to 27° 0" N latitude and 88° 4" to 89° 53" E longitude. The Terai and Duars region politically constitute the plains of Darjeeling, whole of Jalpaiguri and Alipurduar District in West Bengal. The Laurel flora of Terai-Duars region has been prepared through random sampling in three different seasons for five consecutive years, 2008 to 2012. From the present survey the occurrence of 26 species covering 9 genera of Laurels were reported growing in Terai-Duars region. Artificial Dichotomous Keys for the recorded genera and species ware constructed based on significant reliable and easily observable vegetative, flower and fruit characters. All these species were enumerated alphabetically accompanied by local names, salient features, exsiccatus, availability status, flowering and fruiting periods, occurrence in Terai & Duars region and world distribution.

The species of Laurels are economically very important mainly these are used as medicinal resources. Along with these species are utilised in various function like aromatic, edible, spice, timber and many being used in various domestic purposes as well as industrial uses. Although these are economically very important, where as the species of Lauraceae remains poorly recognized and are difficult to identify. In Terai-Duars region eight economically important plants are abundant. Morphologically these eight species are more or less similar. But in every time it is not possible to dependent on morphological characteristics due to both flowers and fruits are used in most generic keys and since specimens almost never bear flowers and fruits, identification is often almost impossible. A drastic remedy for this problem would be to use the different parameters like anatomy, leaf architecture and chemotaxonomy.

After collection and identification of the plant, an attempt was made for study the anatomical characteristics of eight Laurels (*Viz. Cinnamomum bejolghota, C. camphora, C. tamala, C. verum, Litsea assamica, L. glutinosa, L. laeta, L. monopetala*) which are available in Terai and Duars region. All observations were performed on hand-made transverse sections of well-developed stem, petiole and lamina with mid-vain. Double staining method was used for this study. This is the first anatomical

report on the members of Lauraceae from this part of the country. The structural differences are found in several parts like stem, leaf and petiole. In the present study most of the characters are similar in both the genera but distributions of stone cells, air cavities, distribution of sclerenchyma are dissimilar. On the basis the differentiations and similar anatomical characters Higher Archival clustering were drawn. This study concluded that there are two constant clustered groups in these two genera based on similarity in anatomical characters. These groups are: (1) *Litsea monopetala, L. glutinosa, L. leata* and *L. assamica* and (2) *C. bejolghota, C. tamala, C. verum* and *C. camphora*, share a wealth of anatomical characters.

It is well known that leaf architecture is another technique for plant identification. A number of works have been performed on leaf architecture to identify the species successfully and established the relationship between plant species. In the present study, minor venation pattern are distinctly different in *Litsea* and *Cinnamomum,* notable differences in the size and number of areoles were observed. Similarly, the observation of F.E.Vs is also parallel to above results. Various types of stomata were observed in these two genera like anisocytic, diacytic, cyclocytic, anomocytic and anomotetracytic. A dendrogram was produced with the use of such characters showed that *Litsea* can be easily distinguished from *Cinnamomum*.

Chemical constitutions of the plants are stable like structural (morphological and anatomical) features of plant parts. Therefore, for better outcome of identification and relationship of eight species, antioxidant activity, polyphenol content as well as thin layer chromatography were performed.

In this study the antioxidant activity of the different parts (leaf and bark) of eight Laurels were investigated by using DPPH scavenging, reducing power, metal chelating, superoxide scavenging and nitric oxide scavenging assay of the extracts. It is well known that phytochemicals were responsible for antioxidant capacity, therefore quantitative estimation of phytochemicals like total phenol and total flavonoid content as well as qualitative estimation like glycosides, cardiac glycosides, phytosterol, triterpenoids, tannins, alkaloids and amino acids were carried out. In this study, eight different Laurels showed the capacity to reduce oxidation due to presence of high amount of antioxidants. With the help of antioxidant activity (of leaf and bark), two different dendrograms were prepared. However the leaf and bark of eight plants were collected from same plants but the dendrogram was slight dissimilar, because the deposition pattern of the secondary metabolites in bark are stable than leaf. Therefore, in further study we have selected bark of these plants.

The essential oils of Laurels are directly related with the cosmetics and food additives industries. Therefore, the antioxidant activities of the essential oil of eight Laurels were determinate and established relationship within these species. When comparing these species with the cladogram based on the antioxidant profile of essential oil, it was found that *Litsea* genus is separated from the genus of *Cinnamomum*, which is parallel to the grouping developed by the morphological characteristics.

As we have already known that thin layer chromatography is another chemotaxonomic study constitute one of the most important methods of determining the taxonomic positions of taxa. Therefore, with different secondary metabolites like flavonoids, antraquinones, bitter principles, phenolics, essential oils and free radical scavenging screening with TLC figure printing of eight Laurels were performed. By calculating the hR*f* values with different coloured band, a dendrogram was constructed through Agglomerative Hierarchical Clustering (AHC) method.

In all represented phylogeny of the eight species of Lauraceae were different from each other. So, for obtaining more reliable results all data like morphological, anatomical and chemical numerical data

were applied. After the application of these data, an ultimate dendrogram was found where two genera i.e. *Litsea* and *Cinnamomum* were separated.

In conclusion it can be seid that, the methods which were used in present study can help in clustering for solving phylogenic problems. Any family or any kind of plant group like Lauraceae can be easily identify and classify by the above methods. Chemotaxonomy is the process where only needs any kind of plant part for this experiment. So, it is much easier than morphology because of unavailability of flowers and fruits, which are basic requirements for morphological studies. So, chemotaxonomy can be a reliable method for identification of plants.

PREFACE

Floristically, Eastern Himalaya is one of the richest regions in the world and is literally considered as Botanist's Paradise that has attracted plant lovers and hunters equally at least for the last three centuries. Some scientists treat this region as the treasure house of diversified plant species. The Northern part of the Indian State of West Bengal, touching the feat of Eastern Himalaya is generally referred as Teraiand Duars. The Terai and Duars regions are politically represent the plains of Darjeeling and the whole of Jalpaiguri and Alipurduar districts of West Bengal. Famous Wildlife Sanctuaries and National Parks like Mahananda Wildlife Sanctuary, Gorumara National Park, Chapramari Wildlife Sanctuary, Buxa Tiger Reserve and Jaldapara National Park are located in this region. The vegetation of Terai and Duars are floristically very rich and covers all major groups of Plant Kingdom including several endemic and RET species. The wide diversity in habitat structure helped numerous plant families to settle in this area. Lauraceae is one of the dominant families of higher plants in this region which is also economically quite important.

Lauraceae covers around 55 genera and 2500 to 3000 species world-wide, mostly from warmer or tropical regions, especially South east Asia and Brazil. Laurels are economically important as sources of medicine, timber, nutritious fruits, spices and perfumes. Bark and the roots of some Laurels are often used in traditional medicines.

Although it is economically very important, the species of Lauraceae remains poorly recognized and are difficult to distinguish taxonomically. The main reason is that many species are tall trees with minute, inconspicuous flowers that are difficult to collect and of considerable small reproductive season. This makes identification of such species uncertain, since most genera are circumscribed by floral characters.

For solving the difficulties of identification of some economically important Laurels of Terai-Duars region different techniques *viz*. anatomy, leaf-architecture, chemotaxonomic approaches (antioxidant, phytochemical screening, thin-layer chromatographic figure print) were utilized in this dissertation. Finally, a dendrogram was also constructed for to illustrate the phylogenetic relationship among these species.

A clear picture of Laurels distribution in Terai-Duars region has been framed through this study. All these species were enumerated along with their local names, salient features, exsiccatae, availability status, flowering and fruiting periods, occurrence in Terai & Duars region and world distribution.

So, the present work provided considerable bulk of data not only for the taxonomy of Laurels of Terai and Duars but has also collected and worked out considerable data for the resources assessment and utilization.

ACKNOWLEDGEMENTS

The successful completion of this work, leading to the writing and submission of a Doctoral thesis goes to a large number of persons who helped me in various capacities and different manners during the course of this project work.

The first and the foremost, I extend my deepest sense of thanks and gratitude to my supervisor Dr. Abhaya Prasad Das, FLS (London), Professor, Department of Botany, University of North Bengal, for his inspiration, encouragement and guidance throughout the period of execution of this work. He lifted me when I was down and this shall always remain incised somewhere deep within me for the rest of my days. Without his basic perception and supervision it was not possible even to take up the projected work.

I would like to express my heartiest gratitude to my co-supervisor Dr. Palas Mandal, Assistant Professor, Department of Botany, University of North Bengal, who acquainted me with different aspects of my research and imparted constant guidance through both happy and difficult moments to see me successfully complete this work.

I extend my special gratitude and respect to Dr. M. Chowdhury, Assistant Professor, Department of Botany, University of North Bengal for his unconditional support and assistance, in field and laboratory during my research.

I would like to convey my heartiest thanks to the Heads of the Department of Botany who looked after the department during my tenure of work for extending all the required facilities.

I am also thankful to all the teachers of the Department of Botany, namely Prof. B. N. Chakraborty, Prof. P. K. Sarkar, Prof. (Mrs.) U. Chakraborty, Dr. A. Sen and Dr. S. C. Roy for their continuous support, help and encouragement. My thanks also go for all the non-teaching staff of the Department of Botany for their various kinds of assistance.

I express my thanks to Dr. Chandra Ghosh, 'didi', Assistant Professor, Gour Mahavidyalaya of Gour Banga University, for her extensive help during the course of my research work.

Also, I convey special thanks to Dr. Chandrani Choudhuri, Assistant Professor, St. Xeviers' College, Jalpaiguri for her inspiration during this period.

It is indeed important to extend my thanks to all my lab-mates, seniors and juniors, namely Dr. Sonam R. Lepcha, Dr. Animesh Sarkar, Dr. Ajita Sarkar, Mr. Rajib Biswas, Mr. Goutam Saha, Mr. Suman Nirola, Mr. Anurag Chowdhury, Mr. Anand Lama, Mr. Sourav Moktan, Mr. Kishor Biswas,

Mr. Amalesh Bijali, Mr. Biman Krishna Saha, Mrs. Baishakhi Sarkar, Ms. Payel Paul, Mr. Sujit Mandal, Ms. Dipika Chhetri, Mr. Saran Kumar Gupta, Ms. Arunika Subba, Ms. Sumira Mukhia, Ms. Suchisree Jha, Mr. Sujay Sen, Mr. Priankar Roy and others who were constant sources of help and encouragements in the two laboratories in which I conducted my research.

I also offer thanks to Mr. Biplob Sarkar, Mr. Manu Mandal, Mr. Kamalesh Roy, Md. Eqramul Ali, Md. Wazed Ali and others staffs of the *NBU Garden of Medicinal Plants* for their support. Thanks are also due to Mr. Binod Singha and Mrs. Sangita Oraon, Lab & Field Assistants, for their help in my works.

I acknowledge with sincere thanks to Mr. Dilip Singha for helping in different aspects of official works during the course of my research work.

I express my gratitude to the University Grants Commission (UGC) for financial support in the form of Junior and Senior Research Fellowships under BSR Scheme and as a Project Fellow under the SAP of the Department of Botany, to complete the work.

I would remain indebted to Mrs. Ruby Das for her motherly love and inspiration and I like to express my love and thanks to Sri Sanwit Das for his good wishes.

At the end, but the most important, I remember my family and express my heartfelt gratitude and respect to my beloved parents Mr. Ratan Kumar Choudhury and Mrs. Sikha Choudhury for their endless inspiration, encouragement, love and affection. I am also grateful to my parents-in-law Mr. Sitaram Ghosal and Mrs. Shila Ghosal for their moral support. Words are not enough for expressing my heartiest gratitude towards my loving wife Mitali for her continuous support throughout the execution of the work. Thanks are also due to my sister, Rupam and her husband Aniruddha for their constant love and affection. I am also thankful to my other family members for their help and encouragement.

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December 04, 2015

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Introduction

The civilization in its applicable form of understanding would not have been possible without the existence of vegetation. Life originated on this, and the only known life-supporting planet around four billion years ago (Brack 1998; Das 2010). Since then innumerable forms of life have been differentiated, evolved, migrated over distant areas, living together and developed sustainable dependency and formed wide range of recognizable vegetation structure through the selection processes of nature and are now occupying different areas all over the globe. The earth surface has passed through regular geographical, geological, glacial, climatic and, later on, biological changes since its formation (Stouffer *et al.* 1994; Mann *et al.* 1998; Negi & Joshi 2004).Those have modified the earth surface, including the water-filled areas, and created innumerable habitat conditions (Kumar 2008). The formation of diverse habitat conditions provided suitable home to different species according to their suitability. Species preferring a particular set of environmental/ habitat conditions started growing together with mutual understanding and formed a local ecosystem. As it is understood, in any ecosystem just one species can't manage or maintain everything required for its normal survival. The functioning of a vegetation is largely dependent on its biological composition (plants, animals and microbes) at the species level (Qian *et al.* 2007).

1.1. THE HIMALAYAS

After the upheaval of the Himalayas during tertiary from the bottom of the now extinct Tethys Sea, the geological and biological pictures of this region have changed abruptly (Raina 2009). Large number of species of all the biological kingdoms started migrating into this newly formed land and established new associations, niches and ecosystems (Walter 1985). After their settlement, the existing species started mixing and modifying leading to local speciation and there on formed a new flora and fauna of its own, - characteristically Himalayan.

After crossing innumerable difficult barriers, the man, basically one African species (https://www.genome.gov), also extended its settlement in the Himalayas at a pre-historic age. This destructive species, to satisfy its unlimited requirements and to accommodate its logarithmically increasing population, started modifying the vegetation around their settlements that, eventually, expressed as destruction of natural habitat and endangering the survival of thousands of species. Man induced changes in the environmental conditions [i.e. temperature, precipitation, elemental balance of air, pollution, etc.] are also causing havoc (Ariztegui *et al.* 2010). These are now well-known facts that the phenomenon of 'global-warming' becoming dangerous not only for some stray species but even for very large ecosystems for their survival (Walker & Steffen 1997; Root *et al.* 2003).

1.2. CONSERVATION AND IUCN

Conservation efforts, round the world, especially under the guideline of IUCN have, temporarily, created little respite, through the establishment of different types of Protected Areas (PA) and Hotspots.

Establishment of PAs like National Parks, Wildlife Sanctuaries, Ramsar Sites, etc. at the narrower levels and Biodiversity Conservation Hotspots at the wider level are, at least theoretically, creating some islands where at least some species can take shelter for their survival (Manoj *et al.* 2013; Das *et al*. 2008).

Just the creation of PAs are now not looking enough for the proper conservation of huge number of species susceptible to changes in habitat conditions. In most of the cases, their migratory routs are blocked, exploitation in the form of ecotourism, (un-) sustainable utilization and extraction, etc. and the changes in environmental conditions are changing the habitat at different levels are forcing the degradation of their population structure.

1.3. RECOGNITION OF HOTSPOTS

So far, IUCN has recognized 35 Hotspots round the world (http://www.conservation.org). As much as four Hotspots are known to cover different parts of the Indian territory, those are (i) *Himalaya*, (ii) *Indo-Burma*, (iii) *Western Ghats & Sri Lanka*, and (iv) *Sundaland*. IUCN also treated India as a '*Megadiversity Country*' in a select list of 17 such countries recognized till date (CBD, 2009).

1.3.1. Himalaya Hotspot

Hottest of the Hotspots, Himalaya is spanning over six countries and covering the houses for almost unlimited diversity of life-forms and of culture. As recorded before 2004, this Hotspot is covering a total area of 741,706 km^2 in which around 185,427 km^2 area of natural habitat is 'somehow' exists. It is, however, expected that further analysis of recent imageries will produce much more serious picture as the anthropogenic pressure on the Himalayas is a continuous and accelerating process. So far, around 112,578 km^2 has been declared as PA of which 77,739 km^2 is under the IUCN categories I – IV. And, it is also important to note that 31.6% of the higher plants of the Himalayan flora are endemics (Myers *et al.* 2000; WWF & ICIMOD. 2001). Along with the progressive degradation of the habitat conditions, it is feared that this number will reduce very soon and many more endemic species will become extinct, covering all major taxa of plants, animals and microbes.

1.4. DARJEELING-SIKKIM HIMALAYAS

Darjeeling-Sikkim parts of the Eastern Himalaya, along with the contiguous thickly vegetated areas of Terai and Duars are also known to be extremely rich in biodiversity and of natural resources (Das & Chanda 1990; Das *et al*. 2010). Over the last 4 – 5 hundred years, especially during the last 150 years, migration of numerous groups of people into the area, from far and near, established their settlements and the human population in the area has increased manifold and is now increasing extremely fast. This is exerting too much pressure on local landscape as all types of existing vegetation forms are changing very fast. Establishment and extension of huge Tea Gardens and other crop fields, network of innumerable army establishments, new villages and towns, industries, communication networks, mostly illegal extraction of forest products (mostly logging), mining operations, etc. are occupying the spaces where, previously, good vegetation cover, supporting wide diversity of biological forms, were existing. The land-cover map of Darjeeling Hills presented by U. Rai (2006) showed the dangerous situation of Darjeeling forests.

1.5. CONSERVATION EFFORTS

Logically, the National and State Governments have taken ample steps to protect and to conserve the biological diversity of this area. A good number of PAs have, so far, been recognized in this region covering Himalayan hills of Darjeeling-Sikkim and of contiguous Terai and Duars (Table 1.1).

Table 1.1. Protected Areas of Darjeeling-Sikkim and Terai-Duars region

Name	District	Location/ Coordinate	Area [km^2]
SIKKIM			
Khangchendzonga BR	N,S,W Sikkim	27° 30′0″N &88° 02′ 0″E	2,620 km²
Khangchendzonga NP	N,S,W Sikkim	27°42′0″N & 88°08′0″E	1,784 km²
Shingba Rhododendron Sanctuary	N Sikkim	27°50′28″N & 88°44′21″E	43 km²
Barsey Rhododendron Sanctuary	W Sikkim	27°11′ N & 88°7′ E	104 km²
Kyongnosla Alpine Sanctuary	E Sikkim	27°22′37″N & 88°44′28″E	31 km²
Fambong Lho WLS	E Sikkim	27°18′40″N & 88°32′1″E	51.76 km²
Maenam WLS	E Sikkim	27°18′50″ N & 88°23′35″E	35.34 km²
Pangolakha WLS	E Sikkim	27º 09′N & 88º 35′E	128 km²
Darjeeling Hills, Terai & Duars [West Bengal]			
Singalila BR [Proposed]	Darjeeling	Yet to finalize	
Singalila NP	Darjeeling	27°07′N & 88°04′E	78.60 km²
Neora Valley NP	Darjeeling	27°04′N & 88°42′E	88 km²
Gorumara NP	Jalpaiguri	26°42′N & 88°48′E	79.45 km²
Jaldapara NP	Alipurduar	26°37′43″N & 89°22′39″E	216.51 km²
Buxa NP	Alipurduar	26° 30' N & 89° 20'E	117.10 km²
Buxa Tiger Reserve	Alipuduar	26°39′0″N & 89°34′48″E	760 km²
Senchal WLS	Darjeeling	26°59′38″N & 88°15′55″E	38.88 km²
Mahananda WLS	Darjeeling	26°28′52″N & 88°15′50″E	158.04 km²
Jore Pukri WLS	Darjeeling	-	0.04 km²
Chapramari WLS	Jalpaiguri	26°53′52″N & 88°51′1″E	9.60 km²

Apparently, total area under conservation is looking quite significant, but on closer look those might not be so appreciable. Absence of transboundary PAs and proper corridors between these PAs for the easy migration of different species and making business over the conserved areas are some of the basic but missing requirements (Das 2011; Das *et al*. 2008).

1.6. VEGETATION

Floristically, the Sikkim-Darjeeling Himalayas [part of the Eastern Himalaya] is one of the richest regions in the world and is literally considered as *Botanist's Paradise*. Some scientists treat this region as the treasure house of diversified plant species (Das 1995; Lama 2004; Rai 2006). None other than Sir Joseph Dalton Hooker (Hooker 1904) introduced the beauty and the floristic richness of this region to the world. The occurrences of a variety of physiographic, climatic and edaphic conditions often aided by biotic factors are responsible for such richness. The configuration of the hills and mountains, pattern of rainfall distribution over the lower, middle and upper elevation ranges and high humidity have a great role for the development of wide diversity of vegetation of this area (Bhujel 1996; Rai 2001; Bhujel & Das 2002). The altitude of the hill ranges varies markedly between the altitude and vegetation. Thus, altitude is one major factor that determines the range of distribution of different plant species and the associations that they form at different elevation ranges. Various workers have put forth the classification of the vegetation of this region *viz.* Gamble (1875), Hooker (1906), Cowan (1929), Champion (1936), Das (1995), Bhujel (1996). These authors have essentially classified the flora and vegetation according to altitudinal ranges, although they differ considerably in detail. Six major types of vegetation can be recognized as in Table 1.2.

Table 1.2. Different types of vegetation along with their altitudinal range in Darjeeling–Sikkim Himalayas

Vegetation types	Altitudinal ranges
Tropical and plains	Plains to 800 m
Sub-tropical	800 – 1600 m
Temperate	1600 – 2400 m
Cold temperate	2400 – 3200 m
Sub-alpine	3200 – 4000 m
Alpine	Above 4000 m

1.6.1. Dominant Taxa

The foothills or tropical region of Darjeeling-Sikkim Himalayas are covered with forest consisting of *Tectona grandis* Carl Linnaeus,*Shorea robusta* Gaertner,*Dalbergia sissoo* A.P. de Candolle, *Dillenia pentagyna* Roxburgh, *Terminalia myriocarpa*van Heurck & Mueller-Arg., *Syzygium cumini* (Linnaeus) Skeels, *Lagerstroemia parviflora* Roxburgh, *Litsea glutinosa* (Loureiro) C.B. Robinson, *Litsea monopetala* (Roxburgh) Persoon, *Artocarpus lacucha* Buchanan-Hamilton, etc. This type of forest is characterized by the presence of a good number of climbers (some of those are liana) such as *Argyreia roxburghii* (Wallich) Arnott *ex* Choisy, *Bauhinia vahlii* Wight & Arnott, *Mikania micrantha* Kunth, *Tetrastigma planicaule* (Hooker *f.*) Gagnepain, *Thunbergia grandiflora* (Roxburgh *ex* Rottler) Roxburgh, *Tinospora sinensis* (Loureiro) Merrill etc (Das *et al.* 2010). The ground cover vegetation is also very rich, which include annuals, perennial herbs, root parasites, saprophytes etc. like *Ageratum conyzoides* (Linnaeus) Linnaeus, *Blumea balsamifera* (Linnaeus) A.P. de Candolle, *Urena lobata* Linnaeus, *Commelina benghalensis* Linnaeus, *Oxalis corniculata* Linnaeus, *Urena lobata* Linnaeus, *Triumfetta rhomboidea* Jacquin etc (Ghosh 2006).

In sub-tropical region the forest chiefly include *Schima wallichii* Choisy, *Castanopsis indica* (Roxburgh *ex* Lindley) A. de Candolle, *Alangium chinense* (Loureiro) Harms,*Callicarpa arborea* Roxburgh, Duabanga grandiflora (A.P. de Candolle) Walpers, etc. *Cryptomeria japonica* (Thunberg ex Linnaeus *f.*) D.Don is introductedand extensively cultivated in these areas. Several Bamboos may also be found near habitations (Chowdhury & Das 2011).

Temperate forests are evergreen with medium sized trees. There are a number of deciduous species but these form only a small proportion. Laurels and Oaks form large patches. The dominant plant species observed in this zone include *Acer acuminatum* Wallich *ex* D. Don, *Salix sikkimensis* Andersson,*Sorbus microphylla* (Wallich*ex* Hooker *f.*) T. Wenzig, *Rhododendron arboreum* Smith, *Magnolia cathcartii* (Hooker*f.* & Thomson) Nooteboom, *Quercus lamelllosa* Smith, *Quercus lineata* Blume, *Eurya acuminata* DC,*Acer sikkimense* Miquel,*Taxus wallichiana* Zuccarini etc. Laurels like *Cinnamomum impressinervium* Meisner, *Machilus gamblei* King *ex* Hooker *f.*, *Machilus duthiei* King, *Litsea cubeba* (Loureiro) Persoon, *Litsea elongata* (Nees) Hooker *f.*, *Litsea laeta* (Nees) Hooker *f.* are common in this zone (Lama 2004).

Cold temperate forest of the upper hill region comprises of trees like *Quercus lamellosa* Smith, *Magnolia campbellii* Hooker *f.* & Thomson, *Lithocarpus pachyphyllus* (kurz) Rehder, *Rhododendron arboreum* Smith, *Rhododendron falconeri* Hooker *f.*, *Acer campbellii* Hooker *f.* & Thomson *ex* Hiern, *Abies spectabilis* (D.Don) Mirbel etc. (Bhujel 1996; Rai 2001).

Sub-alpine region is clearly dominated by different species of *Rhododendron* and Conifers with few patches of other trees. The commonly occurring trees of this region include *Rhododendron arboreum* Smith, *Rhododendron cinnabarinum* Hooker *f.*, *Rhododendron campylocarpum* Hooker *f.*, *Rhododendron campanulatum* D. Don, *Juniperus squamata* Hamilton *ex* Lambert, *Juniperus communis* Linnaeus, *Quercus lineata* Blume, *Acer campbellii* Hooker *f.* & Thomson *ex* Hiern, *Magnolia campbellii* Hooker *f.* & Thomson, *Abies spectabilis* (D. Don) Mirbel, *Betula utilis* D. Don etc (Bhujel 1996; Ghosh 2006).

Alpine zone is lying just below the permanent snowline. Stunted bushy growth of *Juniperus squamata* Hamilton *ex* Lambert, *Rhododendron lepidotum* Wallich *ex* G. Don, *Rhododendron setosum* D.Don, *Salix calyculata* Hooker *f. ex* Andersson, *Berberis concinna* Hooker *f.* occur in this region (Dhar 2002). Herbs such as *Sanguisorba filiformis* (Hooker *f.*) Handel-Mazzetti, *Primula sikkimensis* Hooker, *Primula tibetica* Watt, *Lancea tibetica* Hooker *f.*& Thomson, *Anaphalis xylorhiza* Schultz Bipontinus & Hooker *f.* etc. cover the grounds every years from April to June (Lama 2004).

1.7. LAURELS

The Lauraceae or the Laurel family comprises a group of flowering plants included in the order Laurales in the Magnoliophyta of the kingdom *Plantae* (Cronquist 1981). The Angiosperm Phylogeny Group classification (Chase & Reveal 2009) puts Lauraceae in Laurales under the clade Magnoliids, which is one of the major clades of angiosperms. Different numbers of genera and species have been considered assigning to this family by different authors in different times. Van der Werff & Richter (1996) included a total number of 55 genera and 2500 to 3000 species under the family, distributed world-wide. According to Takhtajan (1997) this family includes 54 genera and 2500 to 3500 species. Cronquist (1981) estimated that around 2000 Laurels are present in the world. Hutchinson (1964) recognized 47 genera and about 1900 species and Judd *et al.* (2002) reported 50 genera and 2500 species as the global representatives.

1.7.1. Diagnosis

Laurels can be identified by their aromatic nature of bark and foliage as well as unique floral morphology. Flowers typically consist of six alternating, trimerous whorls; two whorls of three tepals each, four whorls of three stamens each, adnate to perianth tube and a gynoecium. In the androecium, stamens from the third whorl frequently bear a pair of additional glands and stamens from the fourth whorl are usually reduced to staminodes or are absent. The basifixed anthers are either two celled or four celled and dehisce by flap-like valves opening upward. The pistil is monocarpellary with a single pendulous anatropous ovule and the ovary is generally superior. The fruit is a berry or drupe that is often subtended or completely surrounded by a fleshy cupule.

Most of the Laurels are aromatic deciduous evergreen trees or shrubs, but *Cassytha* Linnaeus is a genus of partial-parasitic vines.

1.7.2. Fossil history

The Lauraceae has a wide fossil history particularly in Asia and America signifying that the family was dominated in the extinct vegetation of these regions (Yang 1998). Most fossils are leaves, flowers and wood from early tertiary era (Ferguson 1974; Wheeler *et al.*1977; Taylor 1988), but Drinnan *et al.*

(1990) discovered Laurel fossils of mid-cretaceous from Maryland, USA. This fossil has provided the earliest evidence of trimerous flower parts in angiosperms. The flower and inflorescence are surprisingly well preserved and described as *Mauldinia mirabilis* by Drinnan *et al.* (1990). The flowers have three small outer and three larger inner tepals and nine 2-celled anthers in three whorls with well-developed staminode like appendages. This unique floral structure is also present in some extant members of Lauraceae. Drinnan *et al.* (1990) hypothesized that *Mauldinia* like Laurels were originated around the mid-cretaceous in North America.

1.7.3. Distribution

The greatest diversity of Laurels is seen in the lowland rain-forests which is the preferred habitat of these plants. But, some species also occur at high altitude areas in tropical mountain forests where they are dominating the vegetation. So, the Lauraceae is widespread in tropical and subtropical regions throughout the world (Cronquist 1981). However, it is most commonly found in tropics of America and Asia. Whereas in Northern Asia Laurels are widely distributed from China to Japan. In south, Lauraceae occurs in Argentina and southern Chile. The family is inadequately represented in most part of the Africa but several species occur in Madagascar. Numerous species of Lauraceae are scattered in Australia while only one genus, *Beilschmiedia* Nees is found in New Zealand (Yang 1998). The Laurels has a wide range of distribution in India stretching from the coastal plains to the subtropical and temperate regions. Unfortunately, few works were performed on these members particularly, in Terai-Duars belt of West Bengal which is falling under the Himalaya Biodiversity Hotspot (Conservation International 2005). No complete floristic database of Lauraceae of this region was obtained through literature survey. As the study on Laurels has not yet completed in the extremely rich and diverse Terai-Duars vegetation, so there are still some confusions of taxonomic representation of this family, thus, the present study will support the taxonomic clarification of this taxon and will enrich the floristic and systematic database for this entire region.

1.7.4. Importance

Laurels are economically very significant as sources of medicine, timber, nutritious fruits (e.g. *Persea americana* Miller), spices (e.g. *Cinnamomum verum* J.Presl, *C. tamala* (Buchanan–Hamilton) T. Nees & Eberm, *Laurus nobilis* Linnaeus), and perfumes [*C. verum* , *C. cassia* (Linnaeus) J. Presl, *C. burmanni* (Nees & T. Nees) Blume]. The fruits of *Actinodaphne* Nees, *Cinnamomum* Schaeffer, *Cryptocarya* Brown, *Lindera* Thunberg, *Litsea* Lamarck, and *Syndiclis* Hooker *f.* contain abundant oils and fats. *Cinnamomum* trees, such as *C. camphora* (Linnaeus) J. Presl, *C. glanduliferum* (Wallich) Meisner, and *C. porrectum* (Roxburgh) Kostermans, yield camphor and essential oils, which are used for making perfumes and medicines. The bark of *C. cassia* and the roots of *Lindera* sp. are famous for traditional medicines against cold and flu in China (Li *et al.* 2008a).

1.7.5. Difficulties in taxonomic delimitation

Although it is economically very important, the species of Lauraceae remains poorly recognized and are difficult to distinguish taxonomically. The main reason is that many species are tall trees with minute, inconspicuous flowers that are difficult to collect (Van der Werff & Richter 1996) and of considerable small reproductive season. This is clearly shown by recent reports of newly discovered species and genera throughout the globe. Three new species of *Ocotea* (*O. disjuncta* Lorea-Hernández, *O. iridescens* Lorea-Hernandez & Van der Werff, and *O. rovirosae* Lorea-Hernandez & Van der Werff) from southern Mexico are described and illustrated by Lorea-Hernandez & Van der Werff in 2002. Yushi

et al.(2006) have described and illustrated a new species (*Cinnamomum purpureaum* H.G. Ye & F.G. Wang) of Lauraceae from Guangdong, China. A new species from Thailand, *Litsea phuwuaensis* Ngernsaengsaruay was described and illustrated by Ngernsaengsaruay in 2004. Like other countries new species of Lauraceae are discovered also from several regions of India. *Litsea beei* N. Mohanan & E.S.S. Kumar, species of Lauraceae from India was described and illustrated by Mohanan and Santhosh Kumar in 2008. Nine new taxa were described and illustrated belonging to the genera *Actinodaphne* Nees, *Beilschmiedia* Nees, *Cinnamomum* Schaeffer and *Cryptocarya* Brown from India and Myanmar by Gangopadhyay in 2008. *Beilschmiedia tirunelvelica* Manickam, Murugan, Jothi & Sundaresan was reported from Western Ghats, India (Manickam *et al.* 2005). *Phoebe hedgei* M. Gangopadhyay & A. Sarmah a new species of Lauraceae from North-East India was identified by M. Gangopadhyay and A. Sarmah in 2007. Kumar *et al.* (2011) recorded *Cinnamomum alexei* Kostermans from India. *Litsea kakkachensis* R. Ganesan a new species from Agasthyamalai, Western Ghats, India was described and illustrated by R. Ganesan in 2011.

1.7.6. Classification

Multiple classification design base on a variety of morphological characteristics have been proposed but most of those are not accepted in their totality. Van der Werff and Richter (1996) provided the most conventional classification regarding Lauraceae. Although, according to Judd *et al*. (2007), the classification of Van der Werff and Richter (1996) is presently the authority but it is not fully accepted by the scientific community because this classification is based mainly on one morphological character i.e. inflorescence structure. But the problem is that for a substantial number of species of Lauraceae the fruits or flowers are not known which makes generic placement of their species uncertain, since most genera are defined by floral characters. Another problem is that attributes of both flowers and fruits are utilized in most generic keys. But the specimens almost never bear both flowers and fruits, so the identification of such specimens often become almost impossible.

For the solving difficulties of identification as well as classification, now a day's many authors worked with different techniques *viz*. anatomy, leaf architecture, chemotaxonomic approaches such as antioxidant, phytochemical screening, thin layer chromatographic figure print etc. Considering this knowledge gap of identification the following objectives has been framed which will enlighten the more accurate taxonomic interpretation among the members of this family.

1.8. OBJECTIVES FOR THE RECENT WORK

- Floristic survey and recognition of economically important Laurel species of Terai and Duars region
- Construction of distribution map of those Laurel species
- Recording of exact flowering and fruiting periods for different Laurels of the area
- Leaf architectural study of same species
- Evaluation of antioxidants of same species
- Extraction of aromatic principles of same species
- Phytochemical screening of some members and profiling through TLC
- Reconstruction of similarity dendrogram and cluster analysis on the basis of various taxonomic characters.

CHAPTER – 2

Literature Review

Literature review

2.1. LAURACEAE IN PLANT KINGDOM

The family Lauraceae was established by A.L. de Jussieu (1789) in Genera Plantarum. The family covers 55 genera and about 2800 species (Van der Werff & Richter 1996) distributed widely in tropical to temperate regions of the world. The name Lauraceae was come from the type genus *Laurus* Linnaeus.

Bentham & Hooker (1862-1883) placed Lauraceae Jussieu in their classification as follows:

Kingdom: Plantae

Subkingdom: Phanerogamia

Class: Dicotyledons

Subclass: Monochlamydeae

Series: Daphnales

Family: Lauraceae

Cronquist (1981) placed Lauraceae in his classification as follows:

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Laurales

Family: Lauraceae

Takhtajan (1997) placed Lauraceae in his classification as follows:

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Laurales

Suborder: Laurineae

Family: Lauraceae

APG III (Chase & Reveal 2009) placed Lauraceae as follows:

Kingdom: Plantae

Clade: Angiosperms

Clade: Magnoliids

Superorder: Magnolianae

Order: Laurales

Family: Lauraceae

2.2. FAMILY CHARACTERS

Deciduous or evergreen shrubs or trees (*Cassytha* Linnaeus a twining, virtually leafless semi-parasitic perennial herb) (Li *et al.* 2008a), often with aromatic bark and leaves. Terminal buds often very large and bounded by several scales which leave behind dense clusters of scars in rings around the twigs (perulate buds). Leaves usually alternate, sometimes opposite or sub-opposite or clustered at branch ends, simple, usually entire, rarely lobed (*Sassafras* Linnaeus), mostly pinnately veined or strongly 3-veined. Stipules absent (Long 1984). Flowers usually small, in panicles, cymes or umbels surrounded by decussate bracts. Flowers generally bisexual, sometimes unisexual, trimerous or rarely dimerous (e.g. *Potameia* Thouin), actinomorphic, greenish, yellowish, or white (Datta 1988). Perianth segments usually six, in two whorls of three, deciduous or persistent; perianth tube usually persisting as a cupule at the base of fruit (Judd *et al.* 2002). Androecium usually of 4 whorls of 3 stamens each, adnate to perianth tube, often with an inner whorl of 3 staminodes; filaments usually free, innermost filaments usually bearing glands; female flowers with 9 or 12 staminodes; anthers basifixed, 2 or 4 celled (Prain 1903). Pistil 1; ovary usually superior, 1-locular; ovule anatropous in parietal placentation; style 1; stigma 1, rarely 2 or 3 lobed (Li *et al.* 2008a). Fruit a drupe usually borne on enlarged cup like remains of perianth, occasionally perianth completely absent or totally surrounded the fruit. Seeds with straight embryo; endosperm absent (Cronquist 1981).

2.3. ECONOMIC IMPORTANCE

The members of Lauraceae are economically important as sources of medicine, timber, nutritious fruits, spices and perfumes (Judd *et al.* 2002). *Persea americana* Miller (avocado) is a highly nutritious fruit, rich in proteins and fats and low in sugar. The total food value of avocado is high; it provides nearly twice the energy of an equivalent weight of meat and an abundance of several vitamins, such as A, B, C, D, and E (Wolstenholme & Whiley 1999). The leaves of the *Laurus nobilis* Linnaeus (bay Laurel) ware dried and used as a flavouring agent for cooking, particularly for meat and fish dishes. A fat extracted from the seeds of this plant was used to make soap (Bergh & Ellstrand 1986). Cinnamon spice is derived from the inner bark of *Cinnamomum verum* J. Presl, the cinnamon tree, a native of Sri Lanka and southern India. Eugenol, one type of oil distilled from the green leaves of *C. verum*, is used as a substitute for clove oil, as an ingredient in some perfumes, and as a flavouring substance for sweets, foods, and toothpaste. Camphor was derived from *Cinnamomum camphora* (Linnaeus) J. Presl. Camphor was one of the raw materials used in making celluloid, which has now been replaced by other plastics. Camphor is employed in pharmaceuticals, especially in liniments, and in insecticides (Seth 2004).

Many other species of *Cinnamomum* Schaeffer have uses as spices and medicines. The essential oil of the leaves of *Cinnamomum tamala* (Buchanan–Hamilton) T. Nees & Eberm known as *Tejpat* oil, is medicinally used as a carminative, antiflatulent, diuretic and in cardiac diseases (Mir *et al.* 2004). *Cinnamomum cambodianum* Lecomte bark is used to make joss sticks, which are burned as incense. Sassafras oil was extracted from the root-bark of *Sassafras albidum* (Nuttal) Nees. This oil once served as a flavouring agent for sweets, medicines, toothpastes, root beer and sarsaparilla (Seth 2004).

It is said that the wood of all trees of Lauraceae is suitable for industrial purposes, a statement that seems to be only a slight exaggeration. Most of the best-known timbers of Lauraceae have been depleted through overexploitation, however, and are not likely to remain economically important in the future unless serious conservation efforts are undertaken. Many species of the widespread genus *Ocotea* Aublet have been utilized for their widely useful timber (*www.woodwindowalliance.com*).

2.4. GENERA UNDER LAURACEAE

The Lauraceae consists of some 30 to 55 genera as recognized by different authors. Of these, about two-thirds of the species are belonging to only 6 genera: *Ocotea* Aublet (over 400 species), *Litsea* Lamarck (over 250 species), *Persea* (about 200 species), *Cinnamomum* Schaeffer (about 200 species), *Cryptocarya* Brown (about 200 species) and *Beilschmiedia* Nees (about 150 species) (Cronquist 1981). According to Heywood (1993) the family is represented by 32 genera, where as Kostermans (1957) reported 55 genera of Lauraceae. J. Hutchinson (1964) distinguished the Lauraceae into 47 genera as presented in Table 2.1.

Table 2.1. Genera of Lauraceae according to Hutchinson (1964)

Actinodaphne Nees	***Micropora*** Hooker *f.*
Aiouea Aublet	***Misantheae*** Chamisso & Schlechtendal
Aniba Aublet	***Nectandra*** Roland *ex* Rottboell
Apollonias Nees	***Neolitsea*** Merrill
Beilschmiedia Nees	***Nobeliodendron*** O. Schmidt
Brassiodendron Allen	***Nothaphoebe*** Blume
Cardiodaphnopsis Airy-Shaw	***Ocotea*** Aublet
Cassytha Linnaeus	***Persea*** Miller
Cinnamomum Schaeffer	***Phoebe*** Nees
Cryptocarya R. Brown	***Phyllostemonodaphne*** Kostermans
Dahaasia Blume	***Pleurothyrium*** Nees
Dicypellium Nees	***Potameia*** Thouin
Dodecadenia Nees *ex* Wallich	***Ravensara*** Sonnerat
Endiandra R. Brown	***Sassafras*** J. Presl
Endlicheria Nees	***Sassafridium*** Meisner
Eusideroxylon Teijsmann & Binnendijk	***Stemmatodaphne*** Gamble
Hypodaphnis Stapf	***Synandrodaphne*** Meisner

Iteadaphne Blume	***Syndiclis*** Hooker *f.*
Laurus Linnaeus	***Systemonodaphne*** Mez
Licaria Aublet	***Thouvenotia*** Danguy
Lindera Thunberg	***Umbellularia*** Nuttal
Litsea Lamarck	***Urbanodendron*** Mez
Machilus Nees	***Valvanthera*** C.T. White
Mezilaurus Taubert	

2.5. DEVIATIONS FROM NORMAL CHARACTERS IN DIFFERENT GENERA

Stems filiform twining and leaves reduced to scales in *Cassytha* Linnaeus; leaves sometimes sub-opposite in *Beilschmiedia* Nees; mostly prominently 3-nerved from the base in *Cinnamomum* Schaeffer (Long 1984); flowers in *Misanteca* Chamisso & Schlechtendal arranged in dense heads; male and female inflorescences dissimilar in *Endlicheria* Nees; ovary inferior in *Hypodaphnis* Stapf; fruits supported on a double-margined receptacle in *Misantheae* Chamisso & Schlechtendal (Hutchinson 1964); inflorescence enclosed up to flowering time by an involucre of bracts in *Litsea* Lamarck (Prain 1903); flowers dimerous in *Potameia* Thouin; receptacle or pedicle of the Malayan genus *Dehaasia* Blume is swollen; calyx persistant and reflexed in the fruit in *Machilus* Nees; outer sepals much smaller than the inner whorl in *Dehaasia* Blume and *Cyanodaphne* Blume (Judd *et al.* 2002); anthers opening by minute pores in *Micropora* Hooker *f.*; annular disk present in *Synandrodaphne* Meisner; mature fruit completely or almost completely enclosed by the accrescent calyx-tube in *Cryptocarya* Brown (Li *et al.* 2008a).

2.6. GEOGRAPHICAL DISTRIBUTION

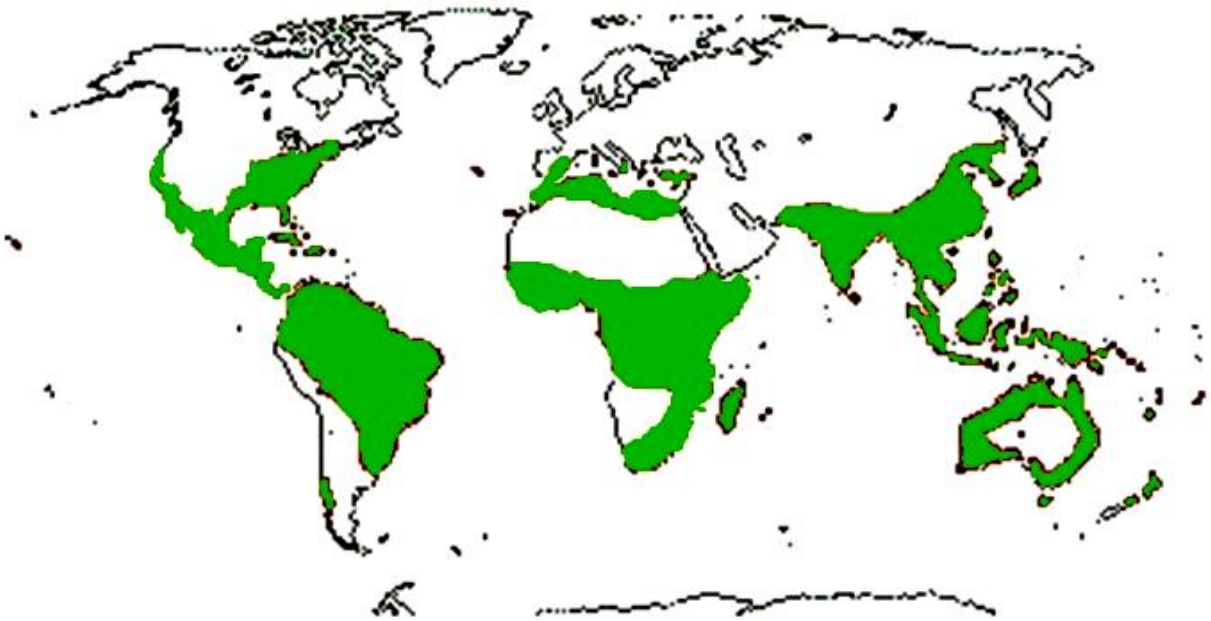


Figure 2.1. Distribution of Laurels in the world [Source: Heywood 1993]

The family Lauraceae was widespread in tropical and subtropical regions throughout the world (Cronquist 1981) (Fig: 2.1). Trees of the Laurel family are predominant in the world's Laurel forests, which occur in a few humid subtropical and mild temperate regions of both the hemispheres, including the southern Japan, Macaronesian islands, central Chile and Madagascar. According to J. Hutchinson (1964) the family is most abundantly represented in the tropics of both hemispheres, with about 18 genera in tropical America and nearly as many in tropical Asia; few genera are common to both the regions. However, the large genus *Cryptocarya* Brown is very widely spread. They are very rare in tropical Africa and Madagascar, though there are about 18 species of the genus. The Atlantic Islands are claimed to be the home for 3 indigenous genera, namely *Persea* Miller, *Laurus* Linnaeus and *Apollonias* Nees. The genus *Apollonias* Nees constituting one of the chief arboreal elements of the evergreen forest belt of the Canary Island and Madeira. There are very few Lauraceae in North-East Australia. Sassafras occurs both in East Asia and Atlantic North America. According to Cronquist (1981) two greatest centers for Lauraceae are in South-East Asia and in Brazil. Long (1984) has recorded fourteen genera for the *Flora of Bhutan*. Among those, *Litsea* Lamarck is the most dominant. Li *et al*. (2008a) reported 25 genera and 444 species for the *Flora of China*. *Machilus* Nees (82 species) *Litsea* Lamarck (74 species) were the widely extending genera in China. Ngernyuang *et al*. (2003) reported 19 species from tropical lower montane forest in northern Thailand**.** In Bangladesh, the family is represented by 13 genera and 46 species (Ara *el al*. 2007). 11 genera and 39 species were recorded earlier from the present Bangladesh areas by Hooker (1886). Heinig (1925) listed 20 species distributed under 9 genera from the Chittagong and Chittagong Hill Tract regions. Sinclair (1955) enumerated 5 species and 3 genera from the area of Cox's Bazar. Alam (1988) recorded 30 species and 9 genera from the forests of Sylhet region of Bangladesh. Three species of *Litsea* Lamarck were reported by Siwakoti and Varma (1999) for Plant Diversity of Eastern Nepal.

2.7. LAURELS IN INDIA

Hooker (1886) reported 15 genera and 204 species of Laurels for *The Flora of British India*. In *Flora Indica*, William Roxburgh (1832) described four genera and nineteen species of Lauraceae. Brandis (1874) recorded eleven species distributed under seven genera from North-West and Central India. Six genera and twenty four species from Agasthyamala were described by Mohanam and Sivadasan (2002). From Chota Nagpur, four genus and six species were described by Hains (1925). Parasitic herb *Cassytha filiformis* Linnaeus was reported from Indian desert by Bhandari (1978). Singh (1986) reported two species of *Cinnamomum* in the *Flora of Patna*. Fifteen genera and sixty eight species were described in *Flora of Assam* (Kanjilal 1940). Hajra (1996) described fourteen species distributed under eight genera from the Namdapha region of Arunachal Pradesh. Eleven genera and fifty species were recorded from Meghalaya by Haridasan & Rao in 1987. Momiyama (1966, 1971, 1975) reported nine genera and twenty nine species from Eastern Himalayan region. Six genus and fifteen species of Lauraceae was reported by Duthie (1915) for *Flora of The Upper Gangetic Plain*. Sanyal (1994) described two genera and two species for *Flora of Bankura District*, West Bengal. Eight genera and twenty four species were described from the pre-independent Bengal by Prain (1903). Cowan & Cowan (1929) listed forty eight species allocated under eight genera from North Bengal. Matthew (1981) enumerated eight genera and seventeen species from the Kurseong sub-division of Darjeeling district. However, some taxonomist have made contribution to the lauraceous flora of Darjeeling-Sikkim Himalaya including Das (1986) enumerated 14 species; P.C. Rai (2001) reported 15 species; U. Rai (2006) listed

twenty one species; Ghosh (2006) enumerated ten species. So, Lauraceous plants are distributed all over the India covering wide range of vegetation structures.

2.7.1. Lauraceae in Terai and Duars of West Bengal

Only few works were done regarding the distribution of Laurels in Terai and Duars region. Banerjee (1993) reported eleven species distributed under six genera from Jaldapara Rhino Sanctuary and are *Actinodaphne obovata* (Nees) Blume, *Beilschmiedia sikkimensis* Hooker *f*., *Cinnamomum bejolghota* (Hamilton) Sweet, *Cinnamomum glaucescens* (Nees) Drury, *Cryptocarya amygdalina* Nees, *Litsea glutinosa* (Loureiro) Robinson, *Litsea monopetala* (Roxburgh) Persoon, *Litsea salicifolia* (Nees) Hooker *f*., *Persea gamblei* (Hooker *f*.) Kostermans and *Persea glaucescens* (Nees) Long. Das *et al.* (2010) reported nine Lauraceous species from three MPCAs (Medicinal Plant Conservation Area) of Terai and Duars region. These MPCAs are situated at Rajavatkhaoa forest, Lataguri forest and North Sevok forest. According to Das *et al.* (2010) the most dominated genus in this area is *Litsea* Lamarck and is followed by *Actinodaphne* Nees, *Cinnamomum* Schaeffer, *Persea* Miller and *Phoebe* Nees.

2.8. GEOLOGICAL HISTORY

Phylogenetic analyses is based on molecular markers and combined with inferences about timing of the phylogenetic events in the group which is based on branch lengths calibrated from fossil records. These fossil records suggested that bulk of the species diversity has accumulated from the time of the Cenozoic for which the family is represented in many fossil floras by leaves, wood, flowers and fruits (Eklund 1999; Chanderbali *et al.* 2001). These analyses further suggest that the basal lineages of Lauraceae, in which few extant species included, were already recognized in the Late Cretaceous (Chanderbali *et al.* 2001).

The fossil record, from the Cretaceous, plays a major role in understanding the early history of angiosperms (Friis *et al.* 2006). Direct fossil evidences of Lauraceae in the Cretaceous are sparse compared to that in the Cenozoic period, but there is nevertheless a growing record of leaves, wood, and reproductive structures which clearly support that the family was present and was also widespread at the early stages of angiosperm evolution. Interestingly, the fossil record of the flowers is much widespread from the Cretaceous than that of the Cenozoic. Particularly several well-preserved fossil flowers are important and have been described during the past two decades. The Cretaceous floral structures assigned to Lauraceae are now known, which are based on 12 taxa from several localities in North America, Europe, and Asia (Drinnan *et al.* 1990; Crane *et al.* 1994; Herendeen *et al.* 1994; Eklund & Kvaeek 1998; Eklund 2000; Takahashi *et al.* 2001; Frumin *et al.* 2004). These fossils provided insights into the floral structure of the Cretaceous representatives of this family. Because of the importance of the floral structure in the systematics of extant Lauraceae, the fossil flowers permitted us to compare the extinct members with those of extant representatives and also help to draw preliminary conclusions about their phylogenetic relationships and distribution over different geological era. These records also provided a new data-set for understanding the biological and geographic history of the family as well as to know their possible phylogenetic and functional differentiation (e.g., floral biology and pollination). According to Arthur Cronquist (1981) some Upper Cretaceous (Maestrichtian) wood from California falls well within the range of variation of the Lauraceae and Eocene wood from Yellowstone National Park is considered to be lauraceous. Von Balthazar *et al.* (2007) reported that Early Cretaceous (Early to Middle Albian) fossil flowers of lauraceous affinity from the Puddledock locality, Virginia, USA.

The flowers have several typical lauraceous structural features but are remarkable for their simple androecium. Nondestructive optical sectioning of single specimen, using synchrotron-radiation x-ray to monographic microscopy (SRXTM) offered crucial information on floral organization and internal structure. This flowers or fragmentary floral material assigned to Lauraceae have already been described from the Puddledock locality (Crane *et al.* 1994), supplied the earliest evidence of the family and also securely establishes its existence in the Early Cretaceous. The material further evidently demonstrated the phylogenetic and functional diversification of Laurales at very early phase of angiosperm evolution (Von Balthazar *et al*. 2007).

2.9. PHYLOGENATIC RELATIONSHIP

Lauraceae is under the order Laurales which is close to Magnoliales (Takhtajan 1981), but more advanced in some respects than the bulk. The perigynous (or even epigynous) flowers, single functional ovule, biaperturate or inaperturate pollen and usually fairly conventional stamens of the Laurales are the all advanced as compared to most of the Magnoliales (Cronquist 1981). Laurales consists of seven families and 3400 species. Major families include Calycanthaceae, Lauraceae, Monimiaceae, Siparunaceae and Hernendiaceae (Judd *et al.* 2002). In 2008, Stevens recorded the molecular and morphological phylogenetic studies in the Laurales. He found that Hernandiaceae, Lauraceae, and Monimiaceae (*sensu stricto*) are belonging to a monophyletic group (Fig. 2.2). Because of the insufficiency of phylogenetically informative substitutions, the relationships among families within this clade remain doubtful. Generally, molecular phylogenies may conflict because of a diversity of factors, including substitution rate dissimilarity among sites and lineages, taxon sampling and base compositional biases (Kubitzki 1981; Renner & Chanderbali 2000).

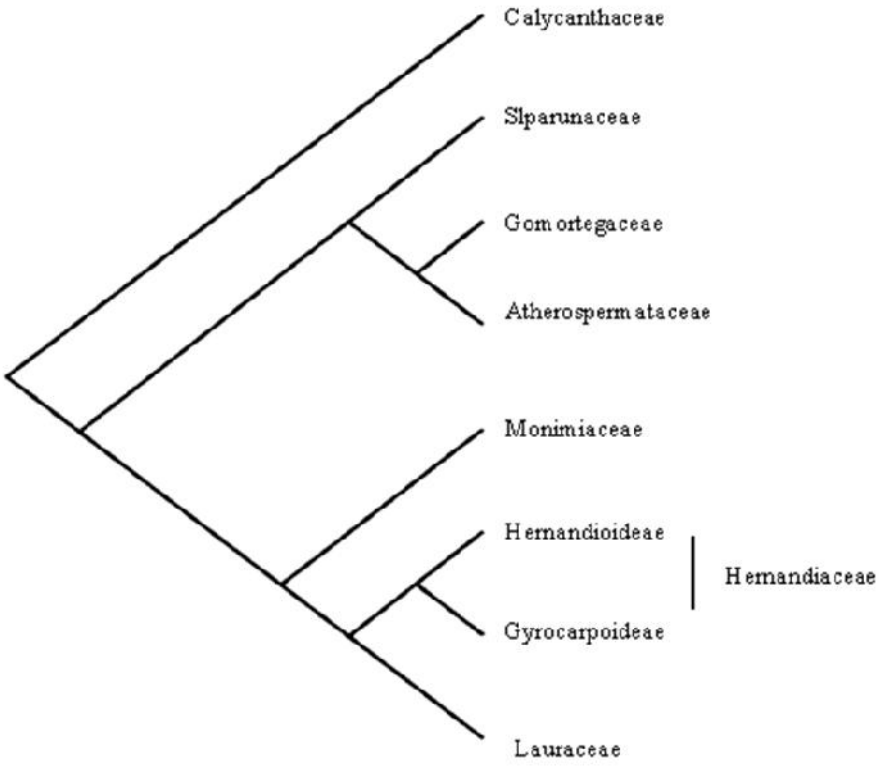


Figure 2.2. Phylogenetic tree of Laurales on the basis of Cronquist and Takhtajan's classification (Stevens, 2008)

According to Hutchinson (1964), Lauraceae is remarkably homogeneous like Sapotaceae. Lauraceae is closely related to Monimiaceae (Renner & Chanderbali 2000). In their study, they analyzed a total of 2846 aligned nucleotides present in a plastid intron, three spacers, and a portion of the nuclear 26S rRNA gene from Hernandiaceae, Lauraceae, and Monimiaceae. Searching of the data indicates that insufficient taxon sampling, fast-evolving outgroups, or biased base composition is very challenging to explain.

Arthur Cronquist (1981) reported that the gynoecium of the Lauraceae is truly monomerous or only pseudomonomerous and derived eventually from 3 carpels. However, Takhtajan (1997) has postulated that the Lauraceae originated from primitive members of the Monimiaceae those had several separate carpels. Now, the question arises, did the evolutionary reduction from numerous separate carpels to a single carpel occur bypassing the formation of a compound pistil, or did the reduction to a particular carpel occur only after a compound pistil evolved in some unknown intermediate ancestor! Sastri (1958, 1962, 1965) has pointed out a significant statement that ontogenetically the gynoecium of a flower develops in from a single conduplicate carpel and the appressed carpellary margins do not unite until a relatively late stage in development. After reviewing the available proofs, Endress (1972) firmly decided that the gynoecium of Lauraceae is truly monomerous, but the argument is probably not ended. Contrasting with the other lauralean families such as Monimiaceae, Calycanthaceae, Trimeniaceae and Hernandiaceae using Chloranthaceae as outgroup, Lauraceae is best for resemble Hernandiaceae embryologically (Heo & Tobe 1995).

Lauraceae and Hernandiaceae share pachychalazal seeds or ovules with raphal vascular tissues ramified at an enlarged chalaza. Close affinities between Hernandiaceae and Lauraceae are also suggested through shared benzyl-isoquinoline (Gottlieb 1972) and micro-fibrilar structure with tubular exine (Kubitzki 1981) and through a cladistic analysis based on morphological characteristics (Loconte & Stevenson, 1991). However, several core lauralean families *viz.* Amborellaceae, Monimiaceae and Gomortegaceae are as yet little known by embryologically, production of these data will probably be helpful for more critical comparison. Certainly, a shared isorhamnetin rather suggested close relationships of Lauraceae with Monimiaceae and Gomortegaceae (Crawford *et al.* 1986). Macromolecular verification from *rbcL* nucleotide sequence analysis suggests that Lauraceae also can be relate to Monimiaceae than to Hernandiaceae (Qiu *et al.* 1993). However, such molecular records are restricted to very few species of Laurales and Lauraceae; when such data will be available, based on more species, better conclusion can be drawn regarding the closest allies of Lauraceae.

2.10. CHALLENGES IN CLASSIFICATION OF LAURACEAE

Classification within the Lauraceae remains unresolved till date, though, numerous classificatory schemes based on morphological characteristics have already been proposed (Van dar Werff & Richter 1996). However, none of these is fully accepted. The knowledge of all the taxa at genus and species levels comprising Lauraceae is still incomplete. Till 1991, approximately 25 – 30 % of neotropical species of Lauraceae had not been described (Rohwer *et al.* 1991). As of 2001, Kimoto & Tobe completed only embryological studies on individuals from 26 genera of this family. Additionally, the huge amount of variation within the family poses a major challenge for developing a reliable classification (Van Dar Werff & Richter 1996; Rohwer *et al.* 1991). Till date, it is impossible to describe even one genus or a tribe by a single well-defined character (Rohwer *et al.* 1991). For this reason, all the proposed classifications rely on a set of characteristics where the combination presents the most frequently observed traits for

the group (Van dar Werff & Richter 1996; Rohwer *et al.* 1991). However, due to an array of molecular and embryological evidences those disagrees with such groupings, kept the scientific community without accepting those classifications in their totality. Their classification is based on inflorescence structures as well as bark and wood anatomy. It splits Lauraceae into two subfamilies *viz*. Cassythoideae and Lauroideae. The Cassythoideae is comprised of a single genus i.e. '*Cassytha*', and is defined by its herbaceous and parasitic habit. The Lauroideae is then divided into three tribes: Laureae, Perseeae, and Cryptocaryeae (Radford *et al.* 1974).

The subfamily, Cassythoideae, is not fully supported. Support has come from "matK" sequences of chloroplast genes (Rohwer 2000), while a questionable placement of *Cassytha* has been concluded from analysis of intergenetic spacers of chloroplast and nuclear genomes (Chanderbali *et al.* 2001). Embryological studies by Heo *et al.* (1998) also supported the creation of this subfamily. It is found that *Cassytha* develops an 'ab initio' cellular type endosperm and rest of the family (with one exception) develops a nuclear type endosperm. Kimoto *et al.* (2006) suggests that *Cassytha* should be placed in the Cryptocaryeae tribe because it shares a glandular anther tapetum and an embryo sac protruding from the nucellus like other members of the Cryptocaryeae.

The Laureae and Perseeae tribes are not well supported by any molecular or embryological studies. Sequences of the "matK" chloroplast gene as well as sequences of chloroplast and nuclear genomes revealed close relationships between the two tribes. Embryological proof does not support a clear division between the two tribes either. Genera such as *Caryodaphnopsis* Airy-Shaw and *Aspidostemon* Rohwer-Richter those share embryological characteristics with one tribe as well as wood and bark characteristics or inflorescence characteristics with another tribe blur the decision to accept these two groups at the rank of Tribe. All available evidence, except for inflorescence morphology as well as wood and bark anatomy, fails to support the segregation of the tribes Perseeae and Laureae.

The tribe Cryptocaryeae is partially supported by embryological and molecular studies. Chloroplast and nuclear genomes supports a tribal grouping that contains all the genera circumscribed by Van der Werff and Richter (1996) as well as three additional genera. Partial support for the tribe is also attained from the "matK" sequences of chloroplast genes as well as from embryology.

2.11. USE OF ANATOMICAL CHARACTERS IN TAXONOMY

Anatomical features have long been used for solving many taxonomic disputes (Agbagwa & Ndukwu 2004; Kharazian 2007). Like other biological characters, anatomical characters also provide preliminary identification of species. For this purpose stem, lamina and petiole anatomy played crucial role (Metcalfe & Chalk 1950).

In woody dicotyledons information of wood elements are extremely significant from taxonomic, systematics and phylogenetic points of view (Metcalfe & Chalk 1950). For the phylogenists, stem anatomy is the most rewarding source of information because of the widespread data can be produced and are already available for most families on comparative basis (Thorne 1976). Lersten & Curtis (2001) indicated that stem anatomical studies support to solve many systematic problems. The wood anatomy of some Laurels like *Beilschmiedia emarginata* (Meisner) Kostermans, *B. rigida* (Mez) Kostermans, *B. taubertiana* (Schwartz *et* Mez) Kostermans and *Anaueria brasiliensis* Kostermans are described by Callado and Costa (1997). The main anatomical differences are: presence and arrangement of secretory cells and the arrangement of the axial parenchyma. In 1981, Weber studied a taxonomic revision of Australian

Cassytha species on the basis of morphology and anatomy. In 2012 Rao *et al.* used anatomical features for the identification of economically important *Litsea glutinosa* (Loureiro) C.B. Robinson.

Not only stem but also the structure of lamina and petiole anatomically shows differences between genera and species (Shaheen 2007; Eric *et al.* 2007). Vaikos (1987) studied foliar epidermis of eight species of Iridaceae. He illustrated epidermal features which were useful for species identification. A notable study on leaf anatomy of 597 species of *Rhododendron* Linnaeus was done by Cowan (1950) and Hayes *et al.* (1951). Foster (1951) has explained the careful description of mesophyll along with detailed leaf anatomy which can yield valuable taxonomic dividends. Like other families anatomical structures of leaf are also very important in Lauraceae (Metcalfe & Chalk 1950). Leaf anatomical investigation was carried out in *Cinnamomum pauciflorum* Nees by Baruah and Nath in 2006. Bhatt and Pundya (2012) examined the leaf anatomy of *Litsea chinensis* Lamarck. According to them uniseriate epidermal layer, anisocytic stomata and presence of abundant mucilage in developed vascular bundle might eventually allow recognition as well as standardization of the species. Thus, useful leaf anatomical characters are determined in designated taxonomical structures of some species.

2.12. USE OF LEAF ARCHITECTURE IN TAXONOMY

Leaf architecture was initially used and described by Hickey to represent the placement of plant species through the expression of leaf structure with leaf shape, gland position, venation pattern and marginal configuration (Hichey 1973; Zetter 1984; Kohler 1993). From earliest to recent times, for fossils to living plant species identification, leaf architecture is used in large scale. In paleobotany, macro-fossils which were showing leaf venation patterns those were extensively employed in identifying fossil taxa (Alvin & Chaloner 1970; Cleal 1981; Walther 1998; Melville 1969). Earlier in 1951, Foster carefully prepared the description of various forms of venation. Pattern of leaf venations are of great significance both in monocots and dicots (Foster 1959). Leaf architecture were studied in several dicotyledonous and monocotyledonous families like Asteraceae (Banerjee & Deshpande 1973), Euphorbiaceae (Sehgal & Paliwal 1974), Berberidaceae (Singh *et al.* 1978), Betulaceae (Frank 1979), Bignoniaceaeb (Jain 1978), Labiatae (Tyagi & Kumar 1978), Rosaceae (Merrill 1978), Solanaceae (Inamdar & Murthy 1978) and Scrophulariaceae (Verghese 1969), Hydrocharitaceae, Taccaceae, Dioscoreaceae, Smilacaceae, Araceae, Alismataceae, Aponogetonaceae (Inamdar *et al.* 1983). In 1974, Sehgal and Paliwal explained the venation pattern of *Euphorbia* sp. On the basis of leaf venation they created categories, groups and subgroups. Micro-morphological characteristics like epidermal hairs played a significant role in plant systematics at generic and specific levels (Hardin 1979). Todzia and Keating (1991) have determined the relationship of Lauraceae and Chlorenthaceae by the data of leaf architecture. Moore *et al.* (2008) analyzed and used the epidermal characters of leaves in *Smilax* (Tournefort) Linnaeus, which is recognized as a successful taxonomic method for distinguishing every individual taxon. Beside these characters, stomata also provide taxonomically many important diagnostic characters, like stomatal index, stomata type and the occurrence of stomata on the adaxial or abaxial leaf surface, etc. (Tripathi & Mondal 2012).

2.13. PHYTOCHEMICAL DATA FOR SOLVING TAXONOMIC PROBLEMS

The subject of chemotaxonomy is concerned with the application of chemical characters to solve the problems of classification and phylogeny. This rapidly expanding discipline of plant taxonomy has been variously called as chemotaxonomy, chemosystematics, biochemical systematic or phytochemistry (Pullaiah 2007). Botanists, of late, have come to the conclusion that evidence for discussing the relationships and

phylogeny must be taken from as many sources as possible. As more comprehensive survey of phytochemistry has become available, taxonomists have shown a growing interest in the application of chemical characters to taxonomic problems (Judd *et al*. 2002). Living organisms produce many types of natural products in unstable amounts, and quite often the biosynthetic pathways responsible for the production of these compounds also differ from one taxonomic group to another. The distribution of these components and their biosynthetic pathways correspond well with existing taxonomic arrangements based on more traditional criteria like morphology (Swain 1963). During evolution it sometimes happens that unrelated groups of plants produce morphologically alike structures. This is called 'convergence' or 'parallel development'. Conversely related plants may give rise to very dissimilar descendants i.e. 'divergence'. These occurrences sometimes cause considerable taxonomic difficulties (Erdtman 1963). Chemical contributions to plant classification are based on the chemical constituents of plants, i.e. on their 'molecular characteristics'. These characteristics are genetically controlled and have the advantage over morphological characteristics (Wink & Waterman 1999). The method of chemical taxonomy is simple in principle and consists of the investigation of the distribution of chemical compounds or groups of biosynthetically related compounds in series of related or supposedly related plants (Mannheimer 1999). Chemical variation has considerable taxonomic value in several ways:

- Confirmation or support of putative classifications derived from other sources of taxonomic characters, such as morphology
- Resolution of problems where relationships based on other evidences are ambiguous or conflicting
- Providing evidence to suggest more natural positioning of anomalous taxa, as well as to separate taxa; often the presence of anomalous taxa in a group is accentuated by their chemical peculiarities
- Detection and confirmation of hybridization
- Providing additional on/off characters for numerical taxonomy by their presence or absence in taxa.

Chemotaxonomy has made rapid progress in the last 40 years because of new instruments and newer techniques (Pullaiah 2007). A crude extract of a plant can be separated into its individual components, especially in the case of micro-molecules, by using one or more techniques of chromatography, including paper, gas, thin-layer, high-pressure liquid chromatography and high performance thin layer chromatography. The resulting chromatogram gives a visual display or "fingerprint" characteristic of a plant species for the particular class of compounds under study (Erdtman 1963; Swain 1963). The individual spots can be further purified and then subjected to one or more types of spectroscopy, such as ultraviolet (UV), infrared (IR) or nuclear magnetic resonance (NMR) or mass spectroscopy (MS) (or both), which may provide information about the structure of the compound. So, for taxonomic purposes, both visual patterns and structural knowledge of the compounds can be compared among different species (Wink & Waterman 1999). Research has shown that there is generally an inverse relationship between the taxonomic distribution of a compound or class of compounds and its biogenetic complexity such that if it is biosynthetically simple and widespread it may be assumed to be primitive, while those are more limited in their distribution and more complex to biosynthesis may be assumed more advanced (Gershenzon & Mabry 1983). Certainly chemotaxonomic investigations have been employed at all levels of the taxonomic hierarchy from sub variety to division (Smith 1978; Stace 1980). Abbott (1886) has made the earliest attempts to correlate chemistry with the phylogenetic level

of development. According to her the saponin containing plants occupied the middle level of Hackel's scheme of plant evolution. The first successful attempt to combine chemical and morphological evidence in the study of a single genus was the work of Baker and Smith (1920) on the essential oils of *Eucalyptus* sp. They recommended that the level of relationship should be in chemical similarities. After collection of the morphological and chemical data from 176 species of *Eucalyptus,* they divided the genus into three groups differing in both morphological structure and chemical constituents.

The subject really came to the age with the publication of several seminal works, especially those of Alston and Turner (1963), Swain (1963, 1966) and Harborne (1964, 1967). Hegnauer had already embarked on his epic series 'Chemotaxonomic der Pflanzen', in which with great carefulness he compiled the current information on occurrence and distribution of metabolites within and between plant families (Hegnauer 1962 – 1990; Hegnauer & Hegnauer 1992 – 1996). By this time, sufficient data had been gathered concerning the occurrence of a wide range of secondary metabolites to allow for generalizations to be made on the taxonomic range of their distribution.

Thin layer chromatography (TLC) is an important technique for plant components identification. To differentiate the diversity of plant species this technique is used frequently. In 2011, Zafar *et al.* compared different category of phytochemicals and classified different groups by preparing cladograms through TLC. In 2009, Choze *et al.* noticed through TLC that the absence or presence of certain compounds in the plant *Augusta longifolia* (Sprengel) Rehder supplied various taxonomic markers with important information about the position of this species in the Rubiaceae. In 1979, Floyd compared the fresh *Pelea* with herbarium samples. He took the both extracts and determined phenolic components through TLC. He obtained the identical result. So, in numerous cases TLC can be used for identification of plant specimens and employed as chemotaxonomic technique.

2.14. COMPOUNDS USEFUL IN PLANT TAXONOMY

Although in theory all the chemical constituents of a plant are potentially valuable to a taxonomist, in practice some sorts of molecules are far more valuable than others. Excluding the inorganic compounds, metabolites which are of relatively little use can be recognized with three broad categories of compounds *viz*. primary metabolites, secondary metabolites and semantides.

2.14.1. Primary Metabolites

Although some primary metabolites are utilized chemo-systematically, as a whole their efficacy is limited. They are intermediates or products of essential metabolic pathways and are present in ubiquitous distribution (e.g. sugars that participate in the Calvin cycle). Occasionally they assemble in high concentrations as unusual storage products and in such instances that they can be used for taxonomic entity (Stace 1980).

2.14.2. Secondary Metabolites

These are very numerous and of more restricted occurrence than primary metabolites, which makes them more valuable as a source of taxonomic evidence (Stace 1980) and a large amount of information on their distribution in angiosperms is available. However, the data was demonstrated that some secondary compounds are more useful than others, particularly on a broad scale (Gershenzon & Mabry 1983). The following are those groups of compounds which were so far established to be most useful:

2.14.2.1. Flavonoids

Jones and Luchsinger (1987) showed that the flavonoids are one of the most useful taxonomic markers for a variety of reasons:

- These demonstrate a wide range of chemical structures, which have a demonstrable genetic basis for their variation
- These are chemically stable, so that analysis of materials can be done by earlier collected materials
- These can be isolated easily and identified even from small amount of plant materials; huge number of plants can easily and rapidly be surveyed for flavonoids using paper, thin-layer or one or two dimensional chromatography and through which, in recent years, many useful results have been achieved (Zafar *et al.* 2011)
- These occur variously but ubiquitously almost in all plants; and
- These can be used at all taxonomic levels in most groups of plants.

Although flavonoids are present in all higher plants (Vickery & Vickery 1981), they are absent in bacteria and the majority of algae. The most primitive group that exhibits them is the Charophyceae (stoneworts), a group of green algae considered to be advanced for a number of reasons. Simple flavonoids have been found in primitive Bryophytes, whereas far more complex ones have been isolated from the most advanced angiosperms (e.g. Orchidaceae).

Flavonoids can be used as a 'fingerprint' in some genera where it has been found that each species has a distinct flavonoids pattern. For example, the *Eaptisra* genus (Fabaceae), 62 different flavonoides are available and each of its 17 species has different characteristic flavonoid pattern. However, closely related species has almost identical pattern of flavonoides (Vickery & Vickery 1981).

Percentage of natural hybrids may be deduced if flavonoids biosynthesis is assumed to be additive (Vickery & Vickery 1981). By recombination, arising from species with differing flavonoids can synthesize compounds found in neither parent. The hybrid was exhibited intermediate characters i.e. exomorphic by this chemical recombination (Davis & Heywood 1973).

2.14.2.2. Terpenoids

Although terpenoids are present in wide range and they show many variation, but they have been used less extensively than flavonoids in taxonomy, possibly due to difficulty of analysis. However, they have been used extensively in the chemosystematics of some groups in which they occur (e.g. mints, umbellifers). Comparison of terpenoid content between plants has been facilitated by gas chromatography, and they have been used to clarify specific and subspecific taxa as well as geographic races and hybrids (Jones & Luchsinger 1987).

2.14.2.3. Alkaloids

A great deal of information on alkaloid occurrence is available and their distribution has contributed to taxonomic studies in various groups. However, these are chemically less stable than flavonoids, and are structurally and biosynthetically more complicated (Jones & Luchsinger 1987). Due to the lack of knowledge as to the relative advancement of the different biosynthetic pathways leading to the various skeletal types, little attempt has been made to compare different alkaloid types phylogenetically (Gershenzon & Mabry 1983). Their contribution to such classifications has thus been restricted. Exceptions among the alkaloids are the benzyl-

isoquinoline alkaloids, which are important taxonomic markers in the angiosperms. Gershenzon and Mabry (1983) attributed this to their biosynthetic uniformity and 'coherent distribution'.

2.14.2.4. Glucosinolates (Mustardoil glucosides)

These, together with the alkaloids, have been used to divide the four families comprising the old order Rhoeadales into two new orders: Capparales, containing the Cruciferae and Capparaceae which produce glucosinolates and Papaverales containing Papaveraceae and Fumariaceae, on the basis of alkaloid pattern. Research has shown that glucosinolate patterns may also be used to document hybridization as well as provide infrageneric characters (Jones & Luchsinger 1987).

2.14.2.5. Iridoids

This group of secondary metabolites is of increasing taxonomic importance (Gershenzon & Mabry 1983; Jones & Luchsinger 1987) and they show promise in the clarification of relationships at various levels. They have also contributed to solving the debate over the ancestral progenitor of the Asteraceae, which do not produce iridoids. Several putative progenitors' cases were weakened when they were shown to produce iridoids, leaving only the Campanulaceae, Araliaceae and Apiaceae, which do not produce iridoids (Jones & Luchsinger 1987).

2.14.3. Semantides

Semantides are the information carrying molecules. DNA is a primary semantide and RNA a secondary semantide, where proteins are tertiary semantides, following from the sequential transfer of the genetic code from the primary genetic information (DNA) (Swain 1963). Stace (1980) mentioned three main methods used in protein taxonomy: electrophoresis, amino-acid sequencing and systematic serology. Electrophoretic techniques enable proteins to be 'fingerprinted' their relative isoelectric point by separating them in variable gel mixtures across a voltage gradient (Stace 1980). Protein profiles produced *via* electrophoretic separation and subsequent staining have been used in various systematic studies investigating polyploid taxa, as well as at interspecific, intraspecific and population levels. Particular care and expertise are required in the use and interpretation by establishing size, charge and of protein profiles (Jones & Luchsinger 1987). Amino-acid sequencing attempts to establish the variation in the precise sequence of amino acids in a single homologous protein throughout a range of organisms (Stace 1980). Sometimes the semantides together with the larger polysaccharides are known as macromolecules and the primary and secondary metabolites as micromolecules (Pullaiah 2007). Most of the micromolecules are shown the antioxidant activity (Hakkim *et al.* 2008; Moura *et al.* 2007; Edreva *et al.* 2008).

So, chemotaxonomy has undoubtedly made a big contribution to taxonomic work in the past and will certainly continue to do so in future. However, given the lack of fossil evidence and the need for live material in some analyses it seems that its contribution to a phylogenetic classification must perforce remain limited. The valuable information it offers is best used in conjunction with other sources of taxonomic evidence and thus a multidisciplinary approach is required in order to establish a system of classification which reflects natural relationships as accurately as possible.

2.15. CHEMOTAXONOMIC IMPLICATION IN LAURACEAE

Economically Lauraceae is an important family of higher plants. But, it is difficult to identify the lauraceous species based on morphological features only, due to short flowering and fruiting period and genera

encompass many species which have shown same type of morphological appearances (Gomes *et al.* 1983). Errors of botanical identification could be prevented by using the information obtained from a comprehensive analysis of the chemical profiles of the putative species. So, crucial value of the chemotaxonomic approach can be seen here. Johns *et al.* (1969) studied an excellent work on the genus *Cryptocarya*. They identified l-benzyl-1,2,3,4-tetra-hydro-isoquinolinea alkaloid from the leaf extract of *Cryptocarya archboldiana* C.K. Allen and also reported several alkaloids from the genus *Cryptocarya* such as the phenanthroquinolizidine alkaloid cryptopleurine from *C. pleurosperma* C.T. White & W.D. Francis, the cryptowolline and dibenzopyrrocoline alkaloids cryptausoline from *C. bowiei* Druce, aporphine alkaloids from *C. angulata* C.T. White, *C. triplinervis* Brown and a benzylisoquinoline alkaloid from *C. konishii* Hayata. In 1989, Lajis *et al.* reported that the leaves of *Alseodaphne perakensis* (Gamble) Kostermans contain one major and a complex mixture of minor alkaloids. The chief component was identified as N-methyl-2,3, 6-trimethoxymorphinandien-7-one through spectroscopic analyses of the parent compound. A chemotaxonomic investigation was carried out with eight species of *Cinnamomum* Schaeffer as part of a biosystematic study on this genus occurring in the state of Kerala by Ravindran *et al.* (1992). They analyzed flavonoids, terpenoids and steroids of these species. The results signified the much chemical variability among those species. According to them chemically *Cinnamomum perrottetii* Meisner, *Cinnamomum verum* J.Presl and *Cinnamomum camphora* (Linnaeus) J. Presl were the most complex, whereas some collections of *Cinnamomum malabatrum* (N.L. Burman) J. Presl were the least complex. They also claimed that *C. camphora, C. verum, C. camphora, C. cassia* (Linnaeus) J. Presl and *C. riparium* Gamble are chemically very distinct. A large amount of infra-specific variability was observed in *C. malabatrum*. The diverse flavonoid pattern in this genus resulted from o-methylation which is considered as an advanced character and flavonols were found to be replaced by flavones. Flavonols and flavones are advanced characters in the evolutionary history of flavonoids. Joshi *et al.* (2009) examined the leaf terpenoid compositions in nine species of Lauraceae, *viz*., *Lindera pulcherrima* (Nees) Hooker *f.*, *Neolitsea pallens* (D.Don) Momiyama & Hara, *Persea duthiei* (King) Kostermans, *Dodecadenia grandiflora* Nees, *Persea gamblei* (King *ex* Hooker *f.*) Kostermans, *Parsea odoratissima* (Nees) Kostermans, *Cinnamomum tamala*, *C. camphora*, and *Phoebe lanceolata* (Nees) Nees collected from the Indian Himalayan region by GC, GC-MS, and NMR analyses in order to determine the similarities and differences among their volatile constituents. They established that Furano-esquiterpenoids were the principal constituents of *N. pallens, D. grandiflora,* and *L. pulcherrima*. Limonene, (E)-Nerolidol, a-pinene and b-pinene were the major constituents of *P. duthiei*; a-pinene, b-caryophyllene and sabinene were noticed predominantly in *P. odoratissima*, while the oils of *P. gamblei* and *P. lanceolata* possessed b-caryophyllene as common major constituent. *C. tamala* and *C. camphora* were marked by the existence of camphor and cinnamaldehyde, respectively. They also made cluster analysis of the oil composition within these nine species of six genera of Lauraceae. Phytochemical investigations on three Brazilian species of Lauraceae from the Cerrado region of Sao Paulo State, *Ocotea elegans* Mez and *O. corymbosa* (Meissner) Mez, *Persea pyrifolia* Nees & Martius *ex* Nees resulted in the isolation of an ester of the 4-*O*-*E*-caffeoylquinic acid, flavonoids, an aromatic sesquiterpene besides furofuran lignans (Batista *et al.* 2010**).** Four aporphine alkaloids from the wood of *Ocotea macrophylla* Kunth were isolated and characterized as (*S*)-3-methoxy-nordomesticine, (*S*)-*N*-formyl-3-methoxy-nordomesticine, (*S*) – *N* – ethoxycarbonyl – 3 – methoxy -nordomesticine and (*S*) – *N* – methoxycarbonyl – 3 - methoxy-nordomesticine; alkaloids 2-4 are reported for the first time by Pabon and Cuca in 2010. Perez *et al.* (2011) could able to differentiate eight Maxican *Litsea* species on the basis of their terpenoid substance. The terpenoids *viz*. 1,8-cineole,

linalool, á-pinene, â-pinene, mcymene, terpinen-4-ol, caryophyllene, á-terpineol and caryophyllene oxide are commonly found in all the *Litsea* species. From the hierarchical classification, three groups of species were recognized: (i) 1,8-cineole group (C-10 terpenes), consisting of *L. glaucescens* Kunth, *L. pringlei* Bartlett, *L. schaffneri* Bartlett and *L. muelleri* Rehder; (ii) limonene-rich group (C-10 oxygenated terpenes), including *L. neesiana* (Schauer) Hemsley and *L. guatemalensis* Mez, (iii) oxygenated sesquiterpenes-rich group (C-15 oxygenated terpenes), includes *L. parvifolia* (Hemsley) Mez.

2.16. SIGNIFICANCE OF ANTIOXIDANTS IN PHYLOGENETIC CLUSTERING

The early classifications of plants were artificial and served practical purposes. After Darwin, botanists proposed 'natural system' in which they were thrashing about for real relationships but it is yet to construct such a true 'phylogenetic system'. The main reason for this is that the vast majority of extinct species is unknown. The natural systems are based essentially on comparative studies of genetically controlled, morphological and anatomical characteristics of plants. Some of these characteristics are of a very general nature and serve for the separation of systemic categories, like divisions, classes, orders, families, genera, subgenera etc. Chemical contributions to plant classification are based on the chemical constituents of plants such as on their 'molecular characteristics'. These characteristics are genetically controlled and have the advantage over morphological characteristics that they can be very exactly described in terms of definite structural and configurational chemical formulae. The greatest virtue of the chemical method is that it is entirely independent of the classical biological methods. Very early in the development of natural products chemistry, many botanists and chemists characterized and classified plants on the basis of their chemical constituents (Smith 1978; Stace 1980). Moreover, botany and chemistry become more and more separated due to increasing specialization of their respective followers. With the growing knowledge of the structure the natural products and their occurrences in plants, it is obvious that the potentialities of chemotaxonomy is now becoming increasing. In 1992 Ravindran *et al.* clustered and made phylogenetic tree of *Cinnamomum* species according to the presence of flavonoids, triterpenoids and steroids which are the potent antioxidant compounds. Kim *et al*. (2009) proved that phenolic compounds, mainly flavonoids are naturally occurring antioxidant components produced by plants. In 2009, Mohy-Ud-Din *et al.* grouped five taxa of *Solanum* on the basis of percentage variation of the flavonoids using GC-MS analysis and 2,4-dinitrophenylhydrazine method. They stated that the significant distance found between *S. americanum* P. Miller, *S. chenopodioides* Lamarck, *S. nigrum* Linnaeus and *S. villosum* P. Miller indicated them as distinct species. But, the noticeable difference is absent in *S. retroflexum* Dunal and therefore hence it is considered as a variety or subspecies of *S. nigrum*.

2.17. ANTIOXIDANT MOLECULES AS CHEMOTAXONOMIC MARKER

Antioxidant molecules are used as marker in chemotaxonomy. For example, anthocyanins are a group of naturally occurring antioxidant compounds related to the colouring of plant parts like fruits and flowers. These pigments are important as quality indicators, as taxonomic markers and for their antioxidant ability. Paola *et al.* (2003) have investigated that the therapeutic efficacy of anthocyanins contained in blackberry extract (cyanidin-3-O-glucoside represents about 80% of the total anthocyanin contents) in an experimental model of lung inflammation induced by carrageenan in rats. Vaccination of carrageenan into the pleural cavity elicited an acute inflammatory response characterized by fluid accumulation which contained a large number of neutrophils as well as an infiltration of polymorpho nuclear leukocytes in lung tissues and subsequent lipid peroxidation, and increased generation of nitrite/nitrate (NOx) and

prostaglandin E2 (PGE2). All parameters of inflammation were prohibited by anthocyanins. The degree of stain accumulation was lowered by anthocyanins treatment. According to them, the anthocyanins contained in the blackberry extract exert multiple protective effects in carrageenan-induced pleurisy. Zarnowski *et al*. (2004) differentiated soft and hard wheat plants utilizing alk(en)ylresorcinols which is a chemotaxonomic marker. Ferrandino and Guidoni (2010) used phenolic compounds such as anthocyanins, flavonols, and hydroxycinnamates as taxonomical markers to discriminate *Vitis vinifera* Linnaeus cv. 'Barbera' clones. Through the analysis of phenolic profiling Siracusa *et al*. (2012) recommended that the secondary metabolites in "Long-storage" tomato (*Solanum lycopersicum* Linnaeus) fruits may be more genetics-dependent than environment-dependent.

A large number of reports concerning the antioxidant ability of essential oils of aromatic plants have been published (Bhargava *et al.* 2013). It is well known that compounds of essential oils are the outstanding marker of chemotaxonomy, therefore the antioxidant activity of these oils are easily used as chemotaxonomic marker. Baker and Smith (1920) worked on *Eucalyptus* spp. They noticed that primitive species are those which have feather-veined leaves and high Pinene content in their essential oils (terpenes), while more advanced types have intermediate venation and contain Pinene and Cineole (Pullaiah 2007). Recently, Basak and Chandan (2013), identified three components of essential oil of *Laurus nobilis* Linnaeus and noticed that these components have significant amount of antioxidant potentiality.

So, antioxidant molecules could be adopted as chemotaxonomic markers in the traceability of this niche product as well as these bioactive components are especially useful for both plant breeders and taxonomists to classify species or cultivars.

The family Lauraceae has been studied worldwide from the botanical and chemical standpoints. Terai and Duars is under the sub-Himalayan region, and its vegetation is enriched with many species of Laurels. Controversies about phylogeny and affinity of species as well as uncertain positioning of different genera are still under consideration. Many researchers showed that this group is enriched with versatile secondary metabolites and these natural products have a wide structural diversity and have been isolated in great scale from plant species; they can be used as taxonomic markers at hierarchical levels. Although there are some controversies on the usage of antioxidant molecules to solve taxonomic problems, it can be said from this study, since a large amount of data will be employed, some positive inferences might be made, when these markers will be utilized. So the utilization of a greater diversity of data will give us safer tools to elucidate at least some existing doubts in the near future concerning the chemical evolution of the family Lauraceae.

CHAPTER - 3
Study Area

Study Area

3.1. INTRODUCTION

The Northern part of the Indian State of West Bengal touching the feat of Eastern Himalaya is generally referred as Terai and Duars. The entire region is made up of sand, gravel and pebbles laid down by the Himalayan rivers like the Teesta, Torsa, Raidak, Jaldhaka, Sankosh and several other small rivulets. The river Teesta has divided the area into two parts, the western part is known as the Terai whereas the eastern part is known as the 'Duars' or 'Doors' (Ghosh 2006). The Terai means 'moist land' and *Duar* means 'door' in both Assamese and Bengali languages and form the gateway to Bhutan and to the North-eastern part of India.

The Terai and Duars are famous for the tea gardens, which were basically developed by the British and much extended after the British Raj left the country. The beauty of the region lies not only in its tea gardens but also in the dense jungles that make up the countryside. Famous wildlife sanctuaries and national park like Jaldapara National Park; Buxa National Park; Gorumara National Park; Chapramari Wildlife Reserve; Baikunthapur Reserve Forests and the Mahananda Wildlife Sanctuary are located in this region. The vegetation of Terai and Duars are very rich and covers all major groups of plants including several members of endemic and RET species (Chatterjee 1940; Das 1986; Kadir 2001; Ghosh & Das 2009). Also, this area is falling under biodiversity hotspot 'Himalaya' (Conservation International 2005). The wide diversity in habitat structure helped numerous plant species to settle in this area (Rai & Das 2008).

3.2. LOCATION AND BOUNDARY

Terai is located between 25° 57" to 26° 36" N latitude and 89° 54" to 88° 47" E longitude; whereas Duars is situated between 26° 16" to 27° 0" N latitude and 88° 4" to 89° 53" E longitude (Das *et al*. 2010; Roy *et al*. 2009). The Terai and Duars region politically constitute the plains of Darjeeling, whole of Jalpaiguri and Alipurduar District in West Bengal. This region is not marked by any natural features. However, its Northern frontier is bounded by Darjeeling hills and Bhutan; east by Assam; Bangladesh, North Dinajpur district of West Bengal and Kishanganj district of Bihar is on south; and in west is by Nepal. The slope of the land is gentle, from north to south. The altitude of this area ranges from 80 to 310 m. (Fig. 3.1 & Fig. 3.2).

3.3. DRAINAGE SYSTEM

Most of the rivers of Terai and Duars are eventually draining southwards. The most important river of Terai is the Tista, originated from the Zemu glacier of north Sikkim and finally flowing into the river Mahananda in Bangladesh (Ghosh 2006). Other important rivers of Terai include rain-fed rivers like Balason, Mahananda and Mechi (Kadir 2001). On the other hand, Torsa is the main river of Duars and other important ones are Jaldhaka, Diana, Karola, Murti, Raidak and Kaljani. It is running down from the Chumbi Valley in TAR (Tibet Autonomous Region of China), where it is known as Machu.

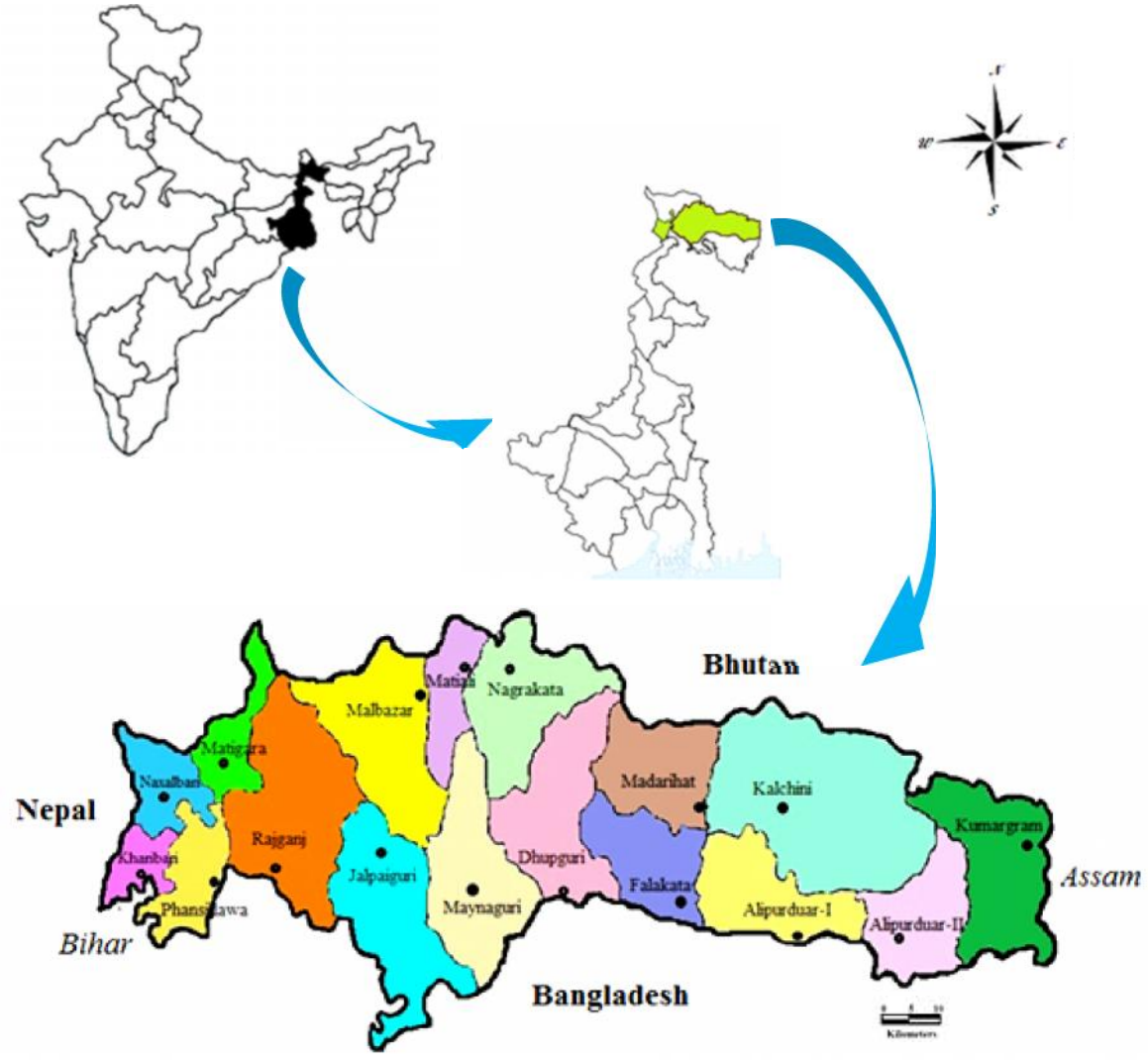


Fig 3.1. Block map of Terai & Duars

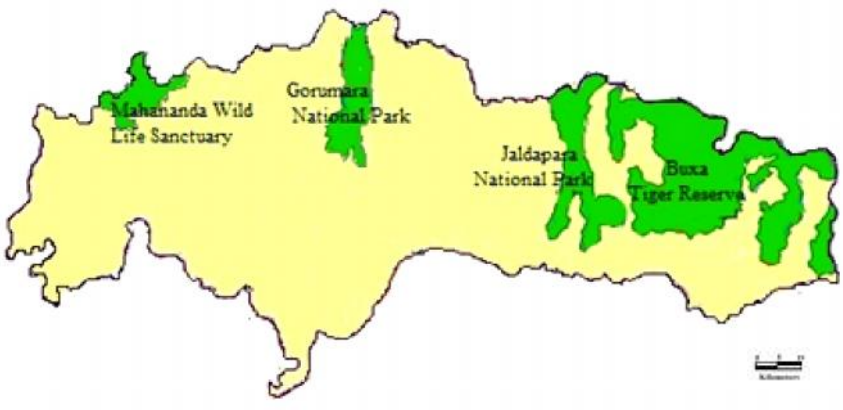


Figure 3.2. Protected areas of Terai & Duars

In addition, there are numerous small and considerable Jhoras or springs in Darjeeling hills. These springs are forming rivulets such as Magurmari, Lachka, Sanjoy etc. at the Sub-Himalayan Terai & Duars region. All these rivulets and their tributaries are rain-fed and remain almost dry in most of their portion when there is no rain in this area.

3.4. SOIL

The geological milieu in Terai and Duars represents the sub-Himalayan or the foothill zone consist almost entirely of the Siwaliks and typical formation of Quaternary and recent sediments (Banerjee *et al*. 2003). The soils are brought down by hill rivers like Tista, Torsa, Mahananda, Jaldhaka, etc. and their tributaries, bringing materials from a height of about 3048 m, and have deposited those layer by layer to form the soil of this area. The depth of the soil vary from 0 – 400 cm in different regions, with texture varying from fine sandy, rocky with moderate organic matter and low Phosphate, Potassium, and other micronutrient contents (State Forest Report 2006 – 2007). The pH of the soil of this region is acidic mainly due to heavy rainfall. The pH ranges between 5.6 to 6.5 in some parts and major portion showing highly acidic soil with pH below 5.5 (Kadir 2001).

3.5. CLIMATE

Climate is one of the basic rudiments in the natural environment. Vegetation of a region mainly depends on the prevailing climate of the area. It is generally defined as the average status of weather over long period. Four climatic seasons can be recognized in the Terai-Duars region and can be referred as Monsoon, Autumn, Winter and Summer. Being situated at the feet of the Himalayas this region experiences somewhat pleasant climate, with no extremes of temperature both in summer and winter (Kadir 2001; Das 1986; Bhujel 1996). The cool wind blowing from the Himalayas provides relief to an otherwise hot and humid climate of the tropical – sub-tropical belt.

3.5.1. Temperature

Temperature is the most significant factor of climate. As Terai-Duars lies in the shadows of Himalayas, it is relatively cooler than the central and southern regions of West Bengal. Temperature of this region varies from a recorded highest of 38°C during summer to about 6°C in the winter. The temperature usually starts increasing from the end of March, and days remain warm till the middle of October and then it falls rapidly throughout the region. In December and January it is colder and May to July it is rather hot when there is monsoon rains (Table 3.1) which helps to maintain a lower ambient temperature.

3.5.2. Precipitation

According to Sahni (1981) this factor is conducive for abundant vegetation. Rainfall of the area usually starts around the middle of April with the arrival of Nor' Westers or locally called 'Kalbaisakhi' and continues till the end of October (Kadir 2001). Rainfall mainly occurs due to south-western monsoon wind. June, July and August are the months of heavy rainfall. A large difference of rainfall can be examined in the climate of this region. The annual rainfall ranges from 2100 mm to 4000 mm in different years. The heavy rainfall occurs in the months of July and August (Table 3.2); extreme rainfall up to 200 mm per day has been recorded in the past (State Forest Report 2006 – 2007). Sometimes water logging occurs in some places of Duars.

Table 3.1. Month wise mean maximum and minimum temperature during 2008 – 2013 in the Study Area (*Source*: Department of Geography and Applied Geography, University of North Bengal)

Month	Mean Temperature (°C)	
	Maximum	Minimum
January	22.03	11.14
February	26.18	13.49
March	29.88	16.82
April	30.47	19.72
May	31.57	22.02
June	32.14	23.48
July	32.07	24.87
August	31.85	24.93
September	31.24	24.35
October	30.92	21.27
November	28.37	16.11
December	26.44	11.64

Table 3.2. Month-wise mean rainfall during 2008 – 2013 in the Study Area (*Source*: Department of Geography and Applied Geography, University of North Bengal)

Month	Mean Rainfall (mm)
January	16.82
February	24.34
March	44.87
April	154.29
May	272.43
June	652.23
July	708.38
August	504.02
September	366.17
October	202.60
November	16.07
December	14.22

However, the formation of dense fog especially during the winter months is a character almost for the entire region. The formation, sometimes, become so dense that it produce a zero-visibility condition. Dissolving of fog also add a good amount of moisture to the upper layers of soil that becomes much helpful for the occurrence and survival of a rich winter flora almost over the entire region.

Another character of the region of recurrence of flood over wide regions in different parts of the region, which affects in both way, bad and good. While some terrestrial vegetation are degraded in one hand, it also form temporary wetland hosting large number of ephemeral plant species and maintain perennial wetlands.

3.5.3. Relative Humidity

An important climatic characteristic of this region for the development of abundant vegetation in this region is the maintenance of higher Relative Humidity throughout the year. May to September, i.e. the period of heavy rainfall, is damp and humid with relative humidity ranging from 85 – 95%

(Table 3.3). But, during the end of winter it is comparatively less, being around 60 % in the morning and 45 % in the afternoon.

Table 3.3. Month-wise mean maximum and minimum relative humidity during 2008 – 2013 in the Study Area (*Source*: Department of Geography and Applied Geography, University of North Bengal)

Month	Relative Humidity	
	Morning	Evening
January	93.40	72.81
February	91.72	67.72
March	88.22	64.60
April	88.90	73.24
May	89.34	75.72
June	93.64	82.11
July	92.40	83.98
August	91.94	80.12
September	92.30	81.23
October	91.17	77.68
November	90.79	72.15
December	92.32	69.56

3.6. VEGETATION

Due to suitable climatic factors and soil characters, the Terai-Duars region represents one of the richest botanical regions in India. So, several plant surveyors both from India as well as from distant countries (Gamble 1878; Hooker 1886; Prain 1903; Brandis 1906; Cowan & Cowan 1929; Champion & Seth 1968; Sikdar 1984; Banerjee 1993) have frequently studied the vegetation of this region. Among them, Gamble (1878) has classified the vegetation of Terai and Duars into four types, Sal forest, Khair and Sissu forest, Savannah forest and Mixed plains forest.

According to Champion & Seth (1968) Vegetation of this region is mainly Northern Tropical Semi-Evergreen and North-Indian Tropical Moist Deciduous forest type, which is further classified into four sub types: Wet Sal forest, Riverine forest, Dry mixed forest and Wet mixed forest

3.6.1. Wet Sal forest

Wet Sal vegetations are mostly tropical forests. *Shorea robusta* Gaertner is the dominant species of this vegetation. Additional associates of these kinds of forests include *Duabanga grandiflora* (DC.) Walpers, *Lagerstroemia parviflora* Roxburgh, *Litsea salicifolia* (Roxburgh *ex* Nees) Hooker *f., Sterculia villosa* Roxburgh, *Terminalia bellirica* (Gaertner) Roxburgh, *Schima wallichii* Choisy etc. Besides those, some shrubby species like *Asparagus racemosus* Willdenow, *Clerodendrum infortunatum* Linnaeus, *Phlogacanthus thyrsiflorus* Nees, *Coffea benghalensis* Heyne *ex* Schultesetc. and few grasses like *Centotheca lappacea* (Linnaeus) Desvaux, *Microstegium ciliatum* (Trinius) A. Camus etc. are quite common.

3.6.2. Riverine forest

The Riverine forests are found in small patches in the elevated river-beds and in the lands raised after shifting of rivers. The vegetation remain occupied by grasses and perennial plants mostly shrubs and

climbers. Main grass species of these forests are *Phragmites karka* (Retzius) Trinius *ex* Steudel, *Saccharum arundinaceum* Retzius, *Saccharum spontaneum* Linnaeus, *Themeda villosa* (Lamarck) A. Camus etc. Whereas *Buddleja asiatica* Loureiro, *Clerodendrum japonicum* (Thunburgh) Sweet, *Clerodendrum infortunatum* Linnaeus etc. are dominating shrubs of such vegetation. Some common species of trees like *Acacia lenticularis* Bentham, *Albizia lebbeck* (Linnaeus) Bentham, *Bischofia javanica* Blume, *Bombax ceiba* Linnaeus with *Acacia catechu* (Linnaeus *f.*) Willdenow and *Dalbergia sissoo* A.P. de Candolle occur as distinct patches in these forests.

3.6.3. Dry mixed forest

Though the annual precipitation is high and with prevailing high atmospheric humidity for most of the period, the dominance of deciduous species of trees is quite prominent in these foothill vegetation formations. This type of vegetation is characterized by the presence of deciduous trees like *Artocarpus lacucha* Buchanan-Hamilton, *Bombax ceiba* Linnaeus, *Cryptocarya amygdalina* Nees, *Gmelina arborea* Roxburgh, *Lagerstroemia parviflora* Roxburgh, *Litsea glutinosa* (Loureiro) C.B. Robinson, *Litsea salicifolia* (Roxburgh *ex* Nees) Hooker *f.*, *Sterculia villosa* Roxburgh, *Wrightia arborea* (Dennstedt) Mabberley, etc. The undergrowth flora includes *Eragrostis unioloides* (Retzius) Nees *ex* Steudel, *Lepidagathis incurve* Buchanan-Hamilton *ex* D. Don, *Lygodium flexuosum* (Linnaeus) Swartz, *Solanum indicum* Linnaeus, *Urena lobata* Linnaeus, etc.

3.6.4. Wet mixed forest

Just opposite of the Dry Mixed Forests, the Wet Mixed Forest is dominated by evergreen and semi-evergreen trees along with a very low frequency of deciduous trees. In addition a large number of shrubs, climbers and herbs are inhabitants in this type of forested vegetation. This zone is also rich in epiphytes and stem-parasites. The major trees of these forests include *Actinodaphne obovata* (Nees) Blume, *Castanopsis tribuloides* (Smith) A. DC., *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet, *Cryptocarya amygdalina* Nees, *Knema erratica* (Hooker *f.* & Thomson) Sinclair, *Litsea glutinosa* (Loureiro) C.B. Robinson, *Litsea monopetala* (Roxburgh) Persoon, *Machilus duthiei* King, *Mesua ferrea* Linnaeus, *Terminalia myriocarpa* Heurck & Mueller, *Syzygium cumini* (Linnaeus) Skeels etc. This type of forest is characterized by the presence of a good number of climbers (some of those are liana) such as *Argyreia roxburghii* (Wallich) Arnott *ex* Choisy, *Bauhinia vahlii* Wight & Arnott, *Mikania micrantha* Kunth, *Tetrastigma planicaule* (Hooker *f.*) Gagnepain, *Thunbergia grandiflora* (Roxburgh *ex* Rottler) Roxburgh, *Tinospora sinensis* (Loureiro) Merrill, etc. The ground cover vegetation is also very rich, which include annual and perennial herbs, root parasites, saprophytes etc. like *Ageratum conyzoides* (Linnaeus) Linnaeus, *Blumea balsamifera* (Linnaeus) A.P. de Candolle, *Commelina benghalensis* Linnaeus, *Oxalis corniculata* Linnaeus, *Urena lobata* Linnaeus, *Triumfetta rhomboidea* Jacquin, etc.

3.7. LAUREL HABITAT

Laurels are mostly terrestrial plants and its different species, mainly shrubs and trees, occur both inside and outside the forested vegetation. Quite often Laurels grow in completely open condition or in grasslands. Savannah type of grasslands are common in the Duars and some species of Lauraceae found to raise their umbrella like top portions over such grasses.

CHAPTER - 4

Materials and Methods

Materials and Methods

4.1. FLORISTIC SURVEY

4.1.1. Collection and Preservation of Materials

The Laurel flora of Terai-Duars region has been prepared through random sampling in three different season *viz.* winter, pre-monsoon and post-monsoon for five consecutive years, 2008 to 2012. Several places in Terai-Duars region, mainly Wildlife Sanctuaries and National Parks like Mahananda Wildlife Sanctuary, Gorumara National Park, Buxa Tiger Reserve, Chapramari Wildlife Sanctuary, Raja Bhatkhawa Reserve Forests and Jaldapara National Park were visited regularly for the collection of all relevant field data including local uses, time of flowering, fruiting etc.

During numerous field trips, round the year, mainly healthy twigs with flowers and /or fruits and generally with leaves were collected in triplicates. In case, flowering and/or fruiting twigs were not available, specimens with healthy and mature leaves were collected. The specimens were tagged and recorded in the *Field Note Book*, and temporarily kept in air tight polythene pouches. On return to the field camp or to the laboratory specimens were cleaned, trimmed and 10% formalin were added at nodes and other soft joints to save the specimens from shattering. Then, the specimens were transferred to a heavy wooden plant press between blotting papers or old newsprints for drying. After proper drying specimens were poisoned by soaking with saturated solution of Mercuric Chloride [generally 4 – 6%] in rectified ethanol and then dried again using blotters. After proper drying and poisoning, specimens were mounted on standard herbarium sheets, labeled and temporarily stored in the Taxonomy & Environmental Biology Laboratory in the Department of Botany of the University of North Bengal. After completion of the work, the main set of herbarium specimens will be deposited at NBU-Herbarium and the duplicates will be deposited CAL. However, all these works were guided by the methodology provided by Jain & Rao (1977).

4.1.2. Field Note Book

The records in the Field Note Book covers specific locations, altitude, dates of collections, availability, habit, habitat, flower colour and such other characters of plants which are not available with dry and mounted herbarium specimens. The field notes were transferred to herbarium labels for ready reference. After completion of the work, the *Field Note Book* was deposited at the NBU-Herbarium.

4.1.3. Identification

The specimens were primarily identified in the Taxonomy & Environmental Biology Laboratory of the Department of Botany, North Bengal University using available literature including Hooker (1886), Brandis (1906), Kanjilal *et al.* (1940), Momiyama (1966), Long (1984), Ara *et al.* (2007), Li *et al.* (2008a) and matching with the available predetermined specimens at the NBU-Herbarium. Finally, for confirmation, specimens were matched at CAL.

4.1.4. Flowering and fruiting calendar

The flowering calendar were prepared by regular monitoring of the vegetation and recording the flowering and fruiting time of different species. All data were derived from direct observation only as the flowering period varies in different species with the change of physiography, altitude, longitude, latitude etc. For detailed methodology Das & Chanda (1987) and Panda *et al.* (1992) have been followed in general.

4.1.5. Enumeration and Description

Different recorded species of Laurels are enumerated below with genera and species under different genera in alphabetic order. For each species the correct name is followed by basionym, if any, and other available or important synonyms. Proper author citation, protologue references and references to the record in other relevant floras of nearby regions are also cited. This is followed by the local name(s). A short description of each species has been given. To cite the voucher specimen, the place of collection with altitude, collector's names, field number and date of collection have been provided separately for the specimens collected from different places. Ranges of flowering and fruiting time have been given. Local distribution provided in the enumeration are based both on collected specimens and observation made during the field trips and is not fully based upon available literature. On the other hand, information related to the general distribution have been determined from published literature and deposited specimen from the different herbaria visited. At the end, a *note* has been provided which mainly covers the local uses and any other interesting observation made during the field study.

4.2. ANATOMICAL STUDIES

4.2.1. Plant samples

For the present study eight species representing two genera of Lauraceae were chosen. Plants were collected from different places in Terai-Duars region of West Bengal. The collected materials and voucher number are given in table no 4.1.

Table 4.1. Laurels with voucher number collected for the anatomical study

Species	**Voucher no. [***Dibakar Choudhury & AP Das]*
Cinnamomum bejolghota (Buchanan–Hamilton) Sweet	064
Cinnamomum camphora (Linnaeus) J. Presl	175
Cinnamomum tamala ((Buchanan–Hamilton) Nees & Ebermaier	006
Cinnamomum verum J. Presl	003
Litsea assamica Hooker *f.*	095
Litsea glutinosa (Loureiro) Robinson	026
Litsea laeta (Nees) Hooker *f.*	090
Litsea monopetala (Roxburgh) Persoon	109

4.2.2. Anatomical work

The materials for anatomical study were fixed in FAA (Formaldehyde: Acetic Acid: Alcohol, 1:1:18 v/v) for 24 hours and then preserved in 70% ethanol (Johansen 1940).All observations were performed on

transverse sections of well-developed stem, petiole and lamina with mid-vain taken by hand. Double staining method was used for this study (Santra *et al*. 1989; Maji 2004). The fine thin sections were dehydrated and double-stained with safranin and light-green through ethanol grades. The cell wall (cutinized / lignified wall) took the colour of safranin; whereas soft tissues were stained with light green and were mounted in Canada balsam (Santra *et al.* 1989; Maji 2004). The sections were studied and photographed from permanent slides with a digital camera attached to an Olympus BX51 light microscope. All measurements and observations were made three times and expressed in micrometer.

All permanent slides were deposited in the Taxonomy and Environmental Biology Laboratory of the Department of Botany, University of North Bengal.

4.3. LEAF ARCHITECTURE

4.3.1. Plant samples

Leaf architectural study has been carried out with same species as selected for anatomical study i.e. *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet, *Cinnamomum camphora* (Linnaeus) J. Presl, *Cinnamomum tamala* ((Buchanan–Hamilton) Nees & Ebermaier, *Cinnamomum verum* J. Presl, *Litsea assamica* Hooker *f., Litsea glutinosa* (Loureiro) Robinson, *Litsea laeta* (Nees) Hooker *f.* and *Litsea monopetala* (Roxburgh) Persoon.

4.3.2. Clearing of leaves

Entire mature leaves were immersed in 10% NaOH at room temperature until soft tissue was discolored as well as dissolved and leaves become fully transparent. Most of the leaves were cleaned within 25 – 30 days, depending upon their thickness. After putting out from the solution, the leaves were washed thoroughly with distilled water to remove the sodium hydroxide and then cleared with a brush. In case the leaves remained opaque after clearing it was then boiled with lactic acid to achieve the desired level of clearing (Lama 2004). After clearing specimens were stained with 2% saffranin prepared in 70% ethanol. The excess stain was washed out with 70% ethanol and finally mounted between two glass plates with DPX mountant (Foster 1952).

4.3.3. Study of Venation

4.3.3.1. Major Venation pattern

The first step in describing venation is to recognize the first two orders of veins. In general the primary and secondary veins are the major structural veins of the lamina which can be easily recognized with naked eye or through a simple magnifying lens. The mid-vein or primary vein is the thickest vein of the lamina and thickness decreases gradually toward the apex. This also includes the study of 2^o vein spacing, 2^o vein angle, inter 2^o veins (veins similar to 2^o s and do not reach the margin), and agrophic veins (comb like complex comprising of lateral 1^o or 2^o veins).

4.3.3.2. Minor venation pattern

The highest orders of veins were identified up to 5° in all cases. Minor venation patterns included several microscopic studies like 3^o vein category, 3^o vein course, 3^o vein angle variability, 3^o vein angle to 1^o, 4^o and 5^o vein categories, marginal ultimate venation etc. Lamina with a vein that form high number of discrete orders or that has regular courses, is considered to be more organized or 'higher rank' leaves. Leaf rank is a semi-quantitative description of the leaf venation system.

For minor venation pattern study the stained segments were observed under 5X objective of a compound microscope using a 10X eye piece. Camera Lucida drawings were made from apical, middle and basal portion including the mid-vein and other tertiary veins. Marginal venations were also studied in this manner. Descriptions were made following the scheme of Hickey (1973) and Leaf architecture working Group (1999).

4.3.4. Study of Areole (vein-islet) & F.E.Vs frequency

Areoles or vein islets are smallest areas of the leaf tissue bounded by veins. These may be of different shape and considered as a significant tool in recognizing a species. Any order of venation can form one or more sides of an areole. F.E.Vs is the freely ending ultimate veins of the lamina. Both, areoles and F.E.Vs can provide important data of taxonomic significance.

Using a stage micrometer and Camera Lucida a rectangular area was made within which areoles along with the mid-vein were drawn. The exact area of the rectangle was measured through proper calculation of magnification. For each species three such drawing were made comparing of one from each of the apex, median and basal portion of the lamina. Frequencies were determined through the following formulas (Chatrath 1992):

Areolar frequency = no. of areoles/mm^2 area

F.E.Vs frequency = no. of F.E.Vs/mm^2 area

In calculation, two incomplete areoles were considered as a complete areole.

4.3.5. Study of Indumentum

For the determination of the location and type of indumentums, fresh lamina and petioles were observed under the low and then high power objectives of the compound microscope. The measurements were made to utilizing the ocular micrometer after proper standardization of the microscope.

4.3.6. Study of Stomata

Several techniques were followed for stomatal study, *viz*-

1. Peeling of lamina mainly from dorsal surface (as stomatal density is much higher in lower surface) with the help of forceps and mounted in 10% glycerin for observation.
2. In impression technique, colorless nail polish was used as an impression material. Impressions of foliar epidermal cells were taken by smearing the nail-polish on the dorsal surface of the leaf and it was allowed to dry completely. Then the thin impression layers were taken out from leaves and placed on glass slides. In this way temporary slides were prepared and studied under compound microscope.
3. In case of thick leaves (such as *C. tamala*, *C. verum*, *C. bejolghota*) where peeling method and impression technique were not producing appreciable results, there the scrapping technique was used. The upper epidermis and mesophylls were scrapped out with the help of a scalpel or a sharp blade. Then the scrapped pieces were dipped into the FAA solution (formalene, glacial acetic acid and 50% ethanol in 1:1:18 ratio) for few minutes. After that, it was placed on clean glass slide and mounted with 10% glycerin. Slides were then studied under the compound microscope.

4. In some cases, leaf samples were boiled approximately for 15 minutes in 15 ml of 10% aqueous HNO_3. After that samples were dipped in lactic acid for 2 minutes to become transparent and finally with the help of forceps small portion was taken out and mounted with 10% glycerin on glass-slide for observation.
5. Boiling technique was also attempted with absolute alcohol till the sample becomes colorless followed by lactic acid treatment for becoming transparent and observed under compound microscope.

Determination of stomatal type was made following the scheme of Leaf architecture working Group (1999). Finally Camera Lucida drawings were made using high power objective. Stomatal Index and Stomatal Frequency were determined through the following formula along with proper measurements (Salisbury 1927):

$$\textbf{Stomatal index (SI)} = \frac{\text{Total number of stomata in each field}}{\text{Number of stomata + Number of Epidermal cells in the field}} \times 100$$

$$\textbf{Stomatal frequency} = \frac{\text{Total number of stomata in each field}}{\text{Area of the field in mm}}$$

4.4. ANTIOXIDANT BASED CHEMOTAXONOMIC APPROACH

4.4.1. Plant samples

Antioxidant activities has been carried out with same species i.e. *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet, *Cinnamomum camphora* (Linnaeus) J. Presl, *Cinnamomum tamala* ((Buchanan–Hamilton) Nees & Ebermaier, *Cinnamomum verum* J. Presl, *Litsea assamica* Hooker *f.*, *Litsea glutinosa* (Loureiro) Robinson, *Litsea laeta* (Nees) Hooker *f.* and *Litsea monopetala* (Roxburgh) Persoon.

4.4.2. Preparation of methanolic plant extracts

Different fresh leaves and barks were surgically separated and were separately crushed with mortar and pestle. Under a soxhlet extractor, crushed samples were individually extracted with methanol for 8h. The methanol was completely removed by vacuum rotary evaporator at 50°C. These crude extracts were freeze-dried. The powder was stored at 4°C and used for further investigation. The extractive value of the plant materials were calculated on dry weight basis from the formula given below:

$$\text{Percent extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

4.4.3. Animal material

Goat liver, used for anti-lipid peroxidation assay, were collected from slaughter house immediately after slay and experiment was conducted within one hour after collection.

4.4.4. Determination of Antioxidant

4.4.4.1. DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-1- picrylhydrazyl) was determined spectrophotometrically. The changes in colour of DPPH free-radical (from deep-violet to light-yellow) were measured at 517 nm wavelength in presence of antioxidants. Radical scavenging activity of extracts was measured by standard method proposed by Blois (1958). Two microliters of each sample, prepared at various concentrations were added to 2 ml of 0.2 mM DPPH solution. The mixture was shaken and allowed to stand for 30 min at 20ºC in dark condition and then the absorbance was measured at 517 nm with UV-VIS spectrophotometer (Systronics, 2201). The percentage inhibition activity was calculated by the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample}) / A_{control} \times 100]$$

Where, $A_{control}$ is the initial concentration of the stable DPPH radical without the test compound and A_{sample} is the absorbance of the remaining concentration of DPPH in the presence of methanol. IC_{50} values (mg/ml) were determined from a plotted graph of scavenging activity against the concentrations of the extracts, where IC_{50} is defined as the total amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

4.4.4.2. Superoxide anions scavenging activity

The superoxide anions generated by phenazine methosulphate (PMN) and reduced nicotinamide-adanine dinucleotide phosphate (NADPH), were detected by the reaction with 2,2´-di-*p*-nitrophenyl-5,5´-diphenyl-(3,3´-dimethoxy-4,4´-diphenylene)di-tetrazoliumchloride, nitroblue tetrazolium (NBT) (Nishikimi *et al*. 1972). Reaction mixture contained 1 ml samples (different concentration), 1 ml of NBT solution (312 µM prepared in phosphate buffer, pH-7.4) and 1 ml of NADH solution (936 µM prepared in phosphate buffer, pH-7.4). Finally, the reaction was accelerated by adding 100 µl PMS solution (120 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixture was incubated at 25ºC for 5 minutes and absorbance at 560 nm was measured against methanol as control. Percentage inhibition and IC_{50} value was calculated using the same formula mentioned above.

4.4.4.3. Nitric oxide activity

Nitric oxide was generated from sodium nitroprusside and was measured by the Greiss reaction (Marcocci *et al*. 1994). In reaction 320 µl methanolic plant extract, 360µl (5mM) sodium nitroprusside-PBS solution, 216 µl Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylene diamine dihydrochloride) was sequentially mixed and incubated at 25ºC for one hour. Lastly 2 ml water was added and absorbance was taken at 546 nm. The IC_{50} value was calculated by the same procedure mentioned above.

4.4.4.4. Metal chelating activity

The chelating activity of the extracts for ferrous ions (Fe^{2+}) was measured according to the method of Dinis *et al.* (1994) with slight modification. To 0.4 ml of methanolic extract, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_2$ (2 mM). After 30 seconds, 0.8 ml ferrozine (5 mM) was added. Then, after 10 min at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated by using the same formula mentioned above.

4.4.4.5. Reducing power

One millilitre of plant extract, 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide (1% w/v) were incubated at 50º C for 20 minutes. The tube was cooled on ice and 2.5 ml 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 minutes to collect the upper layer of solution (2.5 ml) and mixed with distilled water (2.5 ml) and 0.25 ml of $FeCl_3$ (0.1% w/v). Finally, the absorbance was measured at 700 nm against blank sample (Aiyegoro & Okoh 2009).

4.4.4.6. Anti-lipid peroxidation (ALP) assay

The anti-lipid peroxidation activity of the extracts of plants was determined by the standard method followed by slight modification with the goat liver homogenate (Bauchet & Barrier 1998). 2.8 ml of 10% goat liver homogenate, 0.1ml of 50 mM hydrated ferrous sulphate and 0.1 ml extract was mixed. This mixture was incubated for 30 minutes at 37ºC. 1 ml of reaction mixture was taken with 2 ml 10% trichloroacetic acid (TCA) -0.67% thiobarbituric acid (TBA) in acetic acid (50%) for blocking the reaction. Then the mixture was boiled for 1hour at 100ºC and centrifuged at 10,000 rpm for 5 minutes. Supernatant was taken for absorbance at 535 nm. BHT was used for standard. ALP % was calculated by using the following formula:

$$\text{ALP percent} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of control}} \times 100$$

4.4.5. Estimation of Phytochemicals

4.4.5.1. Total phenol estimation

Total phenolic compounds of plant extracts were determined by Folin-Ciocalteu method (Folin & Ciocalteu 1927). For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2 and 0.3 mg/ml methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocalteu reagent (10 times diluted) and 4 ml sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 hour at 20º C and the calibration curve was drawn. 1 ml methanolic fruit extracts (50 mg/ml FWT) was mixed to the same reagent and the mixture was incubated for one hour in room temperature. After 1 hour the absorbance was measured at 765nm.

4.4.5.2. Total flavonoids determination

Spectrophotometric aluminium chloride method was used for flavonoids determination (Sultana *et al*. 2009). Each methanolic leaf and bark extracts (0.5 ml of 100 mg/ml FW) were separately diluted with 4 ml double distilled water. Then the diluted extracts were mixed with 5% (0.3 ml) $NaNO_2$ and 10% aluminium chloride were then added with reaction mixture. After 6 minute 2 ml (1.0 M) NaOH and 2.4 ml double distilled water was added and mixed well. Thereafter, absorbance of the reaction mixture was measured at 510 nm in spectrophotometer. Standard solution of quercetin (0-500 mg L^{-1}) was used as calibration curve.

4.4.6. Phytochemical evaluation of the crude extracts:

The methanolic crude extract (200 mg/ml) of the plant was subjected to various chemical tests in order to determine the secondary metabolites present by employing the use of various methods as follows:

4.4.6.1. Test for resins

0.5ml of extract was evaporated and dissolved in 2ml of petroleum ether; 2ml of 2% cupper acetate solution was then added and the mixture was shaken vigorously and allowed to separate; a green colour indicated the presence of resin (Trease & Evans 1983).

4.4.6.2. Test for amino acid

0.5 ml methanolic plant extracts were treated with few drops of ninhydrin reagent, heated in water bath, a purple colour indicated the presence of amino acids (Kumar *et al*. 2009).

4.4.6.3. Test for anthraquinones

1ml methanolic plant extracts were evaporated and dissolved in 2ml chloroform. 2ml of ammonia was then added. Occurrence of red colour suggested the presence of anthraquinones (Kumar *et al*. 2009).

4.4.6.4. Test for tannin

0.5 ml methanolic extract of each plant part was added with 0.5 ml 1% lead acetate; a yellow colour precipitation indicated the presence of tannin (Kumar *et al*. 2009).

4.4.6.5. Test for triterpenoids

0.5 ml of methanolic plant extracts were evaporated and dissolved in 1ml chloroform. 1ml acetic anhydride was then added and chilled. After cooling, conc. H_2SO_4 was added. If reddish violet colour appeared, the existence of triterpenoids was confirmed (Kumar *et al*. 2009).

4.4.6.6. Test for alkaloids

0.5 ml of each plant extract was added with 0.2 ml of 36.5% hydrochloric acid and 0.2 ml Dragendorff's reagent. Production of orange precipitation denoted the presence of alkaloids (Kumar *et al*. 2009).

4.4.6.7. Test for glycosides

0.5 ml methanolic extracts of plant were added with 2 ml of 50% hydrochloric acid. The mixtures were hydrolyzed for 2 hrs on a water bath. After that, 1 ml pyridine, few drops of 1% sodium nitroprusside solution, and 5% sodium hydroxide solution were added. Pink to red colour designated the presence of glycosides (Kumar *et al*. 2009).

4.4.6.8. Test for steroid

0.5 ml methanolic extracts were evaporated and dissolved in 2 ml chloroform. 2ml of conc. H_2SO_4 was introduced carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroid (Kumar *et al*. 2009).

4.4.6.9. Test for cardiac glycoside

0.5 ml of methanolic plant extracts were evaporated and dissolved in 1 ml glacial acetic acid. One drop of 10% ferric chloride was then added. 1 ml of conc. H_2SO_4 was added by the side of the test tube. Appearance of brown colour ring at the interface indicated of presence of cardiac glycosides (Ngbede *et al*. 2008).

4.5. CHEMOTAXONOMY THROUGH ANTIOXIDAT ACTIVITY OF ESSENTIAL OIL

4.5.1. Plant samples

Antioxidant activities of the essential oil has been carried out with same species i.e. *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet, *Cinnamomum camphora* (Linnaeus) J. Presl, *Cinnamomum tamala* ((Buchanan–Hamilton) Nees & Ebermaier, *Cinnamomum verum* J. Presl, *Litsea assamica* Hooker *f.*, *Litsea glutinosa* (Loureiro) Robinson, *Litsea laeta* (Nees) Hooker *f.* and *Litsea monopetala* (Roxburgh) Persoon.

4.5.2. Extraction of essential oils

The barks of each Laurals (200 g) were placed in a round-bottom flask with 1 litre of deionised water. The solution was steam distilled at 55°C for 3 hrs under reduced pressure. The distillate (900 ml) was extracted with 100 ml of dichloromethane for 6 hrs. After that the extract was dried with anhydrous sodium sulphate. The distillation was stopped when the volume of extract was reduced to approximately 1 ml, and then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.2 ml (Lee & Shibamoto 2000)

The extractive value of the plant materials were calculated on dry weight basis from the formula given below:

$$\text{Percent extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

4.6. TLC BASED CHEMOTAXONOMIC APPROACH

4.6.1. Plant samples

Thin layer chromatography has been carried out with same species i.e. *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet, *Cinnamomum camphora* (Linnaeus) J. Presl, *Cinnamomum tamala* ((Buchanan–Hamilton) Nees & Ebermaier, *Cinnamomum verum* J. Presl, *Litsea assamica* Hooker *f.*, *Litsea glutinosa* (Loureiro) Robinson, *Litsea laeta* (Nees) Hooker *f.* and *Litsea monopetala* (Roxburgh) Persoon.

4.6.2. Extraction of the plant samples

For extraction of different secondary metabolites bark of eight Laurels were used. Bark specimens were surgically separated from plants, washed thoroughly and one gm of each bark material was weighted separately and extracted with 5ml of methanol for 10 min. Methanolic filtrate was concentrated through vacuum rotary evaporator for application on TLC plates.

4.6.3. Extraction of essential oil

Essential oil was extracted by using the method proposed by Lee and Shibamoto (2000). Bark of eight Laurels (200 g) was placed in a 3 litre round-bottom flask with 1 litre of distilled water. The solution was steam distilled at 55 ºC for 8 hrs. Then, 900 ml distillate was fractionated with 100 ml of dichloromethane for 6 h. After the dichloromethane extract was dried over anhydrous sodium sulphate, the solvent was removed until the volume was reduced to 2 ml.

4.6.4. Thin layer chromatography (TLC)

Methanolic bark extracts of each Laurals were subjected to qualitative phytochemical detection as well as DPPH based antioxidant fingerprint through TLC (Wagner *et al.* 1984). It was performed by using silica gel-60 F_{254} chromatographic plates of 8cm x 2cm with 3mm thickness to confirm the presence of secondary metabolites. For the separation of phytochemical compounds, the methanolic bark extracts were spotted manually using micro-pipette. The spotted plates were put in a solvent chamber which contained various solvent systems to detect the suitable mobile phase. After the separation of phytochemicals, various spray reagents specific for detection of special class of secondary metabolites were used to identify the compounds (Wagner & Bladt 1996). The colour of the spots was noted and hR_f values were calculated by using the following formula:

$$\text{Retention factor } (hR_f) = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled the solvent}} \times 100$$

4.7. STATISTICAL ANALYSIS

The data were pooled in triplicate and subjected to analysis of correlation co-efficient matrix using SPSS (Version 12.00, SPSS Inc., Chicago, IL, USA) for drawing the relation between different types of antioxidant attributes and MS Excel 2007 (Microsoft, Redmond, WA, USA) was used for comparing the antioxidant attributes. Different group means were compared by Duncan's Multiple Range Test (DMRT) through DSAASTAT software (version 1.002; DSAASTAT, Peruglia, Italy); $p < 0.05$ was considered significant in all cases. The software package Statistica (Statsoft Inc., Tulsa, OK, USA) was used for analysis of other data. Smith's Statistical Package version 2.5 (prepared by Gary Smith, CA, USA) was used for determining the IC_{50} values of antioxidants and their standard error of estimates (SEE). Thin layer chromatography hR_f values were done through MS-Excel. In order to examine and visualize relationships between different plants in accordance with the presence of phytochemicals and antioxidant capacity, dendrogram were drawn by XLSTAT 2009 software (Levei *et al.* 2013).

CHAPTER - 5
Floristic Survey

Floristic Survey

5.1. INTRODUCTION

Vegetation is the most valuable gift of nature which provides us all kinds of essential requirements for our survival, including food, fodder, medicine, fuel, timber, resins, oils etc. Natural resources survey like floristic study plays important role in the economic improvement of developing country (Ganorkar & Kshirsagar 2013). Beside this, floristic study of a particular region is also produce the picture of natural assemblage of plants, which include total information on numbers of family, genus and species, dominant genera, dominant families and major life-forms occupying a particular habitat (Sasidharan 2002). Likewise, knowledge of the floristic composition of any place is the necessary pre-requisite for the study of various ecosystems. So, floristic study is one of the most important requisites, not only from the taxonomic view point but also to increase of our knowledge and surroundings and also for the benefit of science and the society.

During recent years there has been a revival of interest in floristic studies in different regions in India, especially after knowing that it is one of the seventeen mega diversity countries of the world. Even today, large number new species has been described from various parts of our country (Das 1995, 2004; Eshuo & Chaturvedi 2011; Chowdhury *et al*. 2013a; Rai & Das 2013). This is an expression of many aspects including (i) insufficient previous survey, (ii) processes of evolution are still in progress, and (iii) floristic elements of vegetation changes with time. Occurrences of exotic species in a flora/ vegetation have some impact that in most cases become derogatory for the local species. But, several exotics are gradually naturalizing and are spreading in different parts of India (Das 2002; Chowdhury *et al.* 2013b). On the other hand many native species are gradually becoming endangered and even getting extinct in different floristically rich regions (Das 1986, 2004; Kadir 2001). Further, a large number of species are also extending their distribution in different geographical areas (Kumar *et al*. 2011; Moktan *et al*. 2012). As result of such phenomena the number and the identity of species included in earlier floras are now changing rapidly. This is why it is essential to explore the flora of a region in regular interval. On the other hand, many floristically important regions are not yet explored or quite under explored and this is the high time to explore such areas intensively.

Terai and Duars regions of West Bengal are falling under the IUCN recognized 'Himalaya Biodiversity Hotspot' (Conservation International 2005; Das 2004, 2011, 2013). The vegetation of Terai and Duars are very rich and covers all major groups of plants (Chatterjee 1940; Das 1986; Kadir 2001; Ghosh 2006; Ghosh & Das 2009). Unfortunately, no any complete account of flora is available for this region. So, it is now imminent to record the flora of this region as the natural vegetation in entire Terai and Duars areas are dwindling very fast.

However, Terai and Duars are forming a contiguous and extended part of the rich vegetation of the Darjeeling part of the Eastern Himalaya. Scrutiny through the flora related literature covering East Himalayan region shows that Lauraceae is one of the dominant families in this region (Cowan & Cowan 1929; Momiyama

1971; Matthew 1981; Long 1984; Das 1986; Das & Chanda 1987; Banerjee 1993; Das *et al.* 2010). This family provides a wide collection of valuable economic products including medicines, timber, nutritious fruits, essential oils, spices and perfumes (Judd *et al.* 2002). Unfortunately, there is no detailed floristic work available on these important plants occurring in Terai-Duars belt. So, in the present study an attempt has been made to investigate the distribution of Laurels in Terai and Duars region of West Bengal.

5.2. RESULT

From the present survey the occurrence of 26 species covering 9 genera of Laurels were reported growing in Terai-Duars region of West Bengal (Photo plate I-VI). Artificial Dichotomous Keys for the recorded genera and species ware constructed based on significant reliable and easily observable vegetative, flower and fruit characters. All these species were enumerated below alphabetically accompanied by local names, salient features, exsiccatus, availability status, flowering and fruiting periods, occurrence in Terai & Duars region and world distribution. While referring to voucher specimens, names of different Protected Areas have been written in their abbreviated forms: GNP: Gorumara National Park; MWLS: Mahananda Wildlife Sanctuary; JNP: Jaldapara National Park; BTR: Buxa Tiger Reserve.

Key to the Genera

1a. Bracts forming involucres; flowers unisexual, rarely bisexual, in pseudo umbels or racemes, rarely solitary……. ……. ……	**2**
1b. Bracts not forming involucres; flowers bisexual, rarely unisexual, in panicles or clusters, rarely in pseudo-umbels……. …….	**4**
2a. Leaves usually whorled, rarely opposite or alternate; shoots with conspicuous terminal vegetative buds clustered at branch ends; evergreen trees……. ……. ……. ……. …….	***Actinodaphne***
2b. Leaves alternate, spaced and not clustered; shoots with or without conspicuous terminal vegetative buds; deciduous or evergreen trees……..	**3**
3a. Anthers 2-celled; fruits borne on entire or toothed perianth cup …….	***Lindera***
3b. Anthers 4-celled; fruits borne on enlarged, entire or rarely toothed perianth cup ……. ……. ……. ……. ……. ….	***Litsea***
4a. Anthers 1 or 2-celled ……. ……. ……. ……. …..	**5**
4b. Anthers 4-celled ……. ……. ……. ……. …….	**6**
5a. Terminal vegetative buds absent; minor veins weakly reticulate only on lower surface of lamina; panicles elongate, 15 – 22cm …….	***Cryptocarya***
5b. Terminal vegetative buds present; minor veins strongly reticulate on both surfaces of lamina; panicles short, 1 – 10cm ……. …….	***Beilschmiedia***
6a. Leaves usually triplinerved, sometimes pinnately veined, if pinnately veined then domatia present; perianth tube forming a cup in fruit ……..	***Cinnamomum***
6b. Leaves pinnately veined and domatia lacking; perianth tube does not form any perianth cup……. ……. ……. …….	**7**
7a. Perianth segments not persistent in fruit; leaves not clustered at branch tips	***Persea***
7b. Perianth persistent in fruits; leaves often clustered at branch tips …….	**8**
8a. Perianth lobes soft, longer, loosely clasped at fruit base; fruits globose ….	***Machilus***
8b. Perianth lobes durable, shorter, tightly clasped at fruit base; fruits ovoid to ellipsoid ……. ……. …….. ……. …….	***Phoebe***

ACTINODAPHNE Nees in Wallich, Pl. Asiat. Rar. 2: 61, 68. 1831

Key to the species

1a.	Lamina strongly 3-veined near base, obovate, 6 – 22 cm long ……	***A. obovata***
1b.	Lamina pinnately veined, elliptic, lanceolate to oblanceolate, 2 – 7 cm long ……. ……. ……. ……. …….	**2**
2a.	Lateral veins 8 – 11 on each side; lamina membranous …….	***A. sikkimensis***
2b.	Lateral veins 12 – 16 on each side; lamina coriaceous …….	***A. longipes***

Actinodaphne longipes Kostermans, Reinwardtia 9: 98. 1974; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3:182. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 281. 1984.

Small trees, up to 7 m high; juvenile branches often tomentose. Twigs slender, 2.5 mm in diameter, grey brown or pale brown. Perulate buds present. Leaves coriaceous, usually falsely whorled towards branch the tips; petioles 10–15 mm; lamina narrowly elliptic-lanceolate, 11–15 × 1.5–3 cm, entire, acuminate, base cuneate, lateral veins 12–16 pairs. Umbels on 2–4 mm short-shoots. Male inflorescences consisting of 1–3 sessile umbels, with 6 flowers per umbel; male flowers white, 10 mm long; pedicels 2–4 mm; tepals oblong or narrowly ovate, 4–6 mm; stamens 9, 6-8 mm long. Female inflorescences consisting of 2–3 sessile umbels, with 5–6 flowers per umbel; female flowers 4 mm long; pedicels 3–4 mm; tepals strap-shaped, 2 mm. Fruits broadly ellipsoid, 8–12 mm long, on perianth cups 7–8 mm across.

Flowers: December – January; **Fruits:** February – March

Exsiccatus: Kalijhora, MWLS, 210 m, *Dibakar Choudhury & AP Das 169*, dated 24.02.2010

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; up to 300 m.

General distribution: India **[**West Bengal, Sikkim**]**, Nepal, Bhutan; Endemic to Eastern Himalaya

Note: Timber is used for light construction and interior furnishing.

Actinodaphne obovata (Nees) Blume**,** Mus. Bot. 1: 342. 1851; Cowan & Cowan, Trs. N. Beng. 109. 1929; Kanjilal *et al.*, Fl. Ass. 4: 77. 1940; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 182. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 280. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 51. 1993. *Tetradenia obovata* Nees in Wallich, Pl. Asiat. Rar. 2: 64.1831; Hooker *f.*, Fl. Brit. Ind. 5: 153. 1886.

Local name: *Runchey*, *Runchey Kath*, *Runchepat* (Nepali)

Evergreen trees, up to 20 m high. Twigs stout, 5–10 mm in diameter, blackish, smooth or lenticellate, tomentose. Perulate buds present. Leaves clustered at tips of branchlets; petioles 3–6 cm; lamina obovate, obovate-oblong or elliptic-oblong, 25–50 × 6–22 cm, acute or apiculate, base cuneate, shiny adaxially, lateral veins 5–7 pairs. Flowers in paniculate inflorescences. Male inflorescences 3–5 cm; male flowers yellowish green, 8 mm; pedicels 2–5 mm; tepals broadly ovate, 6 mm; stamens 9, 6–7 mm long. Female inflorescences 3 cm long; tepals 2–3 mm. Fruits ellipsoid, 15–20 mm long on a perianth cup 8 mm across.

Flowers: March – April; **Fruits:** June – July

Exsiccatus: Sevoke, MWLS, 188 m, *Dibakar Choudhury & AP Das 018*, dated 10.04.2009; Sursuti forest, Lataguri, GNP,102 m, *Dibakar Choudhury & AP Das 125*, dated 20.02.2010; North Rajabhatkhawa RF, BTR, 88 m, *Dibakar Choudhury & AP Das 159*, dated 22.03.2010

Status: Frequent in forests

Local distribution: Found in forests of Terai & Duars; up to 200 m.

General distribution: India **[**West Bengal, Sikkim, Assam, Meghalaya, Arunachal Pradesh, Nagaland, Manipur, Tripura**]**, Nepal, Bhutan, Bangladesh, China.

Note: Bark is used to treat fractured bones.

Actinodaphne sikkimensis Meisner, Prodr. 15(1): 213. 1864; Hooker *f*., Fl. Brit. Ind. 5: 147. 1886; Cowan & Cowan, Trs. N. Beng. 108. 1929; Kanjilal *et al.*, Fl. Ass. 4: 77. 1940; Hara, Fl. E. Him. 2: 99. 1966; Matthew, Pl. Kurs. 89. 1981; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 281. 1984.

Local name: *Sik Siki*, *Phurke Sissi*, *Rudilo* (Nepali)

Evergreen trees up to 6 m high. Twigs slender, 2 mm diameter, mid brown, sometimes reddish, smooth, glabrous. Leaves membranous, falsely whorled; petioles 8–10 mm; lamina lanceolate, 10–14 × 2–4 cm, finely acuminate, base cuneate, lateral veins 8–11 pairs. Umbels solitary, on 2–4 mm short-shoots. Male inflorescences 2.5–3 cm long; male flowers yellowish white, 7 mm; pedicels 2–4 mm; Tepals ovate, 7 mm; Stamens 9, 5–6 mm. Female inflorescences 1.5 cm; female flowers 4 mm long; pedicels 2–4 mm; tepals oblong, 2 mm. Fruits ellipsoid, 9–15 mm long on perianth cup 2 mm across.

Flowers: November – February; **Fruits:** April – May

Exsiccatus: Purundibari, Lataguri, GNP, 112 m, *Dibakar Choudhury & AP Das*124, dated 20.02.2010; North Sevoke Forest, MWLS, 214 m, *Dibakar Choudhury & AP Das 070*, dated 26.06.2009

Status: Frequent in forests

Local distribution: Found in Gorumara National Park & Mahananda Wild Life Sanctuary; upto 250 m.

General distribution: India **[**West Bengal, Sikkim, Meghalaya**]**, Nepal, Bhutan, Thiland.

BEILSCHMIEDIA Nees in Wallich, Pl. Asiat. Rar. 2: 61, 69. 1831.

Beilschmiedia assamica Meisner, Prodr. 15(1): 64. 1864; Hooker *f*., Fl. Brit. Ind. 5: 124. 1886; Kanjilal *et al.*, Fl. Ass. 4: 53. 1940; Long in Gierson & Long, Fl. Bhut. 1(2): 256. 1984.

Local name: *Tarsing* (Nepali)

Evergreen trees, up to 20 m high. Twigs glabrous, initially dark reddish brown or blackish, smooth. Terminal buds lanceolate, 8–12 × 2–3.5mm. Leaves opposite or sub-opposite; petioles 5–10 mm; lamina elliptic or elliptic-oblong, 11–18 × 4–8 cm, blunt-acuininate, base cuneate, lateral veins 9–13 pairs. Inflorescence 1–2 cm, glabrous; pedicels 3-4 mm; flowers yellow, 3 mm, glabrous outside; tepals ovate, 3 mm; stamens 1–2 mm, inner whole long; ovary 1 mm, style 1mm, glabrous. Fruits ellipsoid, 3.5 – 4.5 cm in diameter on a 2 cm peduncle.

Flowers: December; **Fruits:** February – March

Exsiccatus: Sursuti**,** GNP,102 m, *Dibakar Choudhury & AP Das 030*, dated 30.05.2009

Status: Less common

Local distribution: Found only in Gorumara National Park; upto 150 m.

General distribution: India **[**West Bengal, Assam, Meghalaya**]**, Bhutan, Bangladesh, Myanmar

Note: Wood is used for making boats and boxes.

CINNAMOMUM Schaeffer, Bot. Exped. 74. 1760, *nom. cons*.

Key to the species

1a. Leaves opposite or sub opposite ……. ……. …….	**2**
1b. Leaves distinctly alternate ……. ……. …….	**5**
2a. Lamina elliptic, 16 – 30 cm long, thickly-leathery, obtuse or acute, base cuneate ……. ……. ……. ……. ….....	***C. bejolghota***
2b. Lamina ovate to oblong-ovate or ovate-lanceolate, 8 – 15 cm long, sub-leathery, smaller, acute or acuminate but not obtuse, base acute or rounded ……. ……. ……. …….	**3**
3a. Fruits ovoid; transvers veins reticulate; acuminate, base acute or rounded……. ……. ……. ……. …….	***C. verum***
3b. Fruits obovoid or ellipsoid; transvers veins undulate; long acuminate, base acute or broadly cuneate …… …….	**4**
4a. Part of perianth segments persistent in fruit; lamina lanceolate or ovate-lanceolate ……. ……. ……. …….	***C. tamala***
4b. Perianth segments not persistent in fruit; lamina elliptic or ovate-elliptic ……. ……. ……. ……. …….	***C. impressinervium***
5a. Perianth glabrous; lamina ovate-elliptic. ……. ……	***C. camphora***
5b. Perienth densely tomentose; lamina elliptic or lanceolate..…….	***C. glaucescens***

Cinnamomum bejolghota (Buchanan-Hamilton) Sweet, Hort. Brit. 344. 1826; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 258. 1984; Das & Chanda, Trans. Bose Res. Inst. 50(4): 109. 1987; Banerjee, Pl. Res. Jal. Rhi. Sanc. 51. 1993; Choudhury *et al*., Pleione 7(2): 443. 2013. *Laurus bejolghota* Buchanan-Hamilton,Trans. Linn. Soc. London. 13(2): 559. 1822. *Cinnamomum obtusifolium* (Roxburgh) Neesin Wallich, Pl. Asiat. Rar. 2: 73. 1831. *Laurus obtusifolia* Roxburgh, Fl. Ind., 2: 302. 1832; Hooker *f*., Fl. Brit. Ind. 5: 128. 1886; Prain, Beng. Pl. 2: 673. 1903; Matthew, Pl. Kurs. 89. 1981; Cowan & Cowan, Trs. N. Beng. 108. 1929.

Local name: *Ram tejpat* (Nepali)

Evergreen trees, up to 25 m high. Twigs pale brown or green, smooth, glabrous. Leaves coriaceous, opposite or sub-opposite, green and shiny adaxial, triplinerved; petioles 1–3 cm; lamina elliptic-oblong or elliptic, 15–30 × 4–9 cm; obtuse or acute, base cuneate. Glands not present in vein axils. Panicles 12–22 cm, glabrous; flowers yellowish white, 3–5 mm, sericeous inside and out; pedicels 3–5 mm; tepals broadly ovate, 3 mm; fertile stamens 9, 1.5–2.5 mm, the innermost whole usually slightly longer; staminodes 1.5 mm; ovary 1 mm, glabrous; style 1.5 mm, glabrous. Fruits ellipsoid, 6–12 mm long; tepals persistent on rim of cupule.

Flowers: February – March; **Fruits:** September – October

Exsiccatus: Garden of Medicinal Plants, NBU, 134 m, *Dibakar Choudhury & AP Das 005*, dated 21.03.2009; Gorumara, 98 m, *Dibakar Choudhury & AP Das 027*, dated 30.05.2009; North Sevoke, MWLS, 190 m, *Dibakar Choudhury & AP Das 064*, dated 26.06.2009; North Rajabhatkhawa, BTR, 88 m, *Dibakar Choudhury & AP Das 158*, dated 22.03.2010

Status: Very common

Local distribution: Throughout forests of Terai and Duars; upto 220 m.

General distribution: India **[**Arunachal Pradesh, Assam, Tripura, Meghalaya, West Bengal, Sikkim, Orissa, Tamilnaru, Madhya Pradesh, Punjab, Himachal Pradesh**]**, Nepal, Bhutan, Bangladesh, China, Myanmar, Thailand, Vietnam.

Note: Leaf and bark are used as condiment.

Cinnamomum camphora (Linnaeus) J. Presl, Prir. Rostlin 2: 36. 1825; Hooker *f.*, Fl. Brit. Ind. 5: 134. 1886; Prain, Beng. Pl. 2: 899. 1903; Kanjilal *et al.*, Fl. Ass. 4: 60. 1940; Momiyama in Hara, Fl. E. Him. 1: 99. 1966; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Choudhury *et al*., Pleione 7(2): 443. 2013. *Laurus camphora* Linnaeus, Sp. Pl. 369. 1753.

Local name: *Karpur* (Bengali)

Evergreen trees, up to 25 m high; whole plant strongly camphor–scented. Twigs blackish brown or green, glabrous, smooth. Perulate buds present. Leaves alternate; petioles 12–25 mm; lamina ovate-elliptic to elliptic, 5–9 × 2.5–5 cm, green or yellow-green and shiny adaxially, shortly acuminate, base cuneate, glabrous on both surfaces; triplinerved sometimes inconspicuously 5-nerved. Panicles 8–10 cm, glabrous; pedicels 2–4 mm; flowers yellow, 2–3 mm long; tepals ovate, 2 mm, tomentose within; fertile stamens 9, 1–1.5 mm, the innermost whorl slightly longer; staminodes 0.5–1 mm; ovary 1 mm, glablous; style 1–1.5 mm, glabrous. Fruits ovoid or subglobose, 6–8 mm in diameter.

Flowers: March – April; **Fruits:** July – August

Exsiccatus: Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 175*, dated 10.05.2012

Status: Rarely planted

Local distribution: Planted in University of North Bengal campus, Sukna etc; upto 200 m.

General distribution: India, China, Japan, Korea, Vietnam; widely cultivated all over the world in warmer and moist regions.

Note: Camphor oil is used in perfume industry and treatment of nervous depression, acne, inflammation, arthritis, cold and fever.

Cinnamomum glaucescens (Nees) Handel-Mazzetti, Oesterr. Bot. Z. 85: 214. 1936; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 259. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 51. 1993; Choudhury *et al*., Pleione 7(2): 443 – 444. 2013. *Laurus glaucescens* Buchanan-Hamilton *ex* Neesin Wallich, Pl. Asiat. Rar. 2: 70. 1831.*Cinnamomum cecidodaphne* Meisner, Prodr. 15(1): 25. 1864; Hooker *f.*, Fl. Brit. Ind. 5: 135. 1886; Cowan & Cowan, Trs. N. Beng. 108. 1929; Kanjilal *et al.*, Fl. Ass. 4: 58. 1940.

Local name: *Malagiri* (Nepali)

Evergreen trees, up to 15 m high. Twigs brown, sometimes reddish,young shoots tomentose, becoming glabrate. Leaves alternate; petioles 6–12 mm; lamina ovate-elliptic, 7–15 × 3.5–8 cm, shortly acuminate, base broadly cuneate or rounded, lateral veins 4–6 pairs. Panicles 12 cm, brownish-tomentose, densely clustered on young shoots; flowers greenish yellow, 3–4 mm long, tomentose; pedicels 2–8 mm; tepals ovate or oblong, 2–3 mm, sericeous or tomentose within; Fertile stamens 9, 1.5–2 mm, the innermost whorl longer; staminodes 1–1.5 mm; ovary 1–1.5 mm, glabrous; Style 1–1.5 mm, glabrous. Fruits globose, 8–10 mm in diameter.

Flowers: January – February; **Fruits:** April

Exsiccatus: Sursuti forest, Lataguri, GNP, 102 m, *Dibakar Choudhury & AP Das 115*, dated 20.02.2010

Status: Rare

Local distribution: Found only in Gorumara National Park – Lataguri area; upto 120m.

General distribution: India **[**Assam, Meghalaya, West Bengal**]**, Nepal, Bhutan, Bangladesh; endemic to Indian subcontinent.

***Note*:** Produce **e**ssential oils to use in perfumery and cosmetic preparations. Locally, it is used in various skin diseases.

Cinnamomum impressinervium Meisner, Prodr. 15(1): 21. 1864. Hooker *f*., Fl. Brit. Ind. 5: 129. 1886; Cowan & Cowan, Trs. N. Beng. 108. 1929; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 258. 1984. Das & Chanda, Trans. Bose Res. Inst. 50(4): 109. 1987; Choudhury *et al*., Pleione 7(2): 445. 2013. *Cinnamomum albiflorum* Hooker *f*. & Thomson *ex* Meisner, Prodr. 15(1): 21. 1864.*Cinnamomum cacharense* R.N. Parker, Repert. Spec. Nov. Regni Veg. 31: 126. 1932.

Local name: *Sissi, Korsane* (Nepali)

Evergreen trees, up to 15 m high. Twigs dark reddish brown, sericeous when young, soon glabrescent, smooth. Leaves opposite or sub-opposite; petioles 7–12 mm; lamina elliptic or ovate-elliptic, 8–20 × 3–5cm, finely acuminate, base cuneate, glossy adaxially with strongly impressed 3 vains. Panicles 6–10 cm, glabrous; pedicels 3–4 mm; flowers whitish yellow, 2–3 mm long; tepals ovate, 2 mm; fertile stamens 9, 1–2 mm long, the innermost whorl slightly longer; staminodes 0.5–1 mm; ovary 1 mm, glablous; style 1.5 mm, glabrous. Fruits ellipsoid, 10–12 mm long.

Flowers: July; **Fruits:** December

Exsiccatus: Sukna, MWLS, 215 m, *Dibakar Choudhury & AP Das 145*, dated 24.02.2010

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; upto 280 m.

General distribution: India **[**West Bengal, Sikkim**]**, Nepal, Bhutan; endemic to Eastern Himalaya.

Note: Bark is used as substitute for or an adulterant of *Cinnamomum verum* J. Presl

Cinnamomum tamala (Buchanan–Hamilton) T. Nees & Ebermaier, Handb. Med.-Pharm. Bot. 2: 426. 1831; Hooker *f*., Fl. Brit. Ind. 5: 128. 1886; Prain, Beng. Pl. 2: 899. 1903; Cowan & Cowan,

Trs. N. Beng. 107. 1929; Kanjilal *et al.*, Fl. Ass. 4: 56. 1940; Momiyama in Hara, Fl. E. Him.1: 99. 1966;Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 183. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 258. 1984; Choudhury *et al*., Pleione 7(2): 445. 2013. *Laurus tamala* Buchanan–Hamilton, Trans. Linn. Soc. London 13(2): 555. 1822.

Local name: *Tejpata* (Bengali), *Tejpat* (Nepali)

Evergreen trees, up to 15 m high. Twigs dark red-brown, sericeous when young, soon glabrescent. Leaves opposite or sub-opposite, thinly leathery, glabrous on both surfaces; petioles 7–13 mm; lamina lanceolate or ovate–lanceolate, 10–15 × 2.5–6 cm, shortly and bluntly acuminate, base cuneate, triplinerved. Panicles 5–10 cm long; flowers white, 5–7 mm long, sericeous; pedicels 4–5 mm; tepals oblong or narrowly ovate, 3–5 mm, sericeous within; fertile stamens 3–4 mm, the innermost whorl usually slightly longer; staminodes 2 mm; ovary 1–1.5 mm, hairy or sparsely hairy; style 2–3 mm, hairy or sparsely hairy. Fruits obovoid or ellipsoid, 8–10 mm long, tepals persistent on rim of cupule.

Flowers: February – April; **Fruits:** July – August

Exsiccatus: Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 002*, dated 21.03.2009; Palashbari near JNP, 64 m, *Dibakar Choudhury & AP Das 006*, dated 24.03.2009

Status: Widely planted for its aromatic leaves; also rarely in wild.

Local distribution: Cultivated several regions of Terai & Duars; up to 160 m.

General distribution: India **[**West Bengal, Assam, Arunachal Pradesh, Meghalaya**]**, Nepal, Bhutan, Tropical and sub-tropical Himalayan regions.

Note: Leaves are used as spice; bark and leaves also used for the treatment of several disease such as diarrhea, colic, vomiting, cardiac disorder etc (Ara *et al.* 2007).

Cinnamomum verum J.Presl, Prir. Rostlin 2: 36. 1823; Choudhury *et al*., Pleione 7(2): 445 – 446. 2013. *Laurus cinnamomum* Linnaeus, Sp. Pl. 1: 369. 1753. *Cinnamomum zeylanicum* Blume, Bijdr. 11: 568. 1825; Hooker *f*., Fl. Brit. Ind. 5: 131. 1886; Prain, Beng. Pl. 2: 899. 1903.

Local name: *Darchini*, *Daruchini*, *Dalchini* (Bengali)

Evergreen trees, up to 10m high. Twigs dark reddish brown, sericeous when young, soon glabrescent; bark black-brown with cinnamic aldehyde flavor. Leaves opposite or sub-opposite; petioles 8–13 mm; lamina ovate, ovate-lanceolate, 8–13 × 4.5–6 cm, green and shiny adaxially; glabrous on both surfaces; triplinerved. Panicle axillary or terminal, 10–12 cm long; flowers white, 3–5 mm long, sericeous; pedicels 2–4 mm; tepals narrowly ovate, 2–4 mm, sericeous within; fertile stamens 9, 2–2.5 mm long, the innermost whorl slightly longer; staminodes 2 mm; ovary 1 mm, hairy; style 2–3 mm, hairy or sparsely hairy. Fruits ovoid, 10–12 mm long, tepals persistent on rim of cupule.

Flowers: March – April; **Fruits:** July – September

Exsiccatus: Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 003*, dated 21.03.2009; Falakata near JNP, 62 m, *Dibakar Choudhury & AP Das 007*, dated 24.03.2009

Status: Less common

Local distribution: Planted at several places in Terai & Duars; upto 150 m.

General distribution: India **[**Cultivated in numerous states**]**, Sri Lanka, China, Myanmar; also cultivated in many other warm countries in Asia.

Note: The dried bark is the source of the important spice 'cinnamon-bark'. It is used medicinally to treat stomachache. The bark and leafy branchlets contain volatile oil (Choudhury *et al.* 2013).

CRYPTOCARYA R. Brown, Prodr. 402. 1810, *nom. cons*.

Cryptocarya amygdalina Nees**,** Pl. Asiat. Rar. 2: 69. 1831; Hooker *f*., Fl. Brit. Ind. 5: 118. 1886; Kanjilal *et al.*, Fl. Ass. 4: 49. 1940; Momiyama in Hara, Fl. E. Him. 1: 100. 1966; Matthew, Pl. Kurs. 89. 1981; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 184. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 253. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993.

Local name: *Patmero* (Nepali)

Trees up to 25 m high. Twigs light brown, minutely tomentose. Leaves alternate, coriaceous; petioles 8–12 mm; lamina elliptic-oblong, 10–25 × 5–9 cm, bluntly apiculate or shortly acuminate, base broadly cuneate or rounded, lateral veins 7–11 pairs. Panicles 15–25 cm, tomentose; pedicels 1–2 mm; flowers yellow, 3–5 mm tomentose outside; tepals narrowly ovate, 1–2 mm; stamens 9, 1.5–2 mm long; staminodes triangular, 1 mm; ovary 1 mm, glabrous; style 1 mm, glabrous. Fruits ovoid, 2–2.5 cm long.

Flowers: April; **Fruits:** May – June

Exsiccatus: Sursuti forest, Lataguri**,** GNP, 102 m, *Dibakar Choudhury & AP Das 120*, dated 20.02.2010; North Sevoke, MWLS, 190 m, *Dibakar Choudhury & AP Das 065*, dated 26.06.2009

Status: Common in forests

Local distribution: Found in Gorumara National Park and Mahananda Wild Life Sanctuary; upto 220 m

General distribution: India **[**West Bengal, Sikkim, Assam, Meghalaya**]**, Nepal, Bhutan, Bangladesh, China.

Note: Timber is used for light construction.

LINDERA Thunberg, Nov. Gen. Pl. 64. 1783, *nom. cons*., *non* Adanson (1763)

Lindera assamica (Meisner) Kurz, Prelim. Rep. For. Veg. Pegu App. A. p. ciii. 1875.Hooker *f.*, Fl. Brit. Ind. 5: 153. 1886; Cowan & Cowan, Trs. N. Beng. 111. 1929; Kanjilal *et al.*, Fl. Ass. 4: 95. 1940; Momiyama in Hara, Fl. E. Him.1: 100. 1966; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 184. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 270. 1984. *Aperula assamica* Meisner, Prodr. 16 (1): 320. 1869.

Local name: *Sanu Pahenle* (Nepali)

Evergreen trees, up to 10 m high. Twigs tomentose, dark brown, sometimes reddish, slightly lenticellate. Leaves thinly coriaceous; petioles 8–15 mm; lamina lanceolate or elliptic-lanceolate, 9–20 × 3–5 cm, acute or shortly acuminate, base cuneate, lateral veins 6–9 pairs. Inflorescences 2–3 cm long with 1–3 umbels on 4–9 mm short shoots. Male umbels with 9–11 flowers; peduncles 9–16 mm; pedicels 3–4 mm, sericeous; flowers 4 mm long; tepals linear, sericeous, 3 mm long; stamens 9, equal in length, 3 mm long. Female umbels with 8–15 flowers; peduncles 5–10 mm; pedicels 2–3 mm, sericeous; female flowers 3 mm long; tepals linear, glabrous, 2 mm; ovary 0.5 mm glabrous; style 1 mm, glabrous. Fruits broadly ellipsoid, 6–10 mm in diameter.

Flowers: February – May; **Fruits:** June

Exsiccatus: Sukna, MWLS, 201 m, *Dibakar Choudhury & AP Das 146*, dated 24.02.2010

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; upto 250 m.

General distribution: India **[**West Bengal, Assam**]**, Nepal, Bhutan, Myanmar; endemic to Indian subcontinent.

Note: Wood is used for constructing houses.

LITSEA Lamarck, Encycl. 3: 574. 1792, *nom. cons*.

Key to the species

1a.	Umbels peduncled, arranged usually in clusters, sometimes solitary or peduncles borne on a short stout axis upto 4 mm long …….	**2**
1b.	Umbels peduncled, arranged in racemes or corymbs with a slender 5 – 70 mm long axis ……. ……. ……. …….	**7**
2a.	Shoots with scaly terminal vegetative bud and ring of bud scale scars	***L. elongata***
2b.	Shoots without scaly terminal vegetative buds and ring of bud scale scars …… …… …….. ……. …….	**3**
3a.	Lamina lanceolate or narrowly elliptic-oblong, 2 – 5 cm broad (up to 8.5 cm broad in *L. salicifolia*) ……. ……. ……. ……	**4**
3b.	Lamina ovate, obovate or broadly elliptic, 6 – 12 cm broad ……..	**6**
4a.	Lateral veins 5 – 7 on each side; leaves coriaceous, pale or yellowish green above when dry ……. ……. ……. ……. …	***L. laeta***
4b.	Lateral veins 8 – 15 on each side; leaves rather membranous, dark green or brown above when dry, ……. ……. ……. ..	**5**
5a.	Lamina lanceolate, glabrous beneath except on veins, lateral veins 8 – 12 on each side ……. ……. ……. ……. …….	***L. cubeba***
5b.	Lamina elliptic–oblong, minutely silky–pubescent beneath, lateral veins 10 – 15 on each side ……. ……. ……. ……. ….	***L. salicifolia***
6a.	Lamina obtuse or apiculate, broadly ovate or obovate to ovate oblong, base rounded, tomentose beneath, lateral veins 6 – 13 on each side ….	***L. monopetala***
6b.	Lamina shortly acuminate, elliptic obovata, base cuneate, pubescent on veins beneath, lateral veins 9 – 15 on each side ……. …….	***L. hookeri***
7a.	Fruits narrow-ellipsoid; lamina 4–12 cm long; petioles 8–14 mm long ...	***L. assamica***
7b.	Fruits globose or depressed globose; lamina 8 – 32 cm long; petioles 10 – 28 mm long ……. ……. ……. ……. ……..	**8**
8a.	Lamina oblong or lanceolate, acuminate or shortly acute …….	***L. panamanja***
8b.	Lamina ovate-lanceolate, obtuse or rounded ……. …….	***L. glutinosa***

Litsea assamica Hooker *f.*, Fl. Brit. Ind. 5: 161. 1886; Kanjilal *et al.*, Fl. Ass. 4: 85. 1940; Choudhury *et al*., Pleione 8(1): 70. 2014.

Local name: *Timur* (Nepali)

Evergreen trees, up to 15 m high. Twigs glabrous, blackish brown. Leaves alternate; petioles 8–14 mm; lamina elliptic, 4–12 × 2.5–6 cm, acute to bluntly acuminate, base cunate, thinly coriaceous, rather glabrous, lateral veins 5–9 pairs. Umbels solitary, 1–1.5 cm long. Male umbels with 8–14 flowers; peduncles length 2–4 mm; male flowers pale yellow, 3–5 mm long, sericeous; pedicels 3–9 mm; tepals oblong, 2.5–3 mm; stamens 9, 1.5–2 mm long. Female umbels with 6–8 flowers; peduncles 2–4 mm; female flowers yellow, 3 mm, sericeous; pedicels 2-3 mm; tepals oblong, 2 mm; staminodes 9; style 0.5 mm, glabrous. Fruits narrowly ellipsoid, 6–9 mm long.

Flowers: May – June; **Fruits:** August – September

Exsiccatus: Chilapata, JNP, 88 m, *Dibakar Choudhury & AP Das 095*, dated 10.11.2009

Status: Less common

Local distribution: Found only in Jaldapara National Park; upto 105m.

General distribution: India **[**West Bengal, North–East India]; Endemic; a new record for West Bengal.

Note: Wood is used for making match boxes.

Litsea cubeba (Loureiro) Persoon, Syn. Pl. 2: 4. 1807; Momiyama in Hara, Fl. E. Him. 1: 101. 1966; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 185. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 274. 1984. Choudhury *et al*., Pleione 8(1): 70 – 71. 2014. *Lauru scubeba* Loureiro, Fl. Cochinch. 1: 252. 1790.

Local name: *Siltimur* (Nepali)

Deciduous shrubs to small aromatic trees, up to 10 m high. Twigs dark, often reddish brown, smooth, glabrous. Leaves alternate; petioles 6–20 mm; lamina lanceolate, 4–14 × 2–4 cm, long acuminate, base cuneate, dark green above when dry, pale beneath, both surfaces glabrous or sericeous-pubescent on veins; lateral veins 8–12 pairs. Inflorescences 1–1.5 cm long, 1–6 umbels densely arranged on 5–8 mm shoots, produced after leaves have emerged. Male umbels with 4–5 flowers; peduncles 4–7 mm; male flowers 4 mm long, glabrous; pedicels 2 mm; tepals oblong, 2.5–3 mm; stamens 9, 2–3 mm long. Female umbels with 3–6 flowers; peduncles 4–6 mm; female flowers yellow, 1.5–3 mm, sericeous; pedicels 1–2 mm; tepals obovata, oblong or ovate 1–2 mm; staminodes 9; style 1 mm, glabrous. Fruits subglobose, 6 – 7 mm.

Flowers: February – March; **Fruits:** July – August

Exsiccatus: Dhupjhora**,** GNP, 127 m, *Dibakar Choudhury & AP Das 113*, dated 20.02.2010; North Sevoke Forest, MWLS, 190 m, *Dibakar Choudhury & AP Das 142*, dated 24.02.2010; Sal Bagan, NBU,143 m, *Dibakar Choudhury & AP Das 155*, dated 15.03.2010.

Status: Less common

Local distribution: Found in Gorumara National Park, Mahananda Wild Life Sanctuary & NBU Campus; upto 230 m.

General distribution: India **[**West Bengal, Sikkim, Assam, Arunachal Pradesh, Meghalaya**]**, Nepal, Bhutan, Myanmar, Java, China.

Note: Fruit oil is added to food for flavouring and is also used as effective bio-pesticide (Agrawal *et al.* 2011).

Litsea elongate (Nees) Hooker *f*., Fl. Brit. Ind. 5: 165. 1886; Momiyama in Hara, Fl. E. Him. 1: 101. 1966; Matthew, Pl. Kurs. 90. 1981; Cowan & Cowan, Trs. N. Beng. 110. 1929; Kanjilal *et al.*, Fl. Ass. 4: 86. 1940; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 185. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 275. 1984; Choudhury *et al*., Pleione 8(1): 71. 2014. *Daphnidium elongatum* Nees in Wallich, Pl. Asiat. Rar. 2: 63. 1831.

Local name: *Thulo pahenlay* (Nepali)

Evergreen trees, robust upto 18 m tall; branchlets often tomentose, brownish. Leaves alternate; petioles 6–16 mm; lamina elliptic to oblanceolate or obovate, 8–18 × 2–6 cm, acute or obtuse, sometimes acuminate, base cuneate, lateral veins 6–13 pairs, much prominent beneath. Umbels solitary, 1–1.5 cm long. Male umbels with 6 flowers; peduncles length 4–5 mm; male flowers pale yellow, 5–7 mm long, sericeous; pedicels 32 mm; tepals ovate or oblong, 3–4 mm; stamens 8–11, outer stamens 5–6 mm long, inner stamens 2.5–4 mm. Female umbels with 3–4 flowers; peduncles 4–6 mm; female flowers 3 mm, sericeous; pedicels 1 mm; tepals narrowly ovate, 2 mm; style 2 mm, glabrous. Fruits ellipsoid 10–14 mm, with minute apical point.

Flowers: July – September; **Fruits:** October – November

Exsiccatus: Sukna, MWLS, 220 m, *Dibakar Choudhury & AP Das 092*, dated 07.10.2009

Status: Less common

Local distribution: Found only in Mahananda Wild Life Sanctuary; upto 300 m.

General distribution: India **[**Himachal Pradesh, West Bengal, Sikkim, Assam, Arunachal Pradesh**]**, Nepal, Bhutan, Myanmar, Tibet, China.

Note: The species is a good fodder for cattle and wood is used for construction works, making furniture, etc.

Litsea glutinosa (Loureiro) C.B. Robinson, Philipp. J. Sci. 6(5): 321. 1911; Matthew, Pl. Kurs. 90. 1981; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 185. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 277. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993; Choudhury *et al*., Pleione 8(1): 71 – 72. 2014. *Sebifera glutinosa* Loureiro, Fl. Cochinch. 2: 638. 1790. *Litsea sebifera* Persoon, Syn. Pl. 2: 4. 1807; Hooker *f*., Fl. Brit. Ind. 5: 124. 1886; Prain, Beng. Pl. 2: 902. 1903; Cowan & Cowan, Trs. N. Beng. 109. 1929; Kanjilal *et al.*, Fl. Ass. 4: 82. 1940.

Local name: *Kawala* (Nepali)

Evergreen, aromatic trees, up to 18 m high. Twigs pale brown to blackish, smooth. Leaves alternate, both surfaces tomentose when young; petioles 10–28 mm; lamina elliptic-oblong or ovate-lanceolate, 7.5–22.5 × 3.5–10 cm, obtuse or rounded, base cuneate, obtuse or rotund, lateral veins 5–12 pairs. Umbels solitary or several on short branchlets. Male umbels with 12 flowers; peduncles length 12–19 mm; male flowers green or yellow, 5–6 mm long, sericeous; pedicels 4 mm; tepals absent; stamens 18, outer stamens 4–5 mm long, inner stamens 3–4 mm. Female umbels with 8–9 flowers; peduncles 4–11 mm; female flowers pale green, 2–3 mm, sericeous; pedicels 1.5–2 mm; tepals absent or strap-shaped, 1 mm; staminodes 12–14; style 1.5–2 mm, glabrous. Fruits globose, 7–9 mm in diameter.

Flowers: March – June; **Fruits:** September – October

Exsiccatus: Lataguri, GNP, 102 m, *Dibakar Choudhury & AP Das 026*, dated 30.05.2009; Rajabhatkhawa, BTR, 80 m, *Dibakar Choudhury & AP Das 051*, dated 09.06.2009; Sevoke,

MWLS, 188 m, *Dibakar Choudhury & AP Das 063*, dated 26.06.2009; NBU campus, 134 m, *Dibakar Choudhury & AP Das 174*, dated 10.05.2012; Salkumar, JNP, 78 m, *Dibakar Choudhury & AP Das 060*, dated 15.09.2009

Status: Abundant

Local distribution: Found throughout the Terai and Duars region; upto 200 m.

General distribution: Pakistan, India **[**almost throughout- Arunachal Pradesh, Assam, Nagaland, Tripura, Meghalaya, West Bengal, Sikkim, Bihar, Jharkhand, Orissa, Andhra Pradesh, Andaman & Nicobar Islands, Karnataka, Maharashtra, Madhya Pradesh, Uttarakhand, Punjab, Himachal Pradesh**]**, Nepal, Bhutan, Sri Lanka, China, Myanmar, Philippines, Thailand, Vietnam.

Note: Bark is used for the treatment of diarrhea, dysentery, rheumatic joint pain etc. and bark powder is used as an adhesive paste in incense stick production (Agrawal *et al.* 2011).

Litsea hookeri (Meisner) Long, Notes Roy. Bot. Gard. Edinburgh. 41: 510. 1984; Long in Gierson & Long, Fl. Bhut. 1(2): 276. 1984; Choudhury *et al*., Pleione 8(1): 72. 2014. *Cylicodaphne hookeri* Meisner, Prodr. 15(1): 209. 1864.

Local name: *Dude Lampate* (Nepali)

Evergreen trees, up to 12 m high. Twigs pale brown, slightly ridged, tomentose. Leaves alternate; petioles 8–15 mm; lamina elliptic-obovate, 12–26 × 6–10 cm, shortly acuminate, base cuneate, pubescent on veins beneath; lateral veins 9–15 pairs. Umbels densely pubescent, clustered on shortest branchlets. Male umbels with 5–8 flowers; peduncles length 10 mm; male flowers green, 5–6 mm long, sericeous; pedicels 2–2.5 mm; tepals oblong or obovata, 2.5–3.5 mm; stamens 12, outer stamens 3 mm, inner stamens 1.5 mm. Female umbels with 5–8 flowers; peduncles 6–8 mm; female flowers yellow, 2–3.5 mm, tomentose outside and glabrous inside; pedicels 2.5–3 mm; tepals ovate, 2 mm; staminodes 9–11; style 2 mm, glabrous. Fruits ellipsoid, 11–17 mm long.

Flowers: May – June; **Fruits:** August – September

Exsiccatus: North Sevoke, MWLS, 214 m, *Dibakar Choudhury & AP Das 009*, dated 10.04.2009; Mahakaldham, Lataguri, GNP, 127 m, *Dibakar Choudhury & AP Das 024*, dated 30.05.2009

Status: Less common

Local distribution: Found in Mahananda Wildlife Sanctuary & Gorumara National Park; upto 240 m.

General distribution: India **[**West Bengal, Assam, Arunachal Pradesh] Bhutan, Thailand.

Note: Timber is used for constructing houses and for making furniture.

Litsea laeta (Nees) Hooker *f.*, Fl. Brit. Ind. 5: 169. 1886; Matthew, Pl. Kurs. 90. 1981; Cowan & Cowan, Trs. N. Beng. 111. 1929*;* Kanjilal *et al.*, Fl. Ass. 4: 88. 1940; Long in Gierson & Long, Fl. Bhut. 1 (2): 275. 1984; Choudhury *et al*., Pleione 8(1): 72 – 74. 2014. *Tetranthera laeta* Nees in Wallich, Pl. Asiat. Rar. 2: 67. 1831.

Shrub or small trees up to 8 m high. Twigs dark, often reddish brown, smooth, glabrous. Leaves alternate, coriaceous; petioles 10–15 mm; lamina oblong-elliptic, 10–20 × 3–5 cm, acute, base cuneate, glabrous; lateral veins 5–7 pairs. Umbels axillary clusters, rarely solitary, 2–5 cm long. Male umbels with 4–6 flowers; peduncles length 9–16 mm; male flowers yellow, 6–8 mm long, sericeous; pedicels 1 mm; tepals oblong, 2.5–4 mm; stamens 9–13, outer stamens 6–8 mm, inner stamens 4–5 mm. Female

umbels with 2–5 flowers; peduncles 5–6 mm; female flowers pale yellow or white, 4 mm, sericeous; pedicels 2 mm; tepals ovate or oblong, 1.5–2.5 mm; staminodes 9 or10; style 2.5 mm, glabrous. Fruits obovoid or subglobose, 5–10 mm long.

Flowers: November – January; **Fruits:** February – April

Exsiccatus: Sukna, MWLS, 220 m, *Dibakar Choudhury & AP Das 090*, dated 07.10.2009; Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 154*, dated 15.03.2010

Status: Less common

Local distribution: Found in Mahananda Wildlife Sanctuary and University of North Bengal campus; upto 280 m.

General distribution: India **[**Andhra Pradesh, West Bengal, Sikkim, Assam, Arunachal Pradesh], Bhutan, Bangladesh.

Note: Seed oil is with high antioxidant activity (Choudhury *et al.* 2013).

Litsea monopetala (Roxburgh) Persoon, Syn. Pl. 2: 4. 1807; Momiyama in Hara, Fl. E. Him. 1: 102. 1966; Matthew, Pl. Kurs. 89. 1981; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 185. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 276. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993; Choudhury *et al*., Pleione 8(1): 74. 2014. *Tetranthera monopetala* Roxburgh, Pl. Coromandel. 2: 26. 1798. *Litsea polyantha* Jussieu, Ann. Mus. Natl. Hist. Nat. 6: 211. 1805; Hooker *f*., Fl. Brit. Ind. 5: 162. 1886; Prain, Beng. Pl. 2: 903. 1903; Cowan & Cowan, Trs. N. Beng. 110. 1929; Kanjilal *et al*., Fl. Ass. 4: 83. 1940.

Local Name: *Bonsum*, *Kutmero* & *Patmero* (Nepali)

Evergreen trees, up to 15 m high, with spreading crown. Twigs reddish brown, densely tomentose, glabrescent. Leaves alternate; petioles 8–20 mm; lamina ovate-oblong, oblanceolate or elliptic-oblong, 7–25 × 6–12 cm, obtuse or apiculate, base rounded, lateral veins 6–13 pairs. Inflorescences 2 cm, with 2–8 umbels densely arranged on stout, produced after leaves have emerged. Male umbels with 6–8 flowers; peduncles length 4–7 mm; male flowers green or yellow, 4 mm long, sericeous; pedicels 1–2 mm; tepals strap shaped, 2–2.5 mm; stamens 9, outer stamens 3 mm, inner stamens 2 mm. Female umbels with 5–9 flowers; peduncles 4–6 mm. Female flowers pale yellow, 3–3.5 mm, sericeous; pedicels 2–3 mm; tepals strap shaped, 2 mm; staminodes 9; style 1.5 mm, glabrous. Fruits globose to ellipsoid, 7–12 mm long; blackish when ripe.

Flower: March – June; **Fruit:** July – August

Exsiccatus: Lataguri, GNP, 98 m, *Dibakar Choudhury & AP Das 109*, dated 20.02.2010; North Rajabhatkhawa Forest, BTR, 88 m, *Dibakar Choudhury & AP Das 050*, dated 09.06.2009; Sevoke, MWLS, 188 m, *Dibakar Choudhury & AP Das 011*, dated 10.04.2009; NBU campus 134 m, *Dibakar Choudhury & AP Das 172*, dated 10.05.2012; Jaldapara National Park, 80 m, *Dibakar Choudhury & AP Das 075*, dated 02.10.2009

Status: Abundant

Local Distribution: Found throughout the Terai and Duars region; upto 200 m.

General Distribution: Pakistan, India [Arunachal Pradesh, Assam, Tripura, Meghalaya, West Bengal, Sikkim, Bihar, Jharkhand, Orissa, Andhra Pradesh, Andaman & Nicobar Islands, Maharashtra, Madhya Pradesh, Uttarakhand], Nepal, Bhutan, China, Myanmar, Thailand, Malaysia, Vietnam, Cambodia.

Note: The leaves are used to treat arthritis and are good food for the larvae of muga-silk moth.

Litsea panamanja (Buchanan–Hamilton *ex* Nees) Hooker *f*., Fl. Brit. Ind. 5: 175. 1886; Prain, Beng. Pl. 2: 903. 1903*;* Kanjilal *et al*, Fl. Ass. 4: 90. 1940; Long in Gierson & Long, Fl. Bhut. 1(2): 277. 1984; Choudhury *et al*., Pleione 8(1): 74. 2014. *Tetranthera panamanja* Buchanan–Hamilton *ex* Nees in Wallich, Pl. Asiat. Rar. 2: 67. 1831.

Local name: *Painle champ* & *Dudhi lampatey* (Nepali)

Evergreen trees, up to 25 m high; Twigs whitish, pale brown, smooth, pubescent and becoming glabrous. Leaves alternate; petioles 13–22 mm; lamina oblong or lanceolate, 15–32 × 3–7 cm, acuminate or shortly acute, base cuneate, both surfaces glabrous, coriaceous; lateral veins 7–11 pairs. Inflorescences 3–9 cm, with 4–15 umbels racemosely arranged on 4–40 mm shoots, produced after leaves have emerged. Male umbels with 6–8 flowers; peduncles length 8–16 mm; male flowers yellow, 7 mm long, sericeous; pedicels 1–2 mm; tepals oblong, 2–3 mm; stamens 11–12, outer stamens 2–3.5 mm, inner stamens 1.5–2.5 mm. Female umbels with 4–6 flowers; peduncles 3–9 mm; female flowers yellow, 2–3 mm, sericeous; pedicels 1 mm; tepals obovate, 1 mm; staminodes 12; style 1 mm, glabrous. Fruits depressed globose, 6–8 mm in diameter.

Flowers: March – April; **Fruits:** April – May

Exsiccatus: Sursuti, GNP, 102 m, *Dibakar Choudhury & AP Das 039*, dated 31.05.2009; North Rajabhatkhawa Forest, BTR, 88 m, *Dibakar Choudhury & AP Das 049*, dated 09.06.2009; Sevoke, MWLS, 210 m, *Dibakar Choudhury & AP Das 140*, dated 24.02.2010

Status: Less common

Local distribution: Found in forest areas throughout Terai and Duars; upto 220m.

General distribution: India **[**Arunachal Pradesh, Assam, Nagaland, Tripura, West Bengal, Sikkim, Andaman & Nicobar Islands**]**, Nepal, Bhutan, Bangladesh, China, Myanmar, Vietnam, Malay Peninsula.

*Note***:** Wood is used for house construction, making furniture and as fire wood.

Litsea salicifolia (Roxburgh *ex* Nees) Hooker *f*., Fl. Brit. Ind. 5: 167. 1886; Prain, Beng. Pl. 2: 903. 1903; Cowan & Cowan, Trs. N. Beng. 110. 1929*;* Kanjilal *et al.*, Fl. Ass. 4: 87. 1940; Momiyama in Hara, Fl. E. Him. 2: 39. 1971; Kostermans & Chater in Hara *et al*., Enum. Fl. Pl. Nep. 3: 186. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 275. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993; Choudhury *et al*, Pleione 8(1): 75. 2014. *Tetranthera salicifolia* Roxburgh *ex* Neesin Wallich, Pl. Asiat. Rar. 2: 66. 1831.

Local name: *Sanu pahenle* (Nepali)

Evergreen trees, up to 10 m high. Twigs dark brown, smooth, glabrescent. Leaves alternate; petioles 8–12 mm; lamina elliptic-oblong, 12–30 × 2.5–8.5 cm, acuminate or acute, base acute, dark brown above when dry, lateral veins 10–15 pairs, prominent beneath. Inflorescences 1.2–2 cm long, with 7–15 umbels in sessile clusters. Male umbels with 4–5 flowers; peduncles length 4–7 mm; male flowers white or whitish green, 4 mm long, glabrous; pedicels 1–1.5 mm; tepals oblong or ovate, 2–2.5 mm; stamens 4–7, outer stamens 4 mm, inner stamens 2.5–4 mm. Female umbels with 4 flowers; peduncles 3–4 mm; female flowers yellow or green, 3 mm, glabrous; pedicels 1 mm; tepals elliptic, 2 mm; staminodes 10; style 2 mm, glabrous. Fruits ellipsoid, 10–11 mm in diameter.

Flowers: February – April; **Fruits:** May – June

Exsiccatus: Dhupjhora, GNP, 127 m, *Dibakar Choudhury & AP Das 112*, dated 20.02.2010; Buxa, BTR, 96 m, *Dibakar Choudhury & AP Das 104*, dated 08.02.2010; Sevoke, MWLS, 190 m, *Dibakar Choudhury & AP Das 013*, dated 10.04.2009; NBU campus, 134 m, *Dibakar Choudhury & AP Das 156*, dated 15.03.2010; Hollong, JNP, 87 m, *Dibakar Choudhury & AP Das 077*, dated 02.10.2009

Status: Frequent in forests

Local distribution: Found in forest areas throughout Terai and Duars; upto 300 m.

General distribution: Pakistan, India **[**Arunachal Pradesh, Assam, West Bengal, Sikkim, Bihar**]**, Bangladesh, Nepal, Bhutan, China, Vietnam, Myanmar.

Note: Seed oil is used as bio-pesticide and leaves are good food for larvae of muga-silk moth.

MACHILUS Rumphius *ex* Nees in Wallich, Pl. Asiat. Rar. 2: 61, 70. 1831.

Key to the species

1a. Leaf midrib adaxially impressed; ovary globose …….	***M. duthiei***
1b. Leaf midrib adaxially concave; ovary subglobose ……..	**2**
2a. Parianth lobes ovate or broadly ovate; leaf base cunneate; petioles 8 – 20 mm; vein-lets abaxially visible ……. ……. …….	***M. glaucescens***
2b. Perianth lobes oblong; leaf base attenuate; petioles 8 – 12 mm; vain-lets inconspicuous ……. ……. ……. …….	***M. gamblei***

Machilus duthiei King, Fl. Brit. Ind. 5: 861. 1890; Momiyama in Hara, Fl. E. Him. 1: 102. 1966. *Persea duthiei* (King) Kostermans, Reinwardtia 6(2): 191. 1962; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 186. 1982; Long in Gierson & Long, Fl. Bhut. 1(2): 266. 1984.

Local name: *Mitsu Shing* (Nepali)

Trees up to 20 m high. Shoots with rings of bud scale scars. Twigs dark reddish brown, smooth, glabrous. Perulate buds present. Leaves coriaceous, minutely silky-pubescent beneath when young; petioles 6–12mm; lamina elliptic, 15–25 × 2.5–4 cm, acuminate, base cuneate or attenuate, lateral veins 7–12 pairs. Panicles 4–16 cm, sericeous; pedicels 4–6 mm; flowers pale greenish-yellow, 5–6 mm long, sericeous outside; tepals oblong or ovate, 4.5–5.5 mm; fertile stamens 3.5–5 mm long; staminodes 1–2 mm; ovary 0.5 mm glabrous; style 1 mm, glabrous. Fruits globose, 9–11 mm in diameter.

Flowers: February – March; **Fruits:** May – June.

Exsiccatus: Sevoke, MWLS, 212 m, *Dibakar Choudhury & AP Das 068*, dated 26.06.2009

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; upto 270 m.

General distribution: Pakistan, India **[**West Bengal, Arunachal Pradesh, Meghalaya**]**, Nepal, Bhutan, China.

Note: Root is used for the treatment of inflammation, asthma, pain, bronchitis, vomiting and blood diseases (Padalia *et al.* 2009).

Machilus gamblei King *ex* Hooker *f.*, Fl. Brit. Ind. 5: 138. 1886; Kanjilal *et al.*, Fl. Ass. 4: 67. 1940; Matthew, Pl. Kurs. 91. 1981. *Machilus bombycina* King *ex* Hooker *f.*,Fl. Brit. Ind. 5: 861. 1890; Prain, Beng. Pl. 2: 900. 1903; Cowan & Cowan, Trs. N. Beng. 107. 1929; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993. *Machilus suaveolens* S.K. Lee, Acta Phytotax. Sin. 8(3): 187. 1963. *Persea bombycina* (King ex Hooker *f.*) Kostermans, Reinwardtia. 6(2): 191. 1962. *Persea gamblei* (King *ex* Hooker *f.*) Kostermans, Reinwardtia. 6(2): 192. 1962; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 186. 1982; Long in Gierson & Long, Fl. Bhut. 1 (2): 267. 1984. *Persea suaveolens* (S.K. Lee) Kostermans, Ann. Missouri Bot. Gard. 77(3): 547. 1990.

Local name: *Kawla* (Nepali)

Trees up to 20 m high; young shoots with densely gray-yellow pubescence, becoming dark reddish brown, glabrate and with rings of bud scale scars, sometimes lenticellate. Leaves thinly coriaceous; petioles 8–12 mm; lamina oblong or oblanceolate, 7–15 × 3–6 cm, acuminate, base cuneate or attenuate, lateral veins 8–10 pairs. Panicles 4–11 cm, sericeous or tomentose; pedicels 4–8 mm; flowers greenish-yellow, 5–8 mm long, sericeous outside; tepals oblong, 5–6 mm; fertile stamens 3–4.5 mm; staminodes 1 mm; ovary 1 mm glabrous; style 1 mm, glabrous. Fruits globose, 8–11 mm in diameter.

Flowers: January – April; **Fruits:** June – July

Exsiccatus: Sukna, MWLS, 210 m, *Dibakar Choudhury & AP Das167*, dated 12.01.2012

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; upto 230 m.

General distribution: India **[**West Bengal, Assam, Meghalaya**]**, Nepal, Bhutan, Bangladesh, China, Myanmar, Thailand, Cambodia, Vietnam.

Note: Produce good quality firewood.

Machilus glaucescens (Nees) Wight, Icon. Pl. Ind. Orient. 5(2): 12. 1852. *Ocotea glaucescens* Nees in Wallich, Pl. Asiat. Rar. 2: 71. 1831. *Laurus villosa* Roxburgh, Fl. Ind., ed. 1832. 2: 310. 1832. *Machilus villosa* (Roxburgh) Hooker *f.*, Fl. Brit. Ind. 5:140. 1886; Prain, Beng. Pl. 2: 900. 1903; Cowan & Cowan, Trs. N. Beng. 106. 1929; Kanjilal *et al.*, Fl. Ass. 4: 65. 1940. *Persea glaucescens* (Nees) Long, Notes Roy. Bot. Gard. Edinburgh. 41(3): 521. 1984; Long in Gierson & Long, Fl. Bhut. 1 (2): 267. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993. *Persea villosa* (Roxburgh) Kostermans, Reinwardtia. 6(2): 194. 1962; Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 187. 1982; *Phoebe glaucescens* (Nees) Nees, Syst. Laur. 100. 1836. *Phoebe villosa* (Roxburgh) Wight, Icon. Pl. Ind. Orient. 5: 11. 1852.

Local name: *Bhale Kaulo* (Nepali)

Trees up to 20 m high. Branch-lets blackish brown, young shoots densely pubescent, becoming glabrate. Leaves coriaceous; petioles 8–20 mm; lamina usually elliptic, 10–20 × 3–7 cm; acuminate, base cuneate, lateral veins 7–10 pairs, very prominent beneath, minor veins reticulate beneath. Panicles numerous, 14–25 cm long, tomentose; pedicels 3–9 mm; flowers greenish-yellow, 4–5 mm long, tomentose outside; tepals ovate, 3–5 mm; fertile stamens 2–2.5 mm; staminodes 1 mm; ovary 1 mm glabrous; style 1.5 mm, glabrous. Fruits globose, 10–12 mm in diameter.

Flowers: January – March; **Fruits:** March – May

Exsiccatus: Sevoke, MWLS, 190 m, *Dibakar Choudhury & AP Das 067*, dated 26.06.2009

Status: Less common

Local distribution: Found only in Mahananda Wildlife Sanctuary; upto 300 m.

General distribution: India **[**Bihar, West Bengal, Sikkim, Assam, Meghalaya, Arunachal Pradesh**]**, Nepal, Bhutan, Bangladesh, China, Myanmar.

Note: Trunk is used as firewood.

PERSEA Miller, Gard. Dict. Abr., ed. 4, 1030. 1754, *nom. cons*.

Persea odoratissima (Nees) Kostermans, J. Sci. Res. (Jakarta) 1: 116. 1942. Kostermans & Chater in Hara *et al.*, Enum. Fl. Pl. Nep. 3: 187. 1982; Long in Gierson & Long, Fl. Bhut. 1 (2): 266. 1984. *Machilus odoratissimus* Nees in Wallich, Pl. Asiat. Rar. 2:70. 1831; Hooker *f*., Fl. Brit. Ind. 5: 139. 1886; Cowan & Cowan, Trs. N. Beng. 105. 1929; Kanjilal *et al.*, Fl. Ass. 4: 64. 1940; Matthew, Pl. Kurs. 91. 1981.

Local name: *Lali Kawla* (Nepali)

Trees up to 10m high. Twigs dark reddish brown or dark brown smooth, sometimes lenticellate. Leaves reddish brown when dry; petioles 8–15 mm; lamina very variable in shape, oblanceolate, oblong-lanceolate, elliptic-oblong or obovate, 8–13 × 3–6cm, shortly acuminate or acute, base cuneate, acute or obtusely narrowed, lateral veins 7–11 pairs. Panicles glabrous, 5–12 cm long; pedicels 3–4 mm; flowers greenish-yellow, 4–6 mm long, sericeous outside; tepals oblong or elliptic, 3–5 mm; fertile stamens 2–3.5 mm; staminodes 1–1.5 mm; ovary 0.5 mm glabrous; style 1 mm, glabrous. Fruits ellipsoid, 12–16 mm long.

Flowers: March – May; **Fruits:** June – July

Exsiccatus: Dhupjhora, GNP, 127 m, *Dibakar Choudhury & AP Das 043,* dated 31.05.2009; Buxa Tiger Reserve, 88 m, *Dibakar Choudhury & AP Das 053*, dated 09.06.2009.

Status: Less common

Local distribution: Found in Gorumara National Park & Buxa Tiger Reserve; upto 140 m.

General distribution: Pakistan, India **[**Punjab, Himachal Pradesh, West Bengal, Sikkim, Assam, Meghalaya**]**, Nepal, Bhutan, Bangladesh, China, Myanmar, Malaysia.

Note: A red dye is prepared from its bark.

PHOEBE Nees, Syst. Laur. 98. 1836.

Phoebe hainesiana Brandis, Hooker's Icon. Pl. 29: t. 2803. 1906; Cowan & Cowan, Trs. N. Beng. 107. 1929; Long in Gierson & Long, Fl. Bhut. 1(2): 262. 1984.

Local name: *Angare* (Nepali)

Trees robust up to 20 m high. Twigs whitish or pale brown, smooth, sericeous and becoming glabrous. Leaves clustered at branch ends; petioles 10–18 mm; lamina oblanceolate or obovate, 11–25 × 4–6 cm, bluntly mucronate, base attenuate, lateral veins 12–16 pairs. Panicles clustered at branch ends, 8–15 cm, sparsely sericeous; pedicels 5–8 mm; flowers pale green or yellow, 6–7 mm long, sericeous outside; tepals broadly ovate, 4.5–5.5 mm, tomentose; stamens 4.5–5 mm; staminodes 3 mm; ovary 2 mm glabrous; style 2.5 mm, glabrous. Fruits broadly ellipsoid, 2.5–3 cm in diameter.

Flowers: May; **Fruits:** December

Exsiccatus: Buxa Tiger Reserve, 96 m, *Dibakar Choudhury & AP Das 106*, dated 08.02.2010

Status: Less common

Local distribution: Found only in Buxa Tiger Reserve; upto 100 m.

General distribution: India **[**West Bengal, Sikkim**]**, Bhutan; endemic to Eastern Himalaya.

Note: Wood is used for making furniture and plywood.

5.3. DISCUSSION

5.3.1. Representation and Distribution

The present study clearly revealed that Lauraceae is one of the well represented families in Terai-Duars region of West Bengal. Among the recorded genera, *Litsea* Lamarck was found to dominate with its 9 species (Fig 5.1). Species diversity of Laurels was highest in Mahananda Wildlife Sanctuary, with the record of 18 species of the total representation of 26 species in the entire study area (Fig 5.2).

5.3.2. New Records

After intensive scrutiny of literature (Prain 1903; Hooker 1886; Brandis 1906; Cowan & Cowan 1929; Banerjee 1993; Das *et al.* 2010) it is revealed that out of the recorded 26 species of Laurels only 10 species viz., *Actinodaphne obovata*, *Cinnamomum bejolghota*, *C. glaucescens*, *Cryptocarya amygdalina*, *Litsea cubeba*, *L. glutinosa*, *L. monopetala*, *L. salicifolia*, *Machilus gamblei* and *M. glaucescens* were reported earlier from the study area. However, in most of the cases without specifying any locality. Accordingly, as much as 16 species *viz.*, *Actinodaphne longipes*, *A. sikkimensis*, *Beilschmiedia assamica*, *Cinnamomum camphora*, *C. impressinervium*, *C. tamala*, *C. verum*, *Lindera assamica*, *Litsea assamica*, *L. elongata*, *L. hookeri*, *L. laeta*, *L. panamanja*, *Machilus duthiei*, *Parsea odoratissima* and *Phoebe hainesiana* are now reported here first time to occur in the Terai and Duars belt of West Bengal. However, apart from *Litsea assamica*, all other species were known to grow from different other localities of the state. The distribution of *Litsea assamica* was earlier known only from North–East India (Kanjilal *et al.* 1940; Bhuinya *et al.* 2009); or, in other words, the species was known earlier as an endemic to that region only. So, the present collection of the species from Terai and Duars is a new record of its occurrence in West Bengal.

5.3.3. Endemics

Out of the recorded 26 species of laurels from the Terai and Duars regions of West Bengal three species, namely *Actinodaphne longipes*, *Cinnamomum impressinervium*, *Phoebe hainesiana* are endemic to Eastern Himalaya and three more species, *viz. Beilschmiedia assamica*, *Cinnamomum glaucescens* and *Lindera assamica* are endemic to north-eastern region of the Indian subcontinent. Also, another important species i.e. *Cinnamomum tamala* is basically a tropical Himalayan plant.

5.3.4. Importance

Present study also indicates that several economically useful species of Lauraceae assets in the vegetation of Terai and Duars belt. More than 92 % species recorded from this region are used for varied purpose

(Fig 5.3). As much as 12 of these species are used as medicine, at least traditionally. In addition, a total of 13 species are used in different other purpose *viz.* packaging, construction, fire wood, bio-pesticide, fragrance and for flavouring substances and as spice.

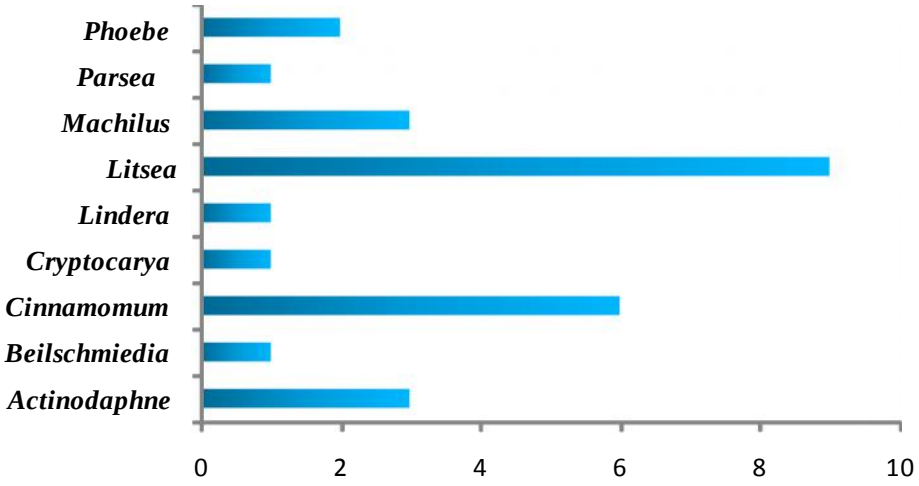


Figure 5.1. Diversity of Laurels in Terai-Duars region of West Bengal

Figure 5.2. Diversity of Laurels in several floristic region of Terai-Duars of West Bengal

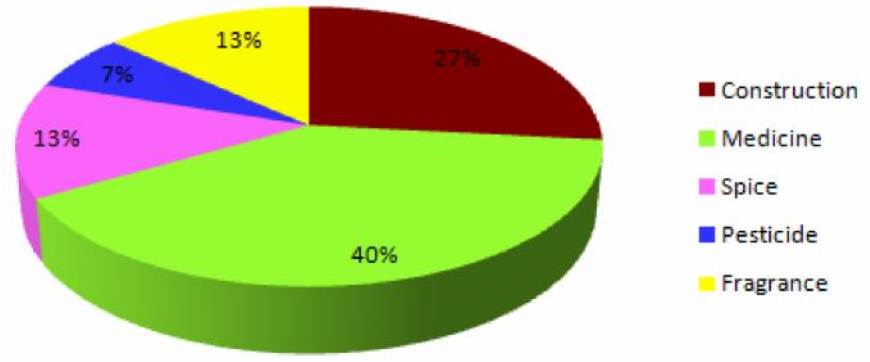


Figure 5.3. Uses of Laurels

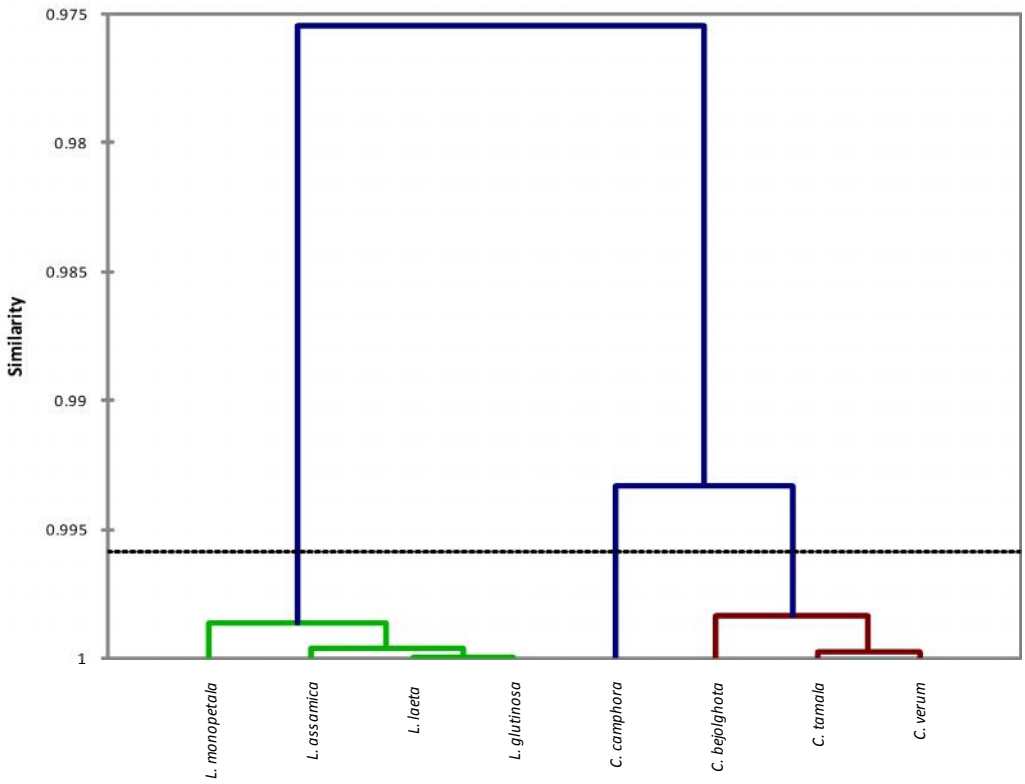
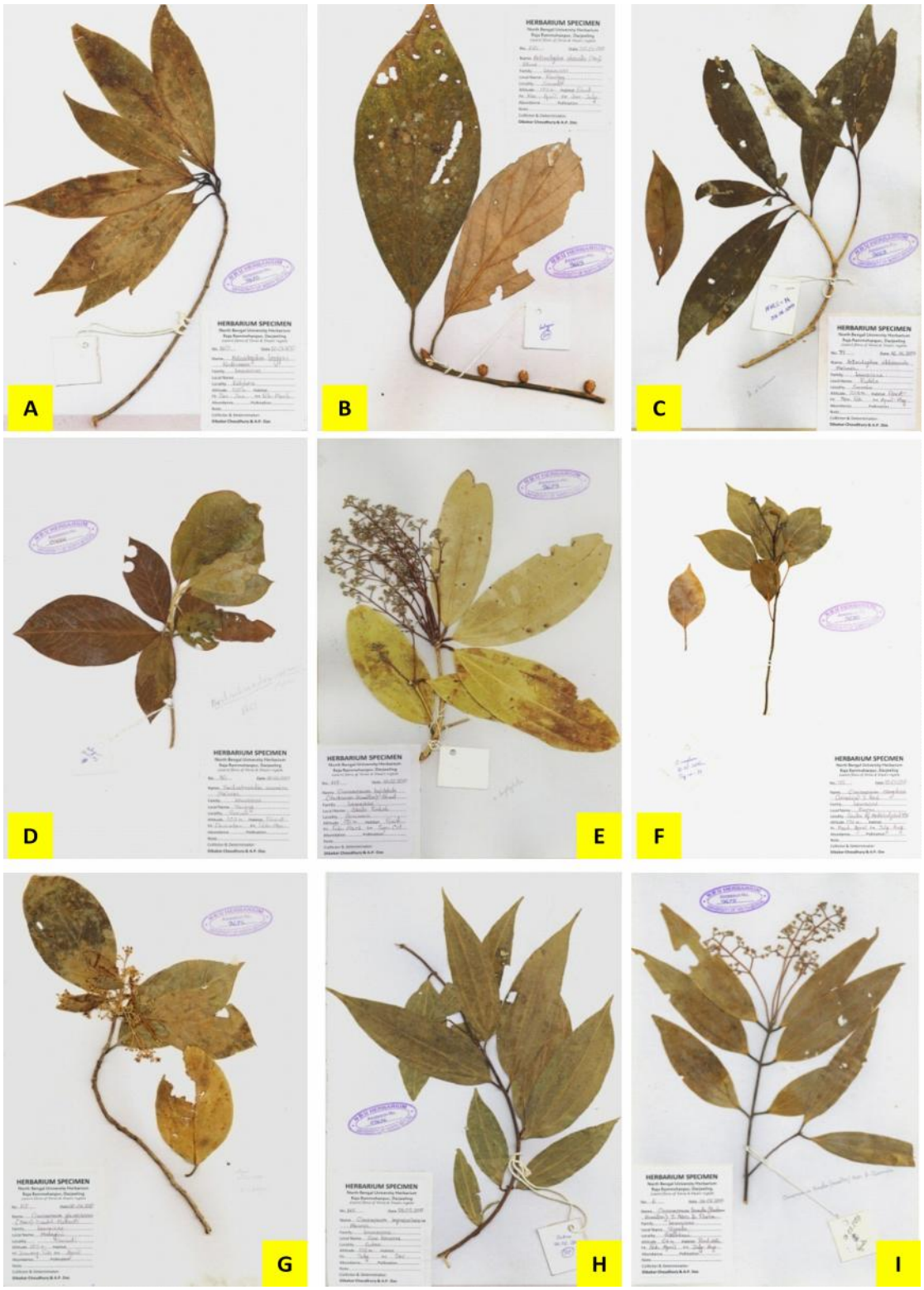


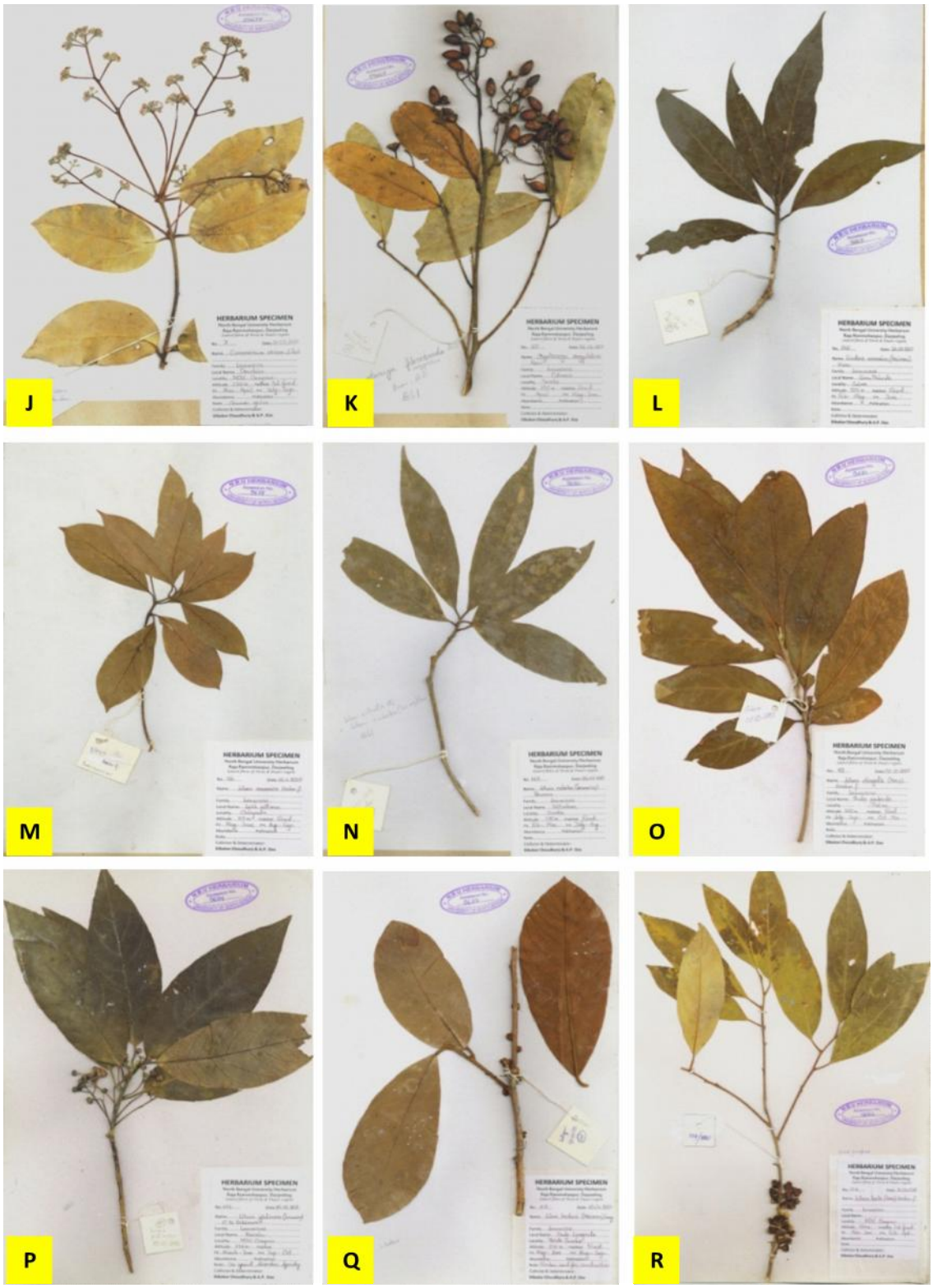
Figure 5.4. Cladistic approach of eight Laurels on the basis of morphological characters

5.3.5. Phylogeny of economically important Laurels

From the recorded 26 Laurels of Terai- Duars region, 8 species of two genera (*Viz. Cinnamomum bejolghota, C. camphora, C. tamala, C. verum, Litsea assamica, L. glutinosa, L. laeta, L. monopetala*) which were abundant as well as economically very important, were taken for phylogenetic study. From the morphological data of these species a dendrogram was prepared where *Litsea* and *Cinnamomum* were distinct in two different branches (Fig 5.4). In case of *Litsea* firstly divided into two branches, one branch represented *L. monopetala* and another branch again divided into three, where *L. glutinosa* and *L. laeta* were correlated than *L. assamica*. On the other hand the branch of *Cinnamomum* is split into two, where *C. camphora* is separated from other three species. These three species were existed in another branch where *C. tamala* and *C. verum* were much linked than *C. bejolghota*.

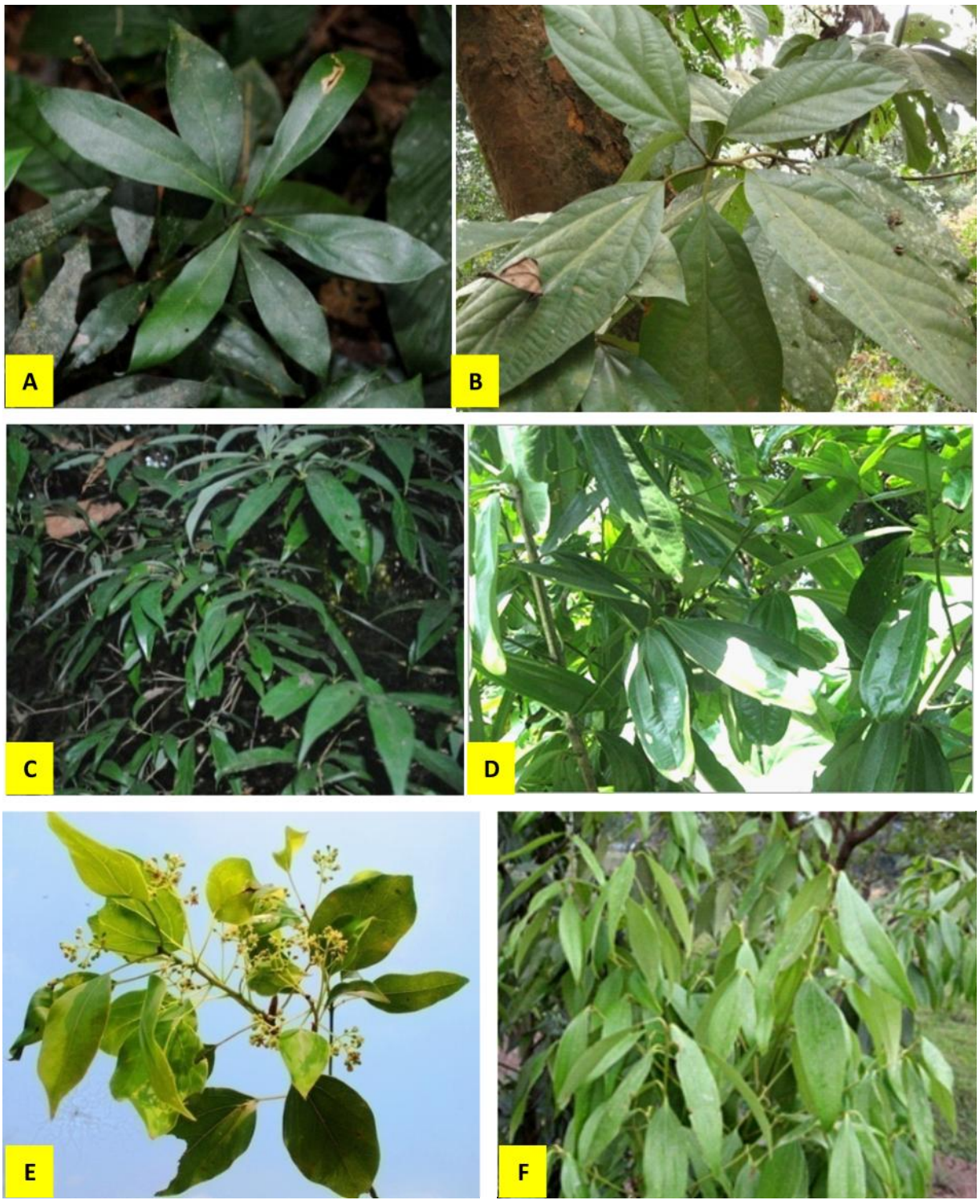


PLATE–I: Photograph of Laurels Herbarium sheet (A–I): A. *Actinodaphne longipes*; **B.** *A. obovata*; **C.** *A. sikkimensis*; **D.** *Beilschmiedia assamica*; **E.** *Cinnamomum bejolghota*; **F.** *C. camphora*; **G.** *C. glaucescens*; **H.** *C. impressinervium* **I.** *C. tamala*



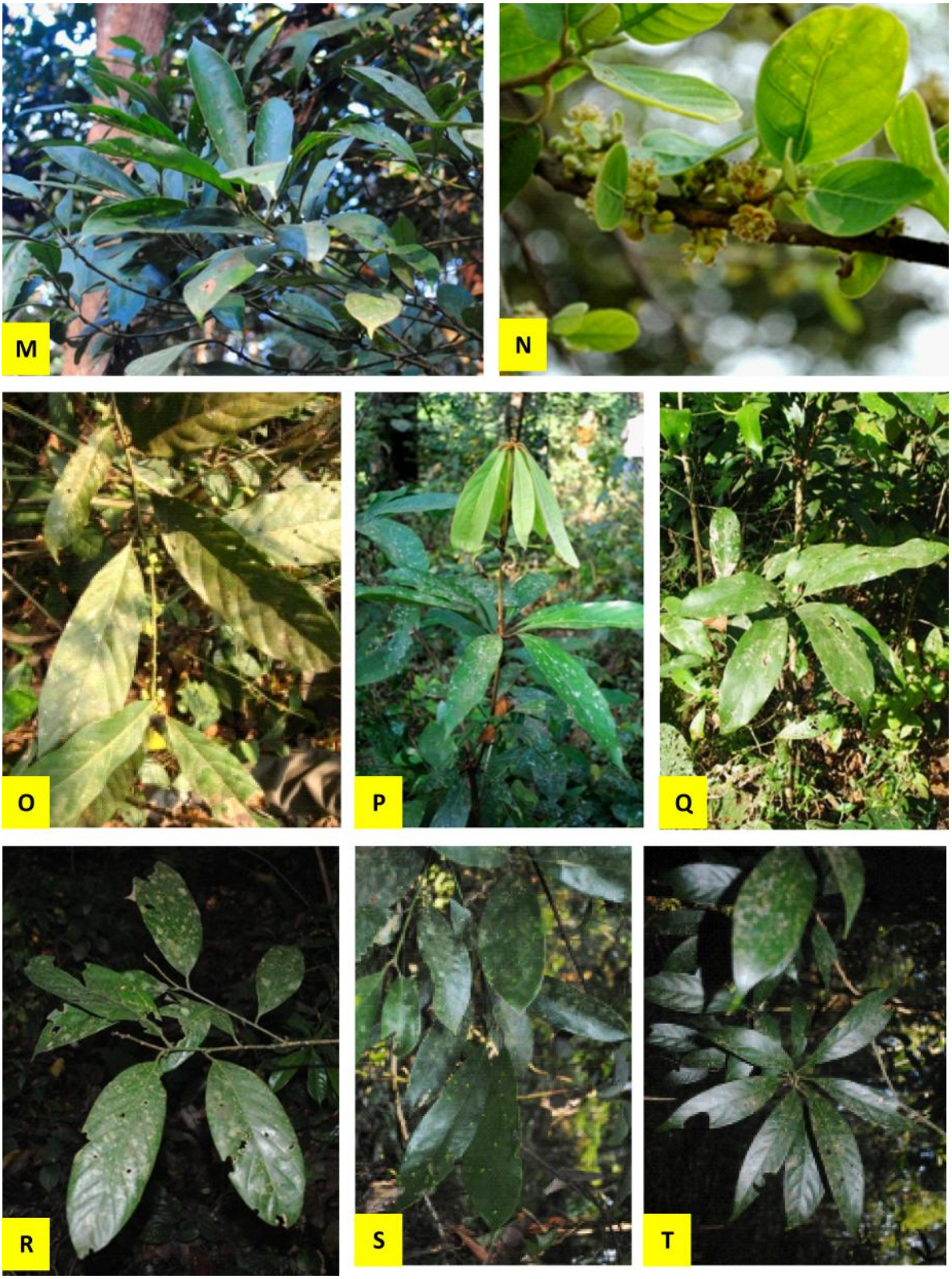
PLATE–II: Photograph of Laurels Herbarium sheet (J–R): J. *Cinnamomum verum*; **K.** *Cryptocarya amygdalina*; **L.** *Lindera assamica*; **M.** *Litsea assamica*; **N.** *L. cubeba*; **O.** *L. elongata*; **P.** *L. glutinosa*; **Q.** *L. hookeri*; **R.** *L. laeta*

PLATE–III: Photograph of Laurels Herbarium sheet (S–Z):S. *Litsea monopetala*; **T.** *L. Panamanja*; **U.** *L. Salicifolia*; **V.** *Machilus duthiei*; **W.** *M. gamblei*; **X.** *M. glaucescens*; **Y.** *Parsea odoratissima*; **Z.** *Phoebe hainesiana*



PLATE–IV: Photograph of Laurels in field (A–F): A. *Actinodaphne longipes*; **B.** *A. obovata*; **C.** *A. sikkimensis*; **D.** *Cinnamomum bejolghota*; **E.** *C. camphora*; **F.** *C. tamala*

PLATE–V: Photograph of Laurels in field (G–L): G. *Cinnamomum verum*; **H.** *Cryptocarya amygdalina*; **I.** *Lindera assamica*; **J.** *Litsea assamica*; **K.** *L. glutinosa*; **L.** *L. hookeri*



PLATE–VI: Photograph of Laurels in field (M–T): M. *Litsea laeta*; **N.** *L. monopetala*; **O.** *L. Salicifolia*; **P.** *Machilus duthiei*; **Q.** *M. gamblei*; **R.** *M. glaucescens*; **S.** *Parsea odoratissima*; **T.** *Phoebe hainesiana*

CHAPTER - 6
Anatomical Studies

Anatomical Studies

6.1. INTRODUCTION

Taxonomy being a multi-disciplinary subject, various parameters contribute to the pool of taxonomic data. The initial and prime need of taxonomy is to identify the species and to understand its relationships. It is the morphological data which has been mostly utilized from the beginning of taxonomic studies for plant identification. Because, plants are enriched with external almost unlimited forms or states of morphological characters which can be easily recognized with naked eye or through a simple magnifying lens. So, morphological characteristics have often represented the basis of taxonomic studies in plants (Adedeji 2005) and are very significant in classification. However, sometimes it becomes difficult to distinguish the closely related species with their too much similar or overlapping morphological characteristics. Under such troubled situation one or more other available techniques need to be used for differentiation and identification. Advanced skills like phytochemistry and DNA sequencing are providing data in recent times; but classical data-sources like anatomical studies have been used successfully since ancient times to clear up innumerable identification problems (Gilani *et al.* 2002; Lande 2009). The most accepted works on anatomical study has been presented in '*Systematic Anatomy of the Dicotyledons'* by Solereder (1908) and '*Anatomy of Dicotyledons*' by Metcalfe & Chalk (1950). Bailey (1951) has published an outstanding paper to justify and for the utilization of anatomical data in classification and for delineating the phylogeny.

Comparative anatomy has proved useful in systematic purposes (Agbagwa & Ndukwu 2004). It has played a crucial role in solving the problems of misplaced and anomalous taxa. By microscopical assessment it has been possible to assign sterile plant specimens to a family or even to a genus. So, anatomy proves very helpful for identifying herbarium or fresh specimens which are not accompanied with any reproductive organ i.e. flowers and/or fruits (Metcalfe & Chalk 1950).Anatomical methods are also used as important tools to provide the botanical identity of commercial samples like medicinal as well as spice plants and thus play an essential aspect in checking adulteration.

On the other hand plasticity of characters is a serious matter (Carlquist 2001). Most of the ecological adaptations have been given an idea about dissimilar divergences among the same species growing in different habitats. However, all these adaptations, must not be supposed to be quite general. If that was so, all species which were belonging to the same ecological condition would acquire the same biological and structural appearance, even if they were from the most widely separated systematic groups. Supplementary to this, habitat and climate do not omit anyone definite type of biological as well as anatomical structure, to be correlated with these factors, upon all the species of a general geographical area. So, it can be said that the species carry definite diagnostic characters, which may vary in extent.

Though data from anatomical studies is continuously added, the information is just fragmentary and we still stand on the first level of information only. On the basis of review of literature it may be concluded that, so far, no anatomical study was done on the members of Lauraceae nor any kind of

observations stated on those growing in Terai-Duars region of West Bengal except their antioxidant values (Choudhury *et al.* 2013a) and external morphological characterization (Choudhury *et al.* 2013b, 2014). Therefore, the main aim of present study is to investigate some anatomical features of some species of Laurels growing in the study area. So, for the present dissertation the anatomical characterization of petiole, leaf and stem of eight economically important Laurels has been taken up, which may contribute to understand the similarities and dissimilarities among those for their effective identification even in the sterile condition.

6.2. RESULTS

The results of anatomical studies on the recorded species of two genera of Lauraceae from the study area are presented below in alphabetical order:

6.2.1. *Cinnamomum bejolghota* (Buchanan–Hamilton) Sweet

6.2.1.1. Anatomy of stem (Figure 6.1.A & Table 6.1.)

a. Epidermis: Single layered oval cell as with cutinized epidermis.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with lignified, thick outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone, where the cells are thin walled, polyhedral and compact. Then the cortex is gradually transformed into phloem. Outer surface of stem is characterised by islands of sclerenchyma in pericyclic region, which are connected by an even band of stone cells in this species. Many secretory cells, tannin, stone cells, oil globules and acicular raphides are present in cortex region.

c. Vascular bundle: Open vascular bundle includes outer thick and continuous cylinder of the phloem. Phloem elements in the outer part are crushed and forming thick dark lines. The Xylem

Table 6.1. Anatomical characteristics of stem of different Laurels

Plants	Microscopic characteristics						
	Plant part	Sclarified tissue	Secretary cells	Stone cells	Blast fibers	Oil globules	Raphides or prismatic crystals
C. bejolghota	Stem	-	+++	-	+	+	++
C. camphora		+	++	-	++	+++	-
C. tamala		++	+++	++++	++	++	+++
C. verum		++	++++	+++	+	+++	++
L. assamica		+++	+	+	++	+	-
L. glutinosa		+++	++	+	++	++	-
L. laeta		+++	++	+	++++	+	-
L. monopetala		++++	+	++	+++	+	-

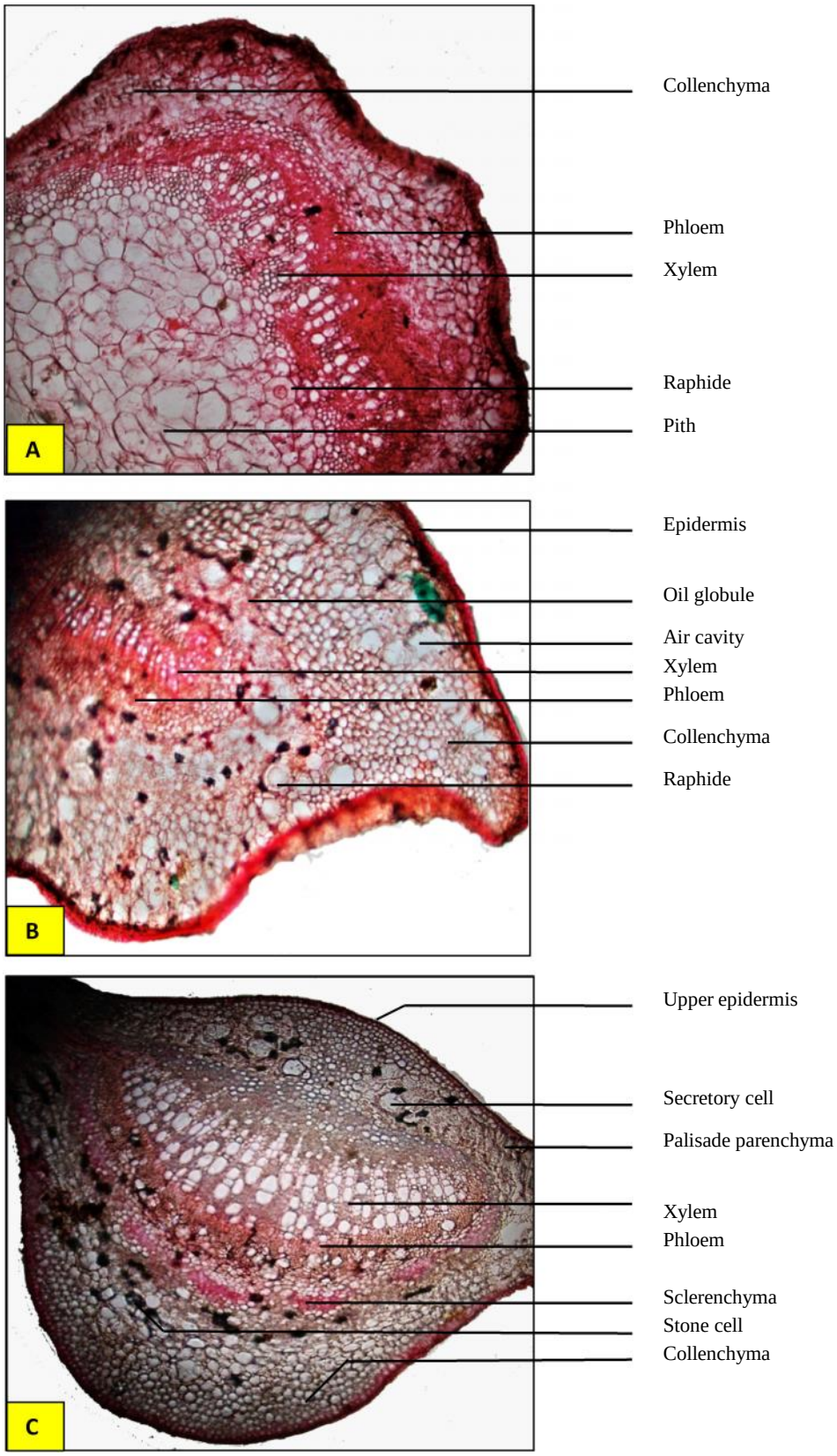


Figure 6.1. ***Cinnamomum bejolghota*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith is occupied by thin walled, compact and circular parenchymatous cells. There are also many secretory cells, oil droplets, acicular raphides and stone cells.

6.2.1.2. Anatomy of petiole (Figure 6.1. B & Table 6.2.)

a. Epidermis: In outline of petiole exhibits convex at adaxial side and abaxial side concave. The cells are sinuous and with many scarifications. The epidermis is of thin-walled small squarish cells.

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semicircular layer of crushed cells. Secretory cells, stone cells, oil globules and raphides or prismatic crystals are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire petiole. Xylem elements are in parallel lines and each line of xylem is having 5 – 8 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

Table 6.2. Anatomical characteristics of petiole of Laurels

Plants	Microscopic characteristics							
	Plant part	Hypodermis	Air cavities	Sclarified tissue	Secretary cells	Stone cells	Oil globules	Raphides or prismatic crystals
C. bejolghota	Petiole	Parenchymatous	++++	++	++++	+++	+++	+++
C. camphora		Colenchymatous	++	+	+++	+++	++++	+++
C. tamala		Parenchymatous	+++	+	+++	+++	++++	++++
C. verum		Parenchymatous	+++	++	++++	++++	++++	+++
L. assamica		Parenchymatous	+	+++	+	+	++	+
L. glutinosa		Parenchymatous	++	+++	++	+	++	++
L. laeta		Parenchymatous	++	++	++	++	+	+
L. monopetala		Parenchymatous	++	++++	++	+++	+	++

6.2.1.3. Anatomy of lamina (Figure 6.1. C & Table 6.3.)

a. Epidermis: Epidermal cells are single layered covered with smooth cuticle, thin on upper surface and thick on lower. These cells are sinuous and stomata are confined to lower.

b. Mesophyll: Uniseriate upper epidermal layer is followed by double layers of chloroplast filled palisade cells. The palisade cells are radially elongated. In between the lower epidermal cells and palisade cells there are several layers of loosely arranged spongy parenchyma cells in the mesophyll with intercellular spaces. These spongy parenchyma cells are also chlorenchymatous.

Abundant deposits of yellow or golden yellow mucilage or secretory cells occur in mesophyll layer. Lysigenous cavities containing oil are present within mesophyll. Oil globules are distributed in laminer cells. Acicular raphides are more common in the cell adjacent to the vascular bundle (stele) and in spongy parenchyma.

c. Vascular bundle: Stele represented by a single shallow crescentic, collateral close vascular bundle; with xylem on the upper side and phloem towards the lower side. The xylem bundles are arranged in approximately 6 – 8 radial rows.

Table 6.3. Anatomical characteristics of lamina of different Laurels

Plants	Microscopic characteristics						
	Plant part	Epidermis	Sclarified tissue	Secretary cells	Stone cells	Oil globules	Raphides or prismatic crystals
C. bejolghota	Lamina	Cell wall Sinuous	++	++++	++++	+++	++++
C. camphora		Cell wall straight	++	++++	+++	++++	+++
C. tamala		Cell wall Sinuous	+++	+	+++	++++	+++
C. verum		Cell wall Sinuous	++	+++	+++	++++	+++
L. assamica		Cell wall Sinuous	++++	++	+	++	+
L. glutinosa		Cell wall Sinuous	++++	+++	++	++	+
L. laeta		Cell wall Sinuous	+++	++	++	+	+
L. monopetala		Cell wall Sinuous	++++	++	++	+	++

6.2.2. *Cinnamomum camphora* (Linnaeus) J. Presl

6.2.2.1. Anatomy of stem (Figure 6.2. A & Table 6.1.)

a. Epidermis: Single layered oval cells as with cutinized epidermis. Epidermal cell walls are slightly curved.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with thick, lignified outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone. Sclerenchymatous patches occur around the vascular bundle. Stone cells and raphides are absent. Few oil globules are present in cortex.

c. Vascular bundle: Open vascular bundle includes outer thick and continuous cylinder of the phloem. Phloem elements in the outer part are crushed and forming thick dark lines. Blast fibres are abundant. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith is occupied by thin walled, compact and semi circular parenchymatous cells.

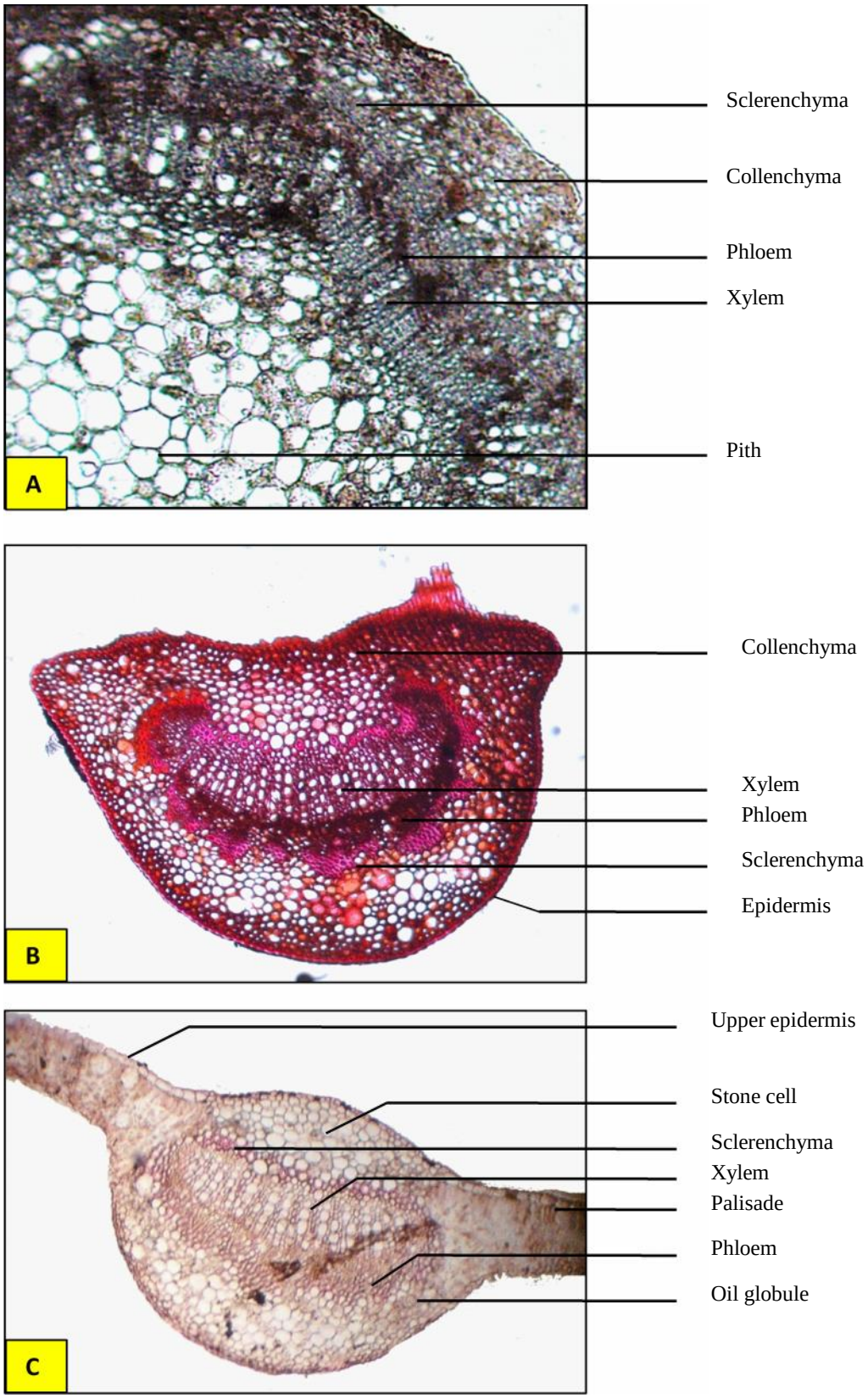


Figure 6.2. ***Cinnamomum camphora*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

6.2.2.2. Anatomy of petiole (Figure 6.2. B & Table 6.2.)

a. Epidermis: T.S. of petiole shows adaxial side with slightly out curving edges, whereas the abaxial side is sharply convex. Epidermis is cutinized and single layered.

b. Mesophyll: In colenchymatous hypodermis several oil, mucilage and tannin cells are found but air cavities are absent.

c. Vascular bundle: The vascular bundle is arc shaped with incurving edges surrounded by a thick continuous zone of sclerenchyma. The xylem is present towards the adaxial side whereas phloem towards the abaxial side that is adjacent to the sclerenchymatous zone.

6.2.2.3. Anatomy of lamina (Figure 6.2. C & Table 6.3.)

a. Epidermis: Cells are single layered covered with smooth cuticle, thin on upper surface and thick on lower. Epidermal cells are tetragonal to polygonal in shape, moderately sinuous and stomata confined to lower surface.

b. Mesophyll: Uniseriate upper epidermal layer is followed by single layer of chloroplast-filled palisade cells. In between the lower epidermal cells and palisade cells there are several layers of loosely arranged spongy parenchyma cells in the mesophyll with intercellular spaces. These spongy parenchyma cells are also chlorenchymatous. Abundant deposits of yellow or golden yellow mucilage or secretory cells occur in mesophyll layer. Lysigenous cavities containing oil are present within mesophyll. Oil globules are distributed in laminar cells. Raphides are absent.

c. Vascular bundle: Stele represented by a single shallow cresentic, collateral and close vascular bundle; with xylem on the upper side and phloem towards the lower side. The xylem bundles are arranged in approximately 10–12 radial rows.

6.2.3. *Cinnamomum tamala* (Buchanan–Hamilton) Nees &Ebermaier

6.2.3.1. Anatomy of stem (Figure 6.3. A & Table 6.1.)

a. Epidermis: Single layered oval cell as with cutinized epidermis.

b. Cortex: The sub-epidermal layer consists of semicircular cells with lignified, thick outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone, where the cells are thin walled, polyhedral and compact. Then the cortex is gradually transformed into phloem. Sclerenchymatous patches are present around the phloem. Many secretory cells, tannin, stone cells, oil globule and acicular raphides are present in cortex region.

c. Vascular bundle: Collateral open vascular bundle includes outer thick and continuous cylinder of the phloem. Phloem elements in the outer part are crushed and forming thick dark lines. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith is occupied by thin walled, compact and circular parenchymatous cells. There are also many secretory cells, oil droplets and stone cells.

6.2.3.2. Anatomy of petiole (Figure 6.3. B & Table 6.2.)

a. Epidermis: In outline of petiole exhibits concave at adaxial side and convex on abaxial side. The cells are sinuous and with many scarifications. The epidermis is of thin-walled small squarish cells.

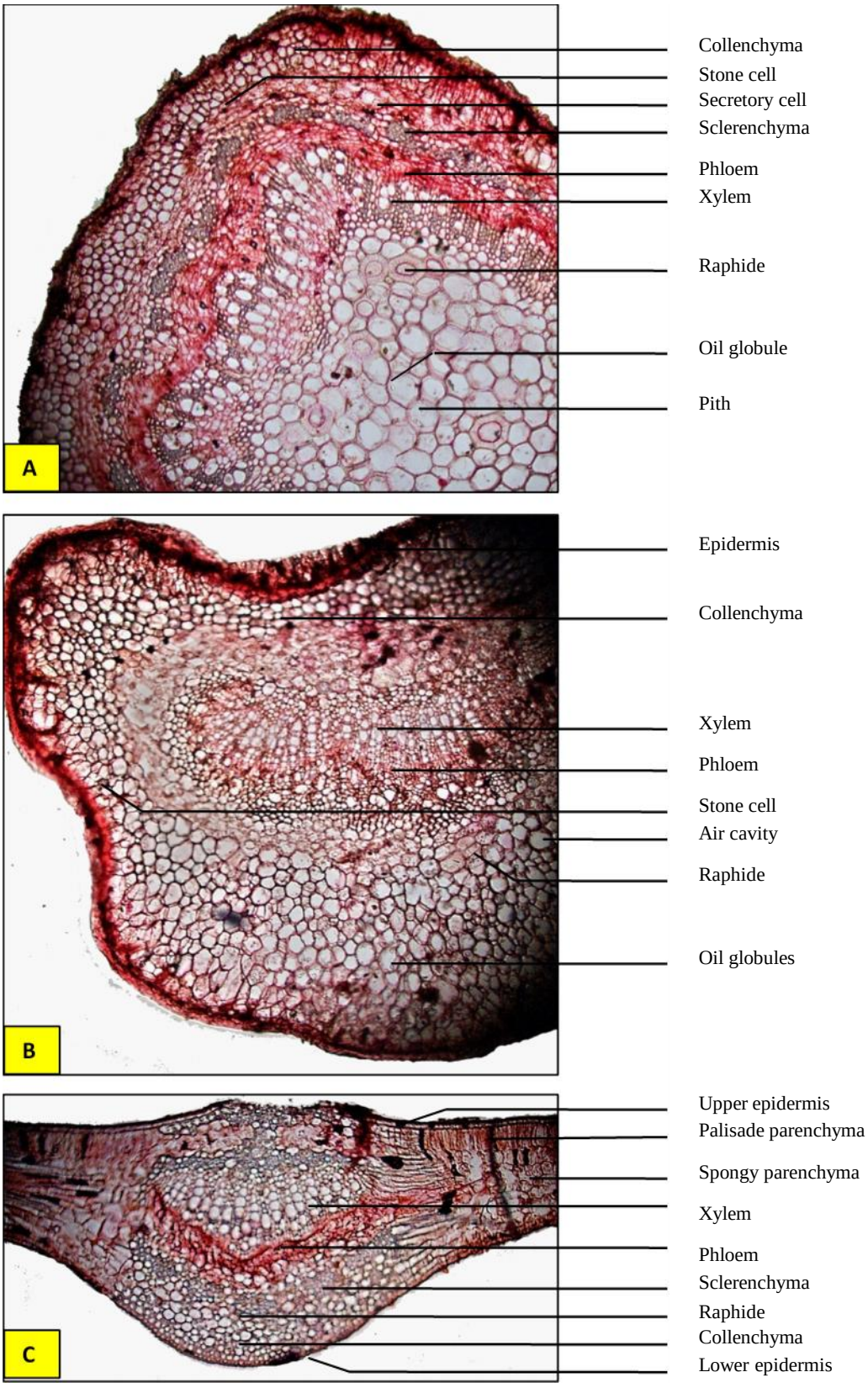


Figure 6.3. ***Cinnamomum tamala*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semi circular layer of crushed cells. Secretory cells, stone cells, oil globules and raphides or prismatic crystals are also present in the ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central region. Xylem elements are in parallel lines and each line of xylem is having 3 – 8 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.3.3. Anatomy of lamina (Figure 6.3.C & Table 6.3.)

a. Epidermis: Cells are single layered covered with smooth cuticle, thick on upper surface and thin on lower. Epidermal cells are sinuous and stomata confined to lower surface.

b. Mesophyll: Uniseriate upper epidermal layer is followed by one layer of chloroplast-filled palisade cells. The palisade cells are radially elongated. In between the lower epidermal cells and palisade cells there are several layers of loosely arranged spongy parenchyma cells in the mesophyll with intercellular spaces. These spongy parenchyma cells are also chlorenchymatous. Abundant deposits of yellow or golden yellow mucilage or secretory cells occur in the mesophyll. Lysigenous cavities containing oil are present within mesophyll. Oil globules are distributed in laminer cells. Acicular raphides are more common in the cell adjacent to the vascular bundle (stele) and in spongy parenchyma.

c. Vascular bundle: Stele represented by a single shallow, crescentic, collateral and close vascular bundles; with xylem on the upper side and phloems towards the lower side. The xylem bundles are arranged in approximately 10 – 12 radial rows.

6.2.4. *Cinnamomum verum* J. Presl

6.2.4.1. Anatomy of stem (Figure 6.4. A & Table 6.1.)

a. Epidermis: Single layered oval cells as with cutinized epidermis.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with lignified, thick outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone, where the cells are thin walled, polyhedral and compact. Then the cortex is gradually transformed into phloem. Outer surface of stem is characterised by islands of sclerenchyma in pericyclic region, which are connected by an even band of stone cells in this species. Many secretory cells, tannin, stone cells, oil globules and acicular raphides are present in cortex region.

c. Vascular bundle: Stele represented by collateral open vascular bundle includes outer thick and continuous cylinder of the phloem. Phloem elements in the outer part are crushed and forming thick dark lines. Blast fibres are rare in *C. verum*. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith occupied by thin walled, compact and circular ground cells. There are also many secretory cells, oil droplets and stone cells.

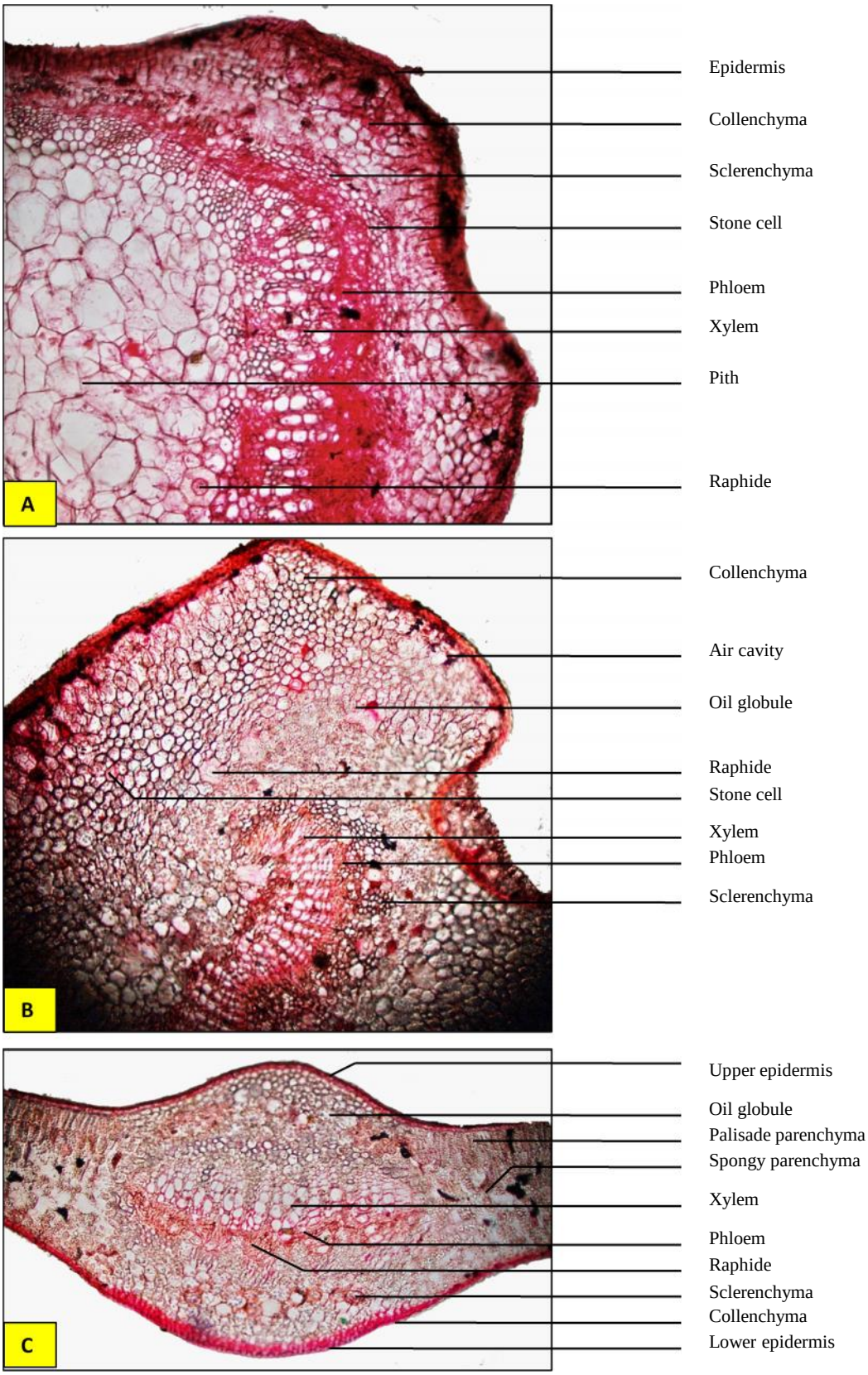


Figure 6.4. ***Cinnamomum verum*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

6.2.4.2. Anatomy of petiole (Figure 6.4. B & Table 6.2.)

a. Epidermis: In outline of petiole exhibits slightly convex at adaxial side and abaxial side enormously convex. The cells are sinuous and with many scarification. The epidermis is of thin-walled small squarish cells.

b. Mesophyll: Hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semi-circular layer of crushed cells. Secretory cells, stone cells, oil globules and raphides or prismatic crystals are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central area. Xylem elements are in parallel lines and each line of xylem having 3 – 8 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.4.3. Anatomy of lamina (Figure 6.4. C & Table 6.3.)

a. Epidermis: Cells are single layered covered with smooth cuticle, thin on upper surface and thick on lower; cells are sinuous and stomata confined to lower surface.

b. Mesophyll: Below the upper epidermis two layers of chlorenchymatous palisade cells and loosely arranged chlorenchymatous spongy cells, with prominent intercellular spaces, between the lower epidermal cells and palisade cells form the ground tissue. Abundant deposits of yellow or golden yellow mucilage or secretory cells occur in the mesophyll. Lysigenous cavities containing oil are present within mesophyll. Oil globules are distributed in laminer cells. Acicular raphides are more common in cells adjacent to the vascular bundle and in spongy parenchyma.

c. Vascular bundle: Stele represented by a single shallow crescentic, collateral close vascular bundle; with xylem in the upper side and phloem towards the lower. The xylem bundles are arranged in approximately 10 – 12 radial rows.

6.2.5. *Litsea assamica* Hooker *f.*

6.2.5.1. Anatomy of stem (Figure 6.5. A & Table 6.1.)

a. Epidermis: Single layered with squarish cells with the heavy cuticle, unicellular hairs few.

b. Cortex: The sub-epidermal layer consists of semicircular cells with thick, lignified outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone, where the cells are thin walled, polyhedral and compact. Then the cortex is gradually transformed into phloem. Sclerenchymatous patches are present around the phloem. Many secretory cells, tannin, stone cells, oil globules and acicular raphides are present in cortex region.

c. Vascular bundle: Open vascular bundle includes outer thick and continuous cylinder of phloem. Phloem elements in the outer part are crushed and they also collapsed into thick dark lines of tannin. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith is occupied by thin walled, compact and circular parenchymatous cells. There are also many secretory cells, oil droplets, acicular raphides and stone cells.

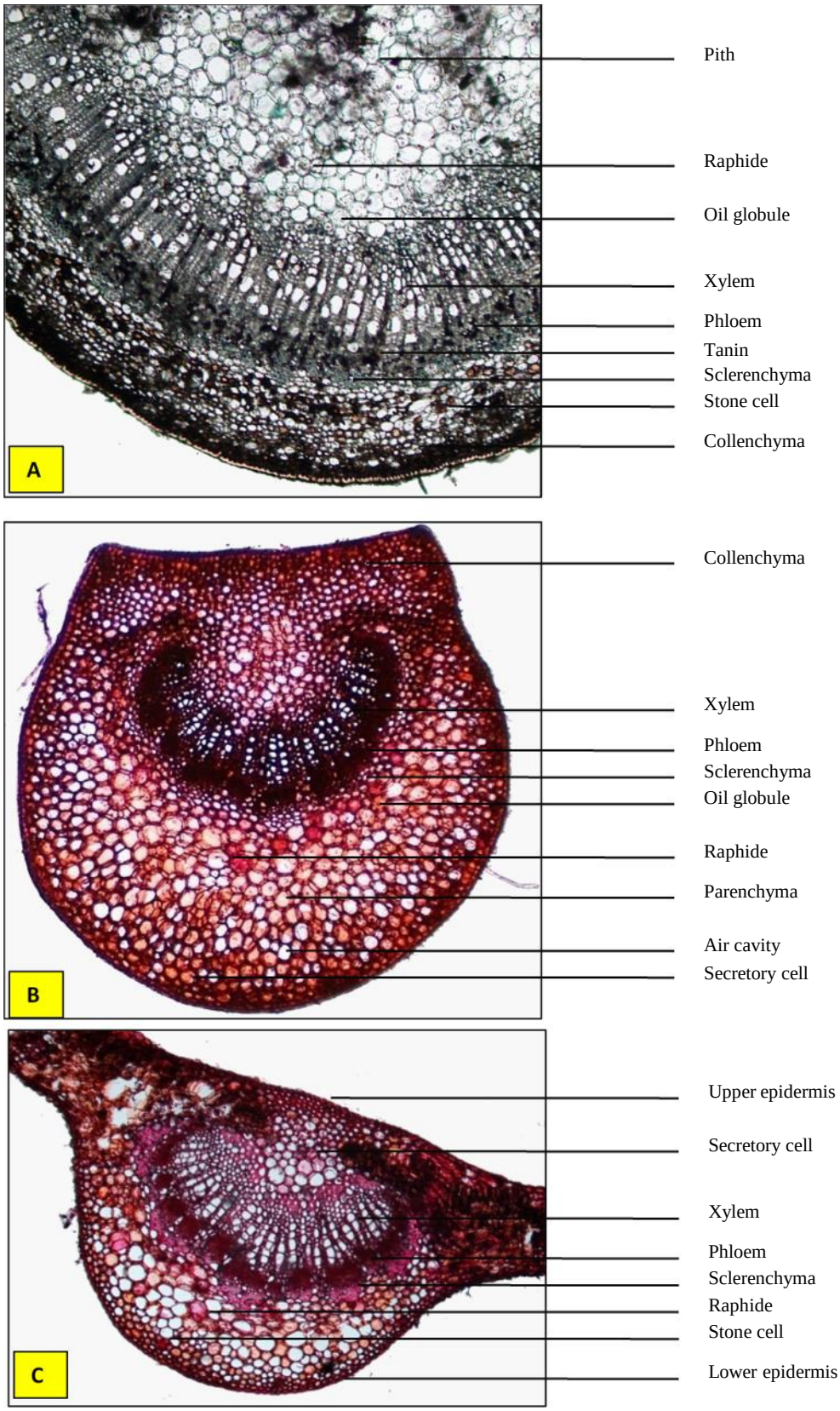


Figure 6.5. ***Litsea assamica*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

6.2.5.2. Anatomy of petiole (Figure 6.5. B & Table 6.2.)

a. Epidermis: Single layered cutinized epidermis with unicellular hair. The epidermal layer of the petiole is thin comprises of small squarish cells. In outline of petiole exhibits almost round in basal portion where, upper portion is flat in shape.

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semi-circular layer of crushed cells. Secretory cells, stone cells, oil globules and acicular raphides are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central region. Xylem elements are in parallel lines and each line of xylem is having 3 – 6 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. Below the phloem zone, wide circular masses of sclerenchymatous fibres are situated. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.5.3. Anatomy of lamina (Figure 6.5 C & Table 6.3.)

a. Eepidermis: The adaxial cells are tabular covered with thick smooth cuticle; where as abaxial cells are rectangular and slightly thicker and undulated. In epidermal wall many scarifications are found.

b. Mesophyll: The mesophyll cells consist of thick region of 2 – 4 layered palisade and 4 or 5 layered of spherical or lobed cells of spongy parenchyma. Some of these cells are modified into circular or four angled secretory idioblasts which are more frequent, distributed randomly in mesophyll tissue. Calcium oxalate crystals or acicular raphides and oil globules are present in the cells adjacent to vascular bundle and in spongy parenchyma.

c. Vascular bundle: Stele is represented by a small collateral close vascular bundle; with xylem on the upper side and phloem towards the lower side and surrounded by the sclerenchymatous bundle sheath. The xylem bundles are arranged in approximately 5 – 12 radial rows.

6.2.6. Litsea glutinosa (Loureiro) Robinson

6.2.6.1. Anatomy of stem (Figure 6.6. A & Table 6.1.)

a. Epidermis: Single layered with squarish cells along with heavy cuticle, unicellular hairs few.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with thick, lignified outer anticlinal walls. Inner to the lignified hypodermal layer, a narrow zone of 2 – 4 layers of periderm is formed. The periderm is followed by fairly 3 – 4 layered parenchymatous cortex.

c. Vascular bundle: The vascular cylinder is thick as well as hollow. It includes outer thick and continuous cylinder of the phloem. Phloem elements in the outer part are crushed and forming thick dark lines. The Xylem cylinder comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick walled.

d. Pith: Wide pith is occupied by thin walled, compact, circular parenchymatous cells and large central lysigneous cavity.

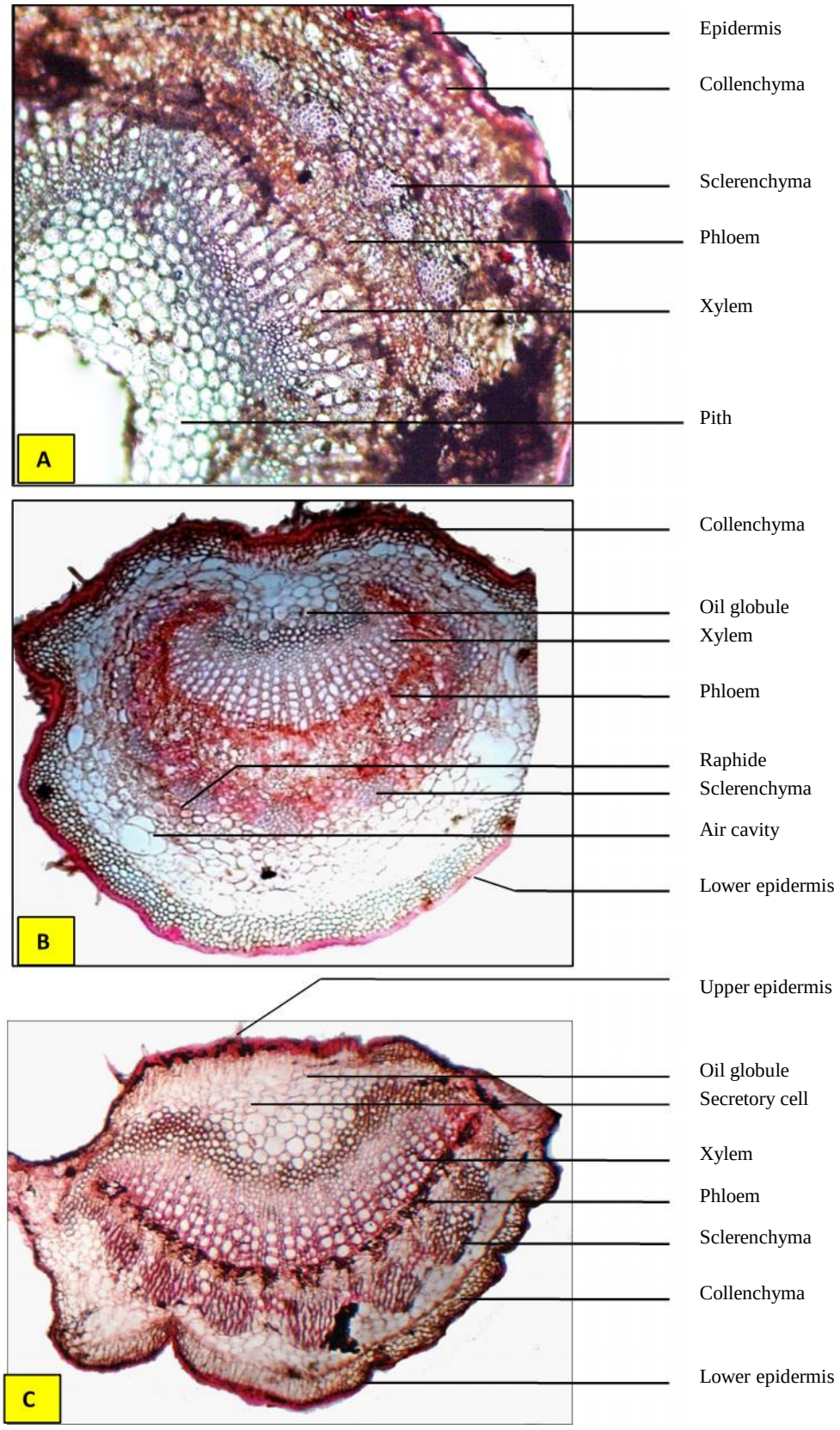


Figure 6.6. ***Litsea glutinosa*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

6.2.6.2. Anatomy of petiole (Figure 6.6. B & Table 6.2.)

a. Epidermis: Single layered cutinized epidermis with unicellular hairs. The epidermal layer of the petiole is thin comprises of small squarish cells. In outline, petiole exhibits slightly concave at adaxial side, while the abaxial side is convex.

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semicircular layer of crushed cells. Secretory cells, stone cells, oil globules and acicular raphides or prismatic crystals are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central region. Xylem elements are in parallel lines and each line of xylem is having 3 – 6 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. Below the phloem zone, about five to six wide circular masses of sclerenchymatous fibres are situated. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.6.3. Anatomy of lamina (Figure 6.6. C & Table 6.3.)

a. Eepidermis: The adaxial cells are tabular covered with thick smooth cuticle; whereas abaxial cells are rectangular, slightly thicker and undulated. In epidermal wall many scarifications are found.

b. Mesophyll: The upper epidermal layer is followed by two layers of pillar like palisade cells. In between the lower epidermal cells and palisade cells there are 4 or 5 layers of spherical spongy parenchyma with inter cellular spaces. Some of these cells are modified into circular or four angled secretory idioblasts which are more frequent, distributed randomly in mesophyll tissue. Calcium oxalate crystals or acicular raphides and oil globules are present in the cells adjacent to vascular bundles and in spongy parenchyma.

c. Vascular bundle: Stele is represented by a small collateral and close vascular bundle; with xylem on the upper side and phloem towards the lower side and surrounded by the sclerenchymatous bundle sheath. The xylem bundles are arranged in approximately 4 – 10 radial rows.

6.2.7. Litsea laeta (Nees) Hooker *f.*

6.2.7.1. Anatomy of stem (Figure 6.7. A & Table 6.1.)

a. Epidermis: Single layered with squarish cells along with heavy cuticle, unicellular hairs few.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with lignified, thick outer anticlinal walls. In between the periderm and phloem, the cortex is present in a narrow zone, where the cells are thin walled, polyhedral and compact. Then the cortex is gradually transformed into phloem. Sclerenchymatous patches are present around the phloem.

c. Vascular bundle: Open vascular bundle includes outer thick and continuous cylinder of phloem. Phloem elements in the outer part. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled with wide lumen.

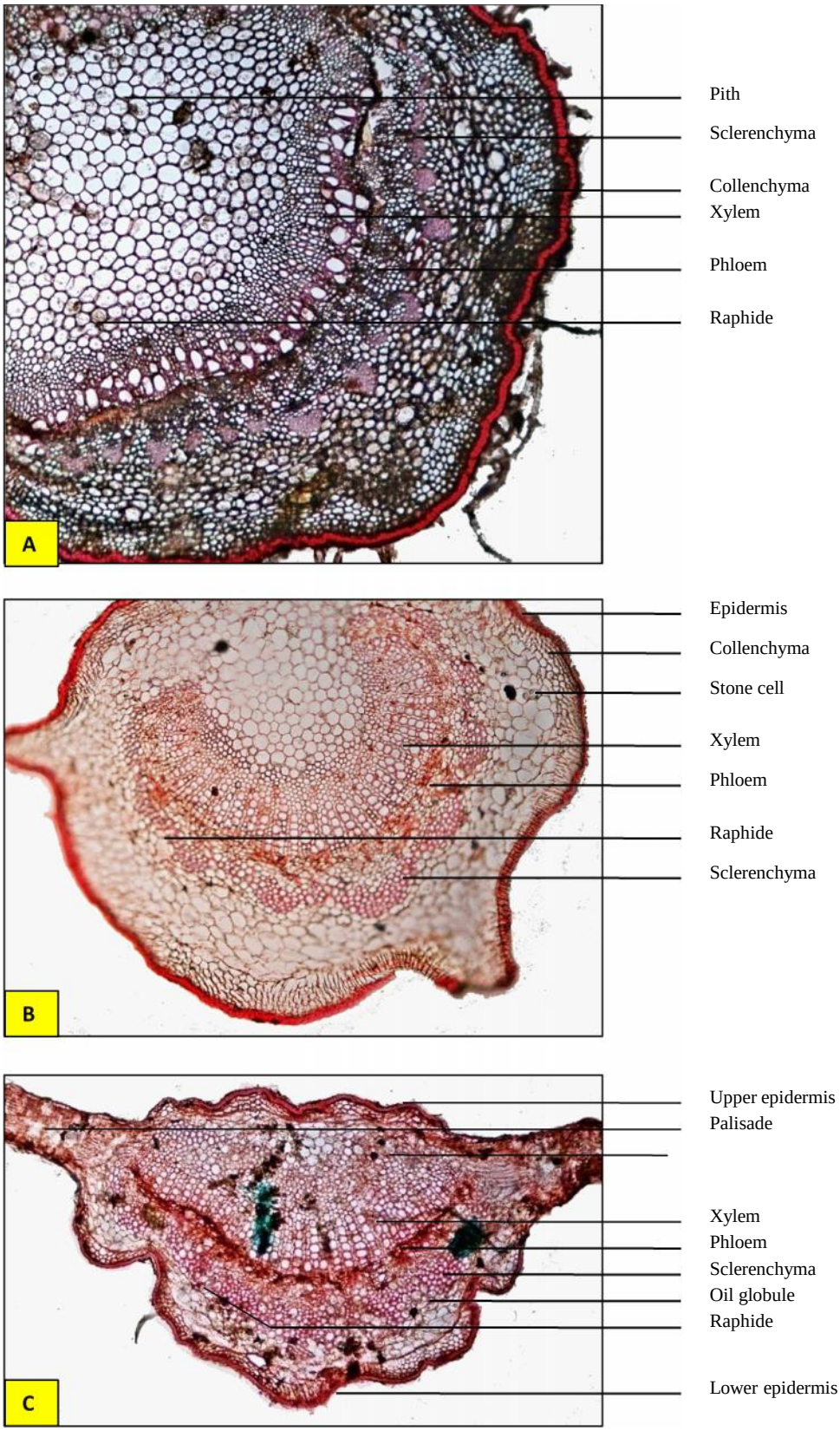


Figure 6.7. ***Litsea laeta*** transverse section of **A**- Stem; **B**- Petiole; **C**- Lamina

d. Pith: Wide pith is occupied by thin walled, compact and circular parenchymatous cells. There are also many secretory cells, oil droplets and stone cells.

6.2.7.2. Anatomy of petiole (Figure 6.7. B & Table 6.2.)

a. Epidermis: Single layered cutinized epidermis with unicellular hair. The epidermal layer of the peliole is thin comprises of small squarish cells. In outline of petiole exhibits almost round with three appendages.

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semi-circular layer of crushed cells. Secretory cells, stone cells, oil globules and acicular raphides or prismatic crystals are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central region. Xylem elements are in parallel lines and each line of xylem is having 3 – 8 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. Below the phloem zone, about five to six wide circular masses of sclerenchymatous fibres are situated. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.7.3. Anatomy of lamina (Figure 6.7. C & Table 6.3.)

a. Eepidermis: The adaxial cells are tabular covered with thick rough cuticle where as abaxial cells are rectangular, slightly thicker and undulated. In epidermal wall many scarifications are found.

b. Mesophyll: The mesophyll cells consist of thick region of 2 – 4 layered palisade and 4 or 5 layered of spherical or lobed cells of spongy parenchyma. Some of these cells are modified into circular or four angled secretory idioblasts which are more frequent, distributed randomly in mesophyll tissue. Calcium oxalate crystals or acicular raphides and oil globules are present in the cells adjacent to vascular bundle and in spongy parenchyma.

c. Vascular bundle: Stele is represented by a small collateral vascular bundle; with xylem on the upper side and phloem towards the lower side and surrounded by the sclerenchymatous bundle sheath. The xylem bundles are arranged in approximately 4 – 10 radial rows.

6.2.8. *Litsea monopetala* (Roxburgh) Persoon

6.2.8.1. Anatomy of stem (Figure 6.8. A & Table 6.1.)

a. Epidermis: Single layered with squarish cells along with heavy cuticle, unicellular hairs many.

b. Cortex: The sub-epidermal layer consists of semi-circular cells with thick, lignified outer anticlinal walls. Inner to the lignified hypodermal layer, a narrow zone of 2 – 4 layers of periderm are formed. The periderm is followed by fairly 3 – 4 layered parenchymatous cortex. Sclerenchymatous patches are scattered in cortex.

c. Vascular bundle: Open vascular bundle includes outer thick and continuous cylinder of phloem. The Xylem cylinder is comprises of vessels and fibres. The vessels are elliptical, wide and thin walled. They occur in long radial multiples or in solitary. Xylem fibres are lignified thick-walled.

d. Pith: Wide pith is occupied by thin walled, compact and circular parenchymatous cells.

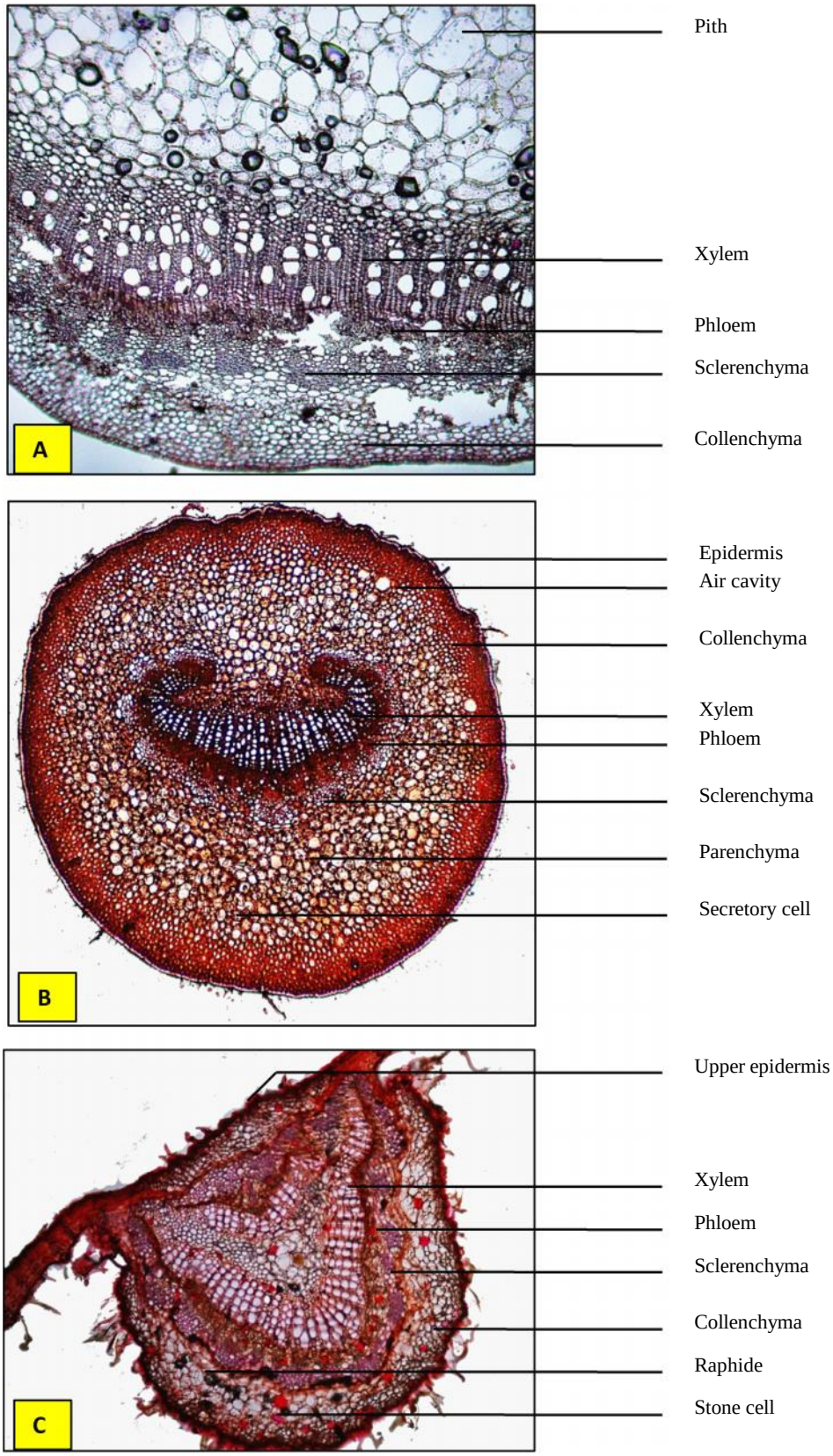


Figure 6.8. ***Litsea monopetala*** transverse section of **A-** Stem; **B-** Petiole; **C-** Lamina

6.2.8.2. Anatomy of petiole (Figure 6.8. B & Table 6.2.)

a. Epidermis: Single layered cutinized epidermis with unicellular hair. The epidermal layer of the petiole is thin comprises of small squarish cells. In outline of petiole exhibits almost round.

b. Mesophyll: The hypodermis is parenchymatous with air cavities present in regular intervals. The ground tissue consists of large sized parenchymatous cells, followed by a thick semi-circular layer of crushed cells. Secretory cells, stone cells, oil globules and acicular raphides or prismatic crystals are also present in ground tissue.

c. Vascular bundle: The arc shaped vascular strand is occupying the entire central region. Xylem elements are in parallel lines and each line of xylem is having 5 – 13 cells. On the lower end of the xylem strands occurs a thin horizontal band of phloem. Below the phloem zone, wide circular masses of sclerenchymatous fibres are situated. It includes a thick collateral vascular bundle with an abaxial horizontal pad of sclerenchyma.

6.2.8.3. Anatomy of lamina (Figure 6.8. C & Table 6.3.)

a. Eepidermis: The adaxial cells are tabular covered with thick smooth cuticle where as abaxial cells are rectangular, slightly thicker. In epidermal wall many scarifications are found.

b. Mesophyll: The upper epidermal layer is followed by four layers of pillar like palisade cells. In between the lower epidermal cells and palisade cells there are 4 or 5 layers of spherical spongy parenchyma with inter cellular spaces. Some of these cells are modified into circular or four angled secretory idioblasts which are more frequent, distributed randomly in mesophyll tissue. Calcium oxalate crystals or acicular raphides and oil globules are present in the cells adjacent to vascular bundle and in spongy parenchyma.

c. Vascular bundle: Stele is represented by a small collateral vascular bundle; with xylem on the upper side and phloem towards the lower side and surrounded by the sclerenchymatous bundle sheath. The xylem bundles are arranged in approximately 4 – 12 radial rows.

6.3. DISCUSSION

In this study, anatomical characteristics of eight economically important Laurels from the Terai-Duars region of West Bengal were examined in order to provide useful as well as additional information to the systematics. This is the first anatomical report on the members of Lauraceae from this part of the country. It is well known that the anatomical characters varies greatly as well as has significant values in many genera of this family such as *Cinnamomum, Laurus, Apollonias* etc. (Kamel & Loutfy 2001; Baruah & Nath 2006; Makbul *et al*. 2006). It is also recorded that, the presence and distribution of secretory cells, main bundles, mesophyll, indumentum (hairs) and surface features are mainly important identical features in different species of *Cinnamomum* (Kamel & Loutfy 2001). In these species of *Cinnamomum* under the present study, the shape of petiolar vascular bundle is a unique characteristic. The two ends of vascular bundle is curved towards the centre and thereby giving a wide arc-shaped appearance.

In the present study, the structural differences are found in several parts like stem, leaf and petiole. In all the studied taxa leaves were hypo-stomatic, the cells of the upper epidermis are different from the lower ones. Two types of cells were observed i.e. angular and sinuate, and the cells are often elongating over the veins. The similar results were observed by Christophel & Rowett (1996) on Australian Laurels.

Chamberlain (1975) reported the parameters of vascular bundles in stem, leaf and petiole, cells of cortex and pith are some of the most important characters in angiosperm classification and phylogeny. It is also well known that the distribution sites and the average number of collenchymatous cells are important in comparative anatomical studies (Özörgücü *et al*. 1991). In the present study most of the characters are similar in both the genera but distribution of stone cells, air cavities, distribution of sclerenchyma are dissimilar. On the basis the differentiations and similar anatomical characters Higher Archival clustering were drawn. This study concluded that there are two constant clustered groups in these two genera based on similarity in anatomical characters (Figure 6.9). These groups are: (1) *Litsea monopetala, L. glutinosa, L. leata* and *L. assamica* and (2) *C. bejolghota, C. tamala, C. verum* and *C. camphora*, share a wealth of anatomical characters. But, it has been noticed that *C. camphora* is different from other *Cinnamomum* spp on the basis of similarity curve.

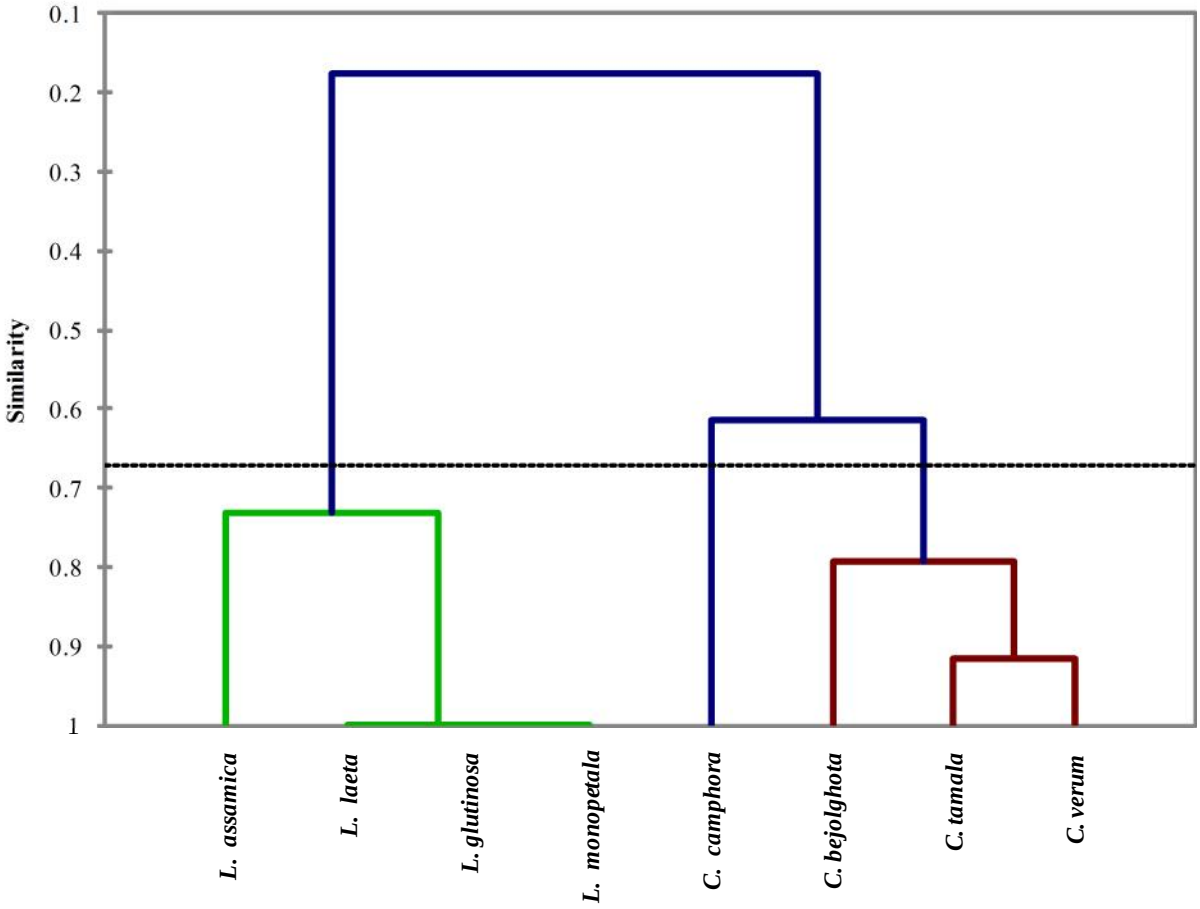


Figure 6.9. Cladistic approach of studied taxa on the basis of anatomical characters of different parts (stem, petiole and leaves)

As a whole, it is needed to be pointed out that the anatomical characters at the species level of these two genera under study found quite helpful at least in their identification and, on the other hand, such characters are also useful to recognize the adulterants of these spices or crud drugs.

6.3.1. An artificial key of the studied taxa based on the investigated anatomical characters is presented as below:

1a. Lamina and petiole not hairy ……. ……. ……. **2**
1b. Lamina and petiole hairy ……. ……. ……. **5**
2a. Raphides or prismatic crystals present in lamina and stem cells; hypodermis of petiole parenchymatous ……. ……. **3**
2b. Raphides or prismatic crystals absent in lamina and stem cells; hypodermis of petiole colenchymatous ……. ……. ***C. camphora***
3a. Crowded oil globules present in stem cells; stone cells present in stem; xylem bundles of lamina arranged in 10 – 12 radial rows……. **4**
3b. Oil globules unusual in stem cells; stone cells absent in stem; xylem bundles of lamina arranged in 6 – 8 radial rows ……. ……. ***C. bejolghota***
4a. Sclarified tissue present around vascular bundle of petiole; blast fibers many in stem ……. ……. ……. ***C. tamala***
4b. Sclarified tissue scattered in vascular bundle of petiole; blast fibers rare in stem ……. ……. ……. ……. ***C. verum***
5a. Medullary cells thin walled, compact and circular ……. … **6**
5b. Medullary region occupied by one large central lysigneous cavity .. ***L. glutinosa***
6a. Cross section of petiole almost circular; phloem elements of stem do not collapsed into thick dark line of tannin ……. …….. **7**
6b. Cross section of petiole flattened above and rounded below; phloem elements of stem collapsed into thick dark line of tannin … ***L. assamica***
7a. Adaxial epidermis of lamina with thick and rough cuticle; vascular bundle in lamina U-shaped ……. ……. ……. … ***L. laeta***
7b. Adaxial epidermis of lamina with thin and smooth cuticle; vascular bundle of lamina triangular ……. ……. ……. ***L. monopetala***

CHAPTER - 7

Leaf Architectural Study

Leaf Architectural Study

7.1. INTRODUCTION

From the ancient time identification and reconstruction of relationships between plants have been based mainly on features of their reproductive organs. The flower and fruit characters have established very useful for identification (Wilkinson 1979), but sometimes these organs are not available for study. For example, leaf impression and compression fossils are the most common macroscopic residues of extinct plants, but they are generally not attached to other plant organs when those are excavated. Because of their abundance and dense stratagraphic occurrence, fossil leaves can provide huge amount of information about the composition and diversity of ancient floras. Tropical botanists need to deal with and to identify and classify plants using mostly vegetative characters because so many long-lived tropical plants flower infrequently and irregularly (Leaf Architecture Working Group 1999). Here leaves are very important as because those are highly polymorphic organs and provide sets of diverse features. The term "leaf architecture" was defined by Hickey (1973) to denote the placement and form of those elements constituting the outward expression and leaf structure, as well as venation pattern, marginal configuration, leaf shape and gland position, etc. The veins and veinlets which form the vasculature, called the 'venation', is an important feature of mature leaves. Venation patterns are important characteristics used to determine many controversies in plant taxonomy (Laraño & Buot 2010). So, leaf architecture like venation pattern were studied in different families, such as: Asteraceae (Banerjee & Deshpande 1973), Berberidaceae (Singh *et al.* 1978), Betulaceae (Frank 1979), Euphorbiaceae (Sehgal & Paliwal 1974), Bignoniaceae (Jain 1978), Labiatae (Tyagi & Kumar 1978), Rosaceae (Merrill 1978), Solanaceae (Inamdar & Murthy 1978) and Scrophulariaceae (Varghese 1969), Hydrocharitaceae, Taccaceae, Dioscoreaceae, Smilacaceae, Araceae, Alismataceae, Aponogetonaceae (Inamdar *et al.*1983).

Beside venation, stomata and epidermal cells also provide taxonomically important diagnostic characters, such as the presence or absence of stomata on the adaxial or abaxial leaf surface and arrangement of epidermal cells adjacent to the guard cells (Tripathi & Mondal 2012). Generally, stomataare known as small openings in the epidermal layers which involve with gaseous exchange among intercellular spaces of sub-epidermal cells and the atmosphere. These are surrounded by guard cells, which organize the pore size. The stoma was first studied by Stresburger (1866) followed by Vesque (1989) who described four basic stomata types as Rannanculaceous, Cruciferous, Rubiaceous and Caryophyllaceous, based on the presence and arrangement of accessory or subsidiary cells along with their mode of development (Ahmad *et al.* 2009). Stace (1980) reported 31 different types of stomata among dicotyledonous plants. Whereas Metcalfe and Chalk in 1950 described main four types of stomata, i.e. Anisocytic, Anomocytic, Diacytic and Paracytic.

Another important character of leaf surface is hair. The micro-morphological characteristics of foliar hair played a significant role in plant systematics, especially of particular groups or smaller taxa at generic and specific levels (Hardin 1979). It was suggested by early investigators that the presence or

absence of peltate hairs and their form, size and colour could be used in distinguishing different genera and species of plants (Cooper 1931; Spring 2000).

So, the present work is undertaken to produce a comprehensive account of the leaf architecture in 8 species under two genera, *Cinnamomum* Schaeffer and *Litsea* Lamarck of the Lauraceae as no such report is available on these two genera.

7.2. RESULTS

Fine leaf architectural attributes of both the studied taxa, i.e. of *Cinnamomum* Schaeffer and *Litsea* Lamarck were summarized in the following tables in which major and minor venation patterns, stomatal and indumentum types along with numerical data were included. Here, the macro-morphological and micro-morphological features of species under the same genus were varied to some extent. It was observed that the leaves of all studied taxa were simple with entire margin and bear symmetric lamina with marginal petiolar attachment. Hairs were entirely absent in all the species of *Cinnamomum.* Lobation was absent and agrophic veins were simple in all species of the two genera.

7.2.1. External Leaf Morphology

7.2.1.1. Shape and Size

***Cinnamomum*:** Opposite phyllotaxy was observed in three species of *Cinnamomum* whereas in *C. camphora* leaves are produced in alternate (spiral) phyllotaxy was found and it contained comparatively longer petiole (Table 7.1). Laminar size varied much in these four species of *Cinnamomum*. It was maximum in *C. bejolghota* and minimum in *C. camphora.* So, these can be categorized under different blade class (Figures 7.1A, 7.2A, 7.3A & 7.4A). Laminar shapes were assorted from ovate to elliptic obovate. Laminar L:W ratio was also considerably diverse (Table 7.1). Apex and base angle was more or less same in all species i.e. acute<90^{o} (Table 7.1). Apex shapes were varied from acute to acuminate whereas base shape was same in all *Cinnamomum* species (Table 7.1; Figures 7.1B & D, 7.2B & D, 7.3B & D, 7.4B & D).

***Litsea*:** Phyllotaxy was alternate in *Litsea* spp. In *L. assamica*, though the leaves are in alternate phyllotaxy but are crowded at branch tip giving an appearance of whorled arrangement (Table 7.1). Petiole length is shortest in *L. assamica* than the other species of *Litsea*. In *L. monopetala* size of lamina was larger than the others and the blade class was mesophyll type (i.e. area of lamina in 4,500–18,225 mm^{2}),whereas smaller lamina was found in *L. assamica* and the blade class was notophyll type (i.e. area of lamina between 2,025–4,500 mm^{2}) (Table 7.1). Laminar shapes were observed almost elliptic in three studied taxa of *Litsea* where as in *L. glutinosa* it was ovate (Figures 7.5A, 7.6A, 7.7A & 7.8A). Laminar L:W ratio was also varied to some extent (Table 7.1). Apex and base angles were noticed more or less similar (apart from *L. monopetala*) in all studied *Litsea* spp i.e. acute<90^{o}. *L. monopetala* was varied from other three *Litsea* species with its rounded base and apex while base shape was cuneate and apex was acute to acuminate in others (Table 7.1; Figures 7.5B & D, 7.6B & D, 7.7B & D, 7.8B & D).

7.2.1.2. Major Venation Pattern

***Cinnamomum*:** The primary vein or 1^{o} vein was categorized and it was slightly different in *C. camphora* than other studied *Cinnamomum* species, where it was pinnate. In rest of other three

Table 7.1. Macro-morphological characteristics of studied taxa

Species	Phyllotaxy	Leaf organization	Petiole features	Lamina length (L) cm	Lamina breadth (W) cm	Laminar size (mm^2)	Blade class	Laminar shape	Laminar symmetry	Laminar L:W Ratio	Base angle	Apex angle	Base shape	Apex shape	Margin type	Lobation
C. bejolghota	Opposite	Simple	Smooth, slightly swollen at node, length 1.5 cm	22	7	10266	Mesophyll	Oblong elliptic	Symmetric	3.14:1	Approx. 63^0 i.e. acute $< 90^0$	Approx. 64^0 i.e. acute $< 90^0$	Cuneate	Acute	Entire	Unlobed
C. camphora	Alternate	Simple	Smooth, not swollen, length 2.5 cm	7.5	3.3	1650	Microphyll	Elliptic	Symmetric	2:1	Approx. 60^0 i.e. acute $< 90^0$	Approx. 60^0 i.e. acute $< 90^0$	Cuneate	Acuminate	Entire	Unlobed
C. tamala	Opposite	Simple	Smooth, not swollen, length 1.5 cm	14	6.7	6253	Mesophyll	Lanceolate	Symmetric	3.3:1	Approx. 65^0 i.e. acute $< 90^0$	Approx. 63^0 i.e. acute $< 90^0$	Cuneate	Acuminate	Entire	Unlobed
C. verum	Opposite	Simple	Smooth, not swollen, length 1.5 cm	10	6	4000	Notophyll	Ovate	Symmetric	2.2:1	Approx. 60^0 i.e. acute $< 90^0$	Approx. 60^0 i.e. acute $< 90^0$	Cuneate	Acute	Entire	Unlobed
L. assamica	Alternate with crowded tip	simple	Base not swollen, length 1.1 cm	9.1	3.6	2184	Notophyll	Elliptic	Symmetric	2.5:1	Approx 55^0 i.e. acute $< 90^0$	Approx 57^0 i.e, acute $< 90^0$	Cuneate	Acuminate	Entire	Unlobed
L. glutinosa	Alternate	Simple	Base slightly swollen, cylindrical, length 2.3 cm	13	5.6	4853	Mesophyll	Oblong elliptic	Symmetric	2.3:1	Approx 73^0 i.e. acute $< 90^0$	Approx 60^0 i.e, acute $< 90^0$	Cuneate	Acute	Entire	Unlobed
L. laeta	Alternate	Simple	Base slightly swollen, cylindrical, length 2.7 cm	12	5.2	4160	Notophyll	Oblong elliptic	Symmetric	2.3:1	Approx 70^0 i.e. acute $< 90^0$	Approx 65^0 i.e, acute $< 90^0$	Cuneate	Acute	Entire	Unlobed
L. monopetala	Alternate	Simple	Base slightly swollen, cylindrical, length 2.5 cm	12	8.5	6800	Mesophyll	Ovate oblong	Symmetric	1.4:1	Approx 95^0 i.e. obtuse $> 90^0$	Approx 109^0 i.e, acute $>90^0$	Round	Obtuse	Entire	Unlobed

species of *Cinnamomum*, it was observed acrodromous i.e. primaries were run in convergent arches toward the leaf apex. The 2° vein category was similar as 1° in their respective taxa but in *C. camphora*, it was weak brochidodromous (as 2° joined together in a series of arches). 2° vein spacing was absent in *C. tamala*, *C. verum* and *C. bejolghota* as there was only one pair of 2° vein, whereas in *C. camphora* it was increased towards the base.Other features were almost similar in all the studied species of *Cinnamomum* (Table 7.2)**.**

Table 7.2. Major venation pattern in lamina of studied taxa

Species	1° vein category	2° vein category	Agrophic veins	2° vein spacing	2° vein angle	Inter 2° veins
C. bejolghota	Acrodromous	Acrodromous (suprabasal)	Simple	Absent	One pair acute basal secondaries	Absent
C. camphora	Pinnate	Weak brochidodromous	Simple	Increasing toward base	Smoothly decreasing toward base	Absent
C. tamala	Acrodromous	Acrodromous (suprabasal)	Simple	Absent	One pair acute basal secondaries	Absent
C. verum	Acrodromous	Acrodromous (suprabasal)	Simple	Absent	One pair acute basal secondaries	Absent
L. assamica	Pinnate	Weak brochidodromous	Simple	Decreasing toward base	Smoothly decreasing toward base	Absent
L. glutinosa	Pinnate	Weak brochidodromous	Simple	Decreasing toward base	Smoothly increasing toward base	Absent
L. laeta	Pinnate	Weak brochidodromous	Simple	Decreasing toward base	Smoothly increasing toward base	Absent
L. monopetala	Pinnate	Weak brochidodromous	Simple	Irregular	Smoothly increasing toward base	Absent

Litsea**:** Here**,** 1° vein category was perceived as pinnate in all the species. 2° vein category was similar in all species, particularly weak brochidodromous. Absence of inter 2° veins was another notable feature. Beside these other features were almost similar (Table 7.2), though 2° vein spacing was irregular in *L. monopetala* and 2° vein angle was smoothly decreased toward the base in *L. assamica*.

7.2.1.3. Minor Venation Pattern

In all studied species 4°s were crossed between tertiaries in alternate percurrent way (i.e. with an abrupt angular discontinuity) and 5°s were regular polygonal reticulate (i.e. veins anastomose with other veins to form polygons of similar size and shape). Beside this, well developed areoles and freely ending ultimate veins were found in all the Laurels studied here (Table 7.3).

Table 7.3. Minor venation pattern in lamina of studied taxa

Species	3° vein category	3° vein course	3° vein angle to 1°	3° vein angle variability	4° vein category	5° vein category	Areolation	F.E.V.S	Highest order	Highest Excurrent	Marginal ultimate venation	Leaf rank
C. bejolghota	Alternate percurrent	Convex	Acute	Uniform	Alternate percurrent	Regular polygonal reticulate	Well developed	2 or more branched	5°	4°	Looped, no teeth	3r
C. camphora	Alternate percurrent	Convex	Acute	Inconsistent	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear, unbranched	5°	3°	Looped, no teeth	3r
C. tamala	Alternate percurrent	Convex	Acute	Uniform	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear, unbranched	5°	3°	Looped, no teeth	3r
C. verum	Alternate percurrent	Convex	Acute	Uniform	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear, unbranched	5°	4°	Looped, no teeth	3r
L. assamica	Mixed opp/alt Percurrent	Sinuous	Obtuse	Inconsistent	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear unbranched	5°	4°	Looped, no teeth	4r
L. glutinosa	Mixed opp/alt Percurrent	Sinuous	Obtuse	Increasing basally	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear, unbranched	5°	4°	Fimbrial vein, no teeth	4r
L. laeta	Mixed opp/alt Percurrent	Sinuous	Obtuse	Increasing basally	Alternate percurrent	Regular polygonal reticulate	Well developed	Curved unbranched	5°	4°	Fimbrial vein, no teeth	4r
L. monopetala	Opposite Percurrent	Straight	Obtuse	Uniform	Alternate percurrent	Regular polygonal reticulate	Well developed	Linear unbranched	5°	4°	Looped, no teeth	4r

***Cinnamomum*:** All the mentioned features up to high order venation were observed more or less similar in all the studied taxa. 3° vein category was alternate percurrent (i.e. tertiaries cross between adjacent secondaries with an offset) in all (Table 7.3). 3° vein angle variability was uniform (here tertiary angles was not varied over the lamina surface) in *C. tamala, C. verum* and *C. bejolghota* but it was inconsistent (i.e. tertiary angle varied randomly over lamina) in *C. camphora*. Highest excurrent vein was 3° in case of *C. camphora* and *C. tamala*, whereas in other two it was 4°. Looped marginal ultimate venation (ultimate veins recurved to form loops) and 3r leaf rank was noticed in case of all the studied species of *Cinnamomum* (Table 7.3).

***Litsea*:** Opposite percurrent type (where tertiaries cross in parallel paths without branching) of 3° vein category was noticed in *L. monopetala*. Whereas other three *Litsea* species were exhibited mixed opposite and alternate percurrent type of 3° vein. 3° vein angle variability was found uniform in *L. monopetala*, increased towards base in *L. laeta* as well as in *L. glutinosa* and in consistent in *L. assamica*. Looped marginal ultimate venation was found in *L. monopetala* and *L. assamica* whereas in *L. glutinosa* and *L. laeta* fimbrial (where higher vein orders were fused into a vein running just inside the margin) marginal ultimate venation was noticed. All the studied species of *Litsea* were categorized under the 4r leaf rank. Other features were almost same in all studied species of *Litsea* (Table 7.3).

7.2.2. Areolation and F.E.Vs

In this study it was found that the maximum areolation frequency in apical part of the lamina and minimum in the basal part for all species of *Cinnamomum* (Figures 7.1G-J, 7.2G-J, 7.3.G-J, 7.4G-J and Table 7.3 & 7.4) and *Litsea* (Figures 7.5 H-K, 7.6H-K, 7.7H-K, 7.8H-K and Tables 7.3 & 7.5). F.E.Vs frequency was same as areoles, but breadth of midrib was gradually decreased towards the apex.

7.2.3. Stomatal Study

Various types of stomata were observed in different taxa such as anisocytic (containing single ring of three cells enclosing the guard cells), diacytic (bearing two subsidiary cells at right angle to the axis of guard cell), cyclocytic (with single ring of five or more cells enclosing the guard cells), anomocytic (five or more cells enclosing the guard cells, same as normal epidermal cells), anomotetracytic (four subsidiary cells enclosing the guard cell in irregular and variable pattern) and numerical data was obtained from camera lucida drawing and finally Stomatal Index and Stomatal Frequency were determined.

Cinnamomum: Stomatal type was different in all species of *Cinnamomum* under the present investigation (Figure 7.1F, 7.2F, 7.3F, 7.4F & Table 7.6). Stomatal frequency and epidermal cells in unit area was found maximum in *C. bejolghota* and minimum in *C. tamala*. Stomatal index was highest in *C. verum*.

***Litsea*:** Anisocytic stomata were observed both in *L. monopetala* and *L. assamica*. The number of epidermal cell per unit area was maximum in *L. assamica*. Stomatal index and stomatal frequency were highest in *L. monopetala* and lowest in *L. assamica* (Figure 7.5G, 7.6G, 7.7G, 7.8G & Table 7.6).

7.2.4. Indumentum (surface Hairs)

Hair is important among various type of indumentum and these can be considered as valuable criteria in the process of species identification. In this study, it was noticed that hairs were entirely absent in all *Cinnamomum* species. But all species of *Litsea* contained simple unicellular hairs, but hair length was different (Figure 7.5F, 7.6F, 7.7F, 7.8F & Table 7.7).

7.2.5. Cluster analysis

The prepared dendrogram clearly illustrated that *Litsea* has dissimilar characters than *Cinnamomum*. *C. camphora* is present in a separate clade from the other plants (Figure 7.9).

Table 7.4. Areolation and F.E.Vs of *Cinnamomum* spp.

Species name →		***C. bejolghota***			***C. camphora***			***C. tamala***			***C. verum***		
Parameter	Leaf part	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean
Areolation frequency /mm^2	base	.032	187.5	**262.55**	.017	1000	**1243.58**	.018	444.44	**545.58**	.021	476.19	**618.68**
	middle	.031	258.06		.013	1230.76		.016	500		.019	684.21	
	apex	.038	342.10		.012	1500		.013	692.30		.023	695.65	
FEVs Frequency /mm^2	base	.032	31.25	**77.76**	.017	117.64	**227.24**	.018	111.11	**176.45**	.021	95.23	**142.34**
	middle	.031	96.77		.013	230.76		.016	187.5		.019	157.89	
	apex	.038	105.26		.012	333.33		.013	230.76		.023	173.91	
Breadth of midrib **(µm)**	base	.032	80.62	**64.86**	.017	72.28	**42.62**	.018	66.72	**53.74**	.021	55.6	**50.04**
	middle	.031	61.16		.013	38.92		.016	55.6		.019	50.04	
	apex	.038	52.82		.012	16.68		.013	38.92		.023	44.48	

Table 7.5. Areolation and F.E.Vs of *Litsea* spp.

Species name →		***L. assamica***			***L. glutinosa***			***L. laeta***			***L. monopetala***		
Parameter	Leaf part	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean	Area (mm^2)	Value /mm^2	Mean
Areolation frequency /mm^2	Base	.015	866.67	**112.6.19**	.015	1200	**1297.43**	.018	722.2	**1031.93**	.021	761.9	**973.2**
	Middle	.012	1083.33		.015	1000		.014	1142.8		.019	894.7	
	Apex	.0091	1428.57		.013	1692.30		.013	1230.7		.019	1263	
FEVs frequency /mm^2	Base	.015	66.67	**169.96**	.015	133.33	**213.67**	.018	111.11	**185.38**	.021	190.47	**274.01**
	Middle	.012	333.33		.015	200		.014	214.28		.019	263.15	
	Apex	.0091	109.89		.013	307.69		.013	230.76		.019	368.42	
Breadth of midrib (µm)	Base	.015	69.5	**41.7**	.015	83.4	**50.04**	.018	111.2	**70.42**	.021	133.4	**86.1**
	Middle	.012	38.92		.015	44.48		.014	72.28		.019	83.4	
	Apex	.0091	16.68		.013	22.24		.013	27.8		.019	41.7	

Table 7.6. Stomatal study of studied taxa

Species	Stomata type	Microscopic Area (mm^2)	No. of epidermal Cell/mm^2	Stomatal Frequency Per mm^2	Stomatal Index(%)
C. bejolghota	Cyclocytic	.000269	137546.46	22304.83	13.95
C. camphora	Anomocytic	.000415	130120.48	21686.74	14.28
C. tamala	Anisocytic	.007023	7119.46	1139.11	13.79
C. verum	Diacytic	.005090	9430.25	2750.49	22.58
L. assamica	Anisocytic	.005619	8556.14	534.75	5.882
L. glutinosa	Anomocytic	.007808	5128.20	897.43	14.893
L. laeta	Anomotetracytic	.005264	5509.11	759.87	12.12
L. monopetala	Anisocytic	.006197	6777.47	1452.31	17.64

Table 7.7. Indumentum study of *Litsea* spp.

Parameters	*L. assamica*	*L. glutinosa*	*L. laeta*	*L. monopetala*
Type	Simple, Unicellular	Simple, unicellular	Simple unicellular	Simple, unicellular
Glandular ornot	Non-glandular	Non-glandular	Non-glandular	Non-glandular
Nature of origin	Few scattered hairs	Borne singly, Numerous hairs	Borne singly, Numerous hairs	Singly borne well scattered hairs
Branched or not	Non branched	Non branched	Non branched	Non branched
Tip shape	Acute	Acute	Acute	Acute
Basal origin	From epidermis	From epidermis	From epidermis	From epidermis
Stalk nature	Stalk unicellular	Stalk unicellular	Stalk unicellular	Stalk unicellular
Shape	Slightly curved, Not so much dense	Long, soft, curved dense	Long, soft, curved dense	Slender, curved long
Length in 10x (O.D)	5-8	20-60	20-40	7-12
Breadth in 10x (O.D)	1	1-2	1	1

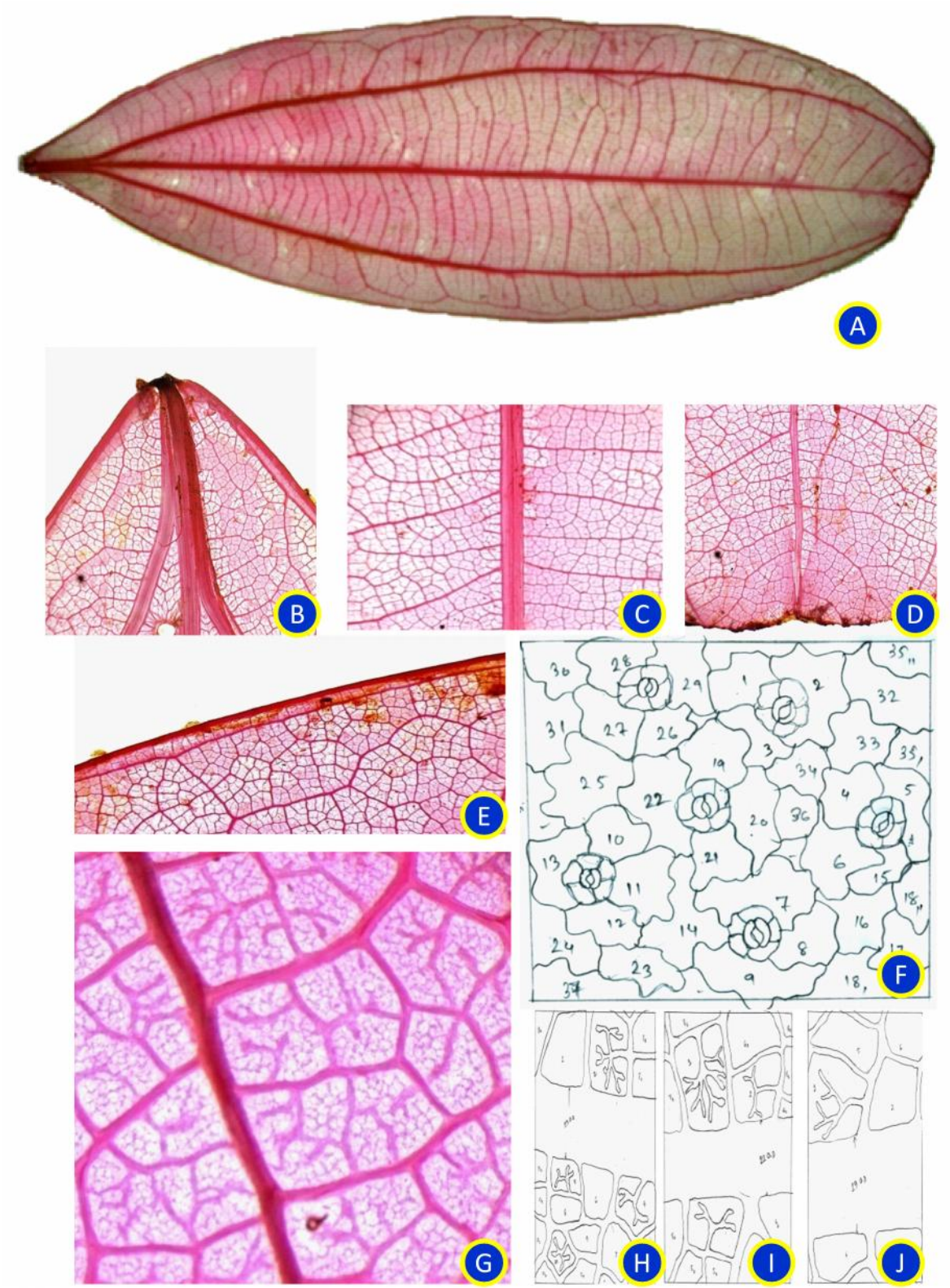


Figure 7.1. ***Cinnamomum bejolghota*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Camera lucida drawing of leaf stomata; **G**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **H**- Apex, **I**- Middle, **J**- Base

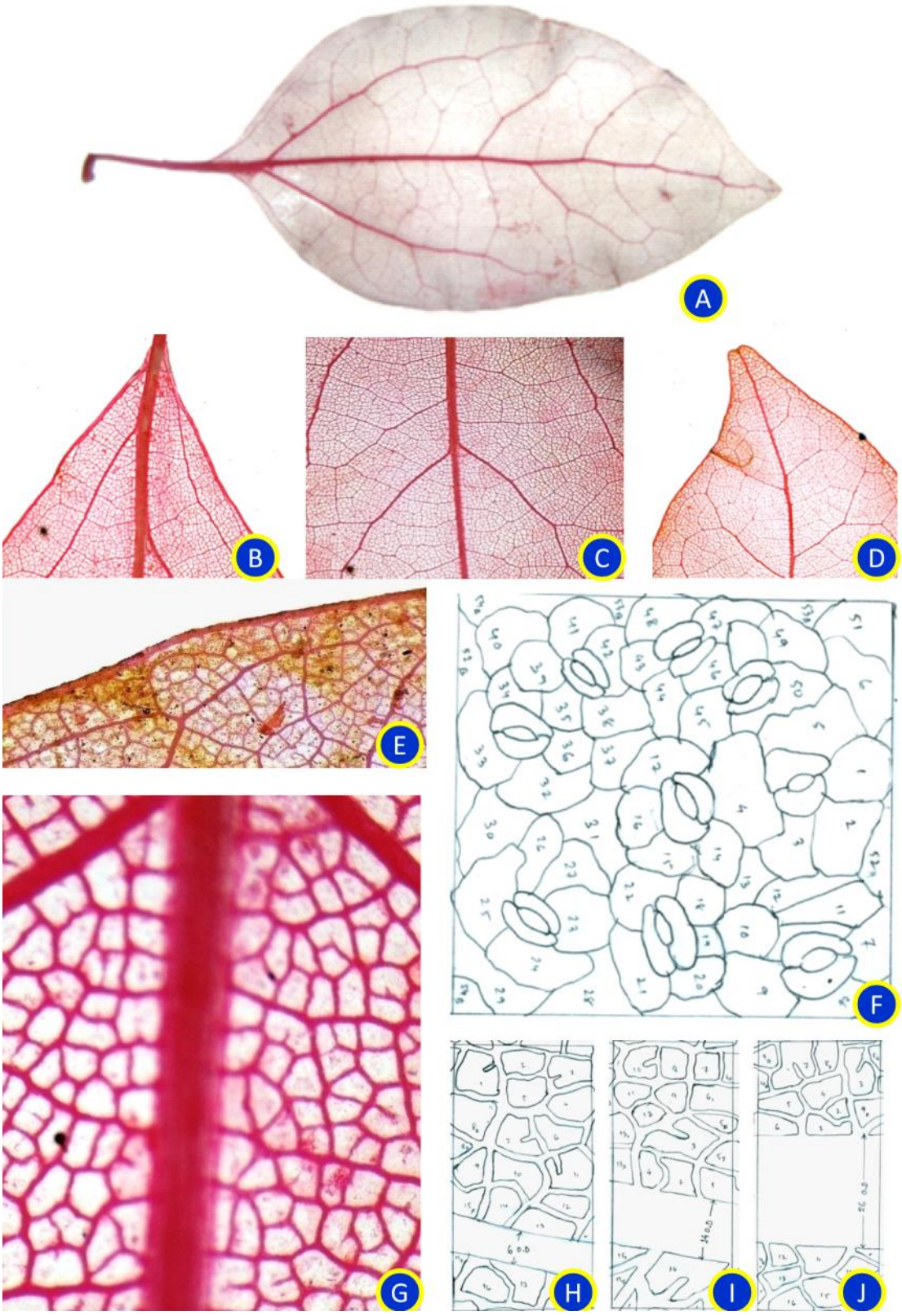


Figure 7.2. ***Cinnamomum camphora*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Camera lucida drawing of leaf stomata; **G**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **H**- Apex, **I**- Middle, **J**- Base

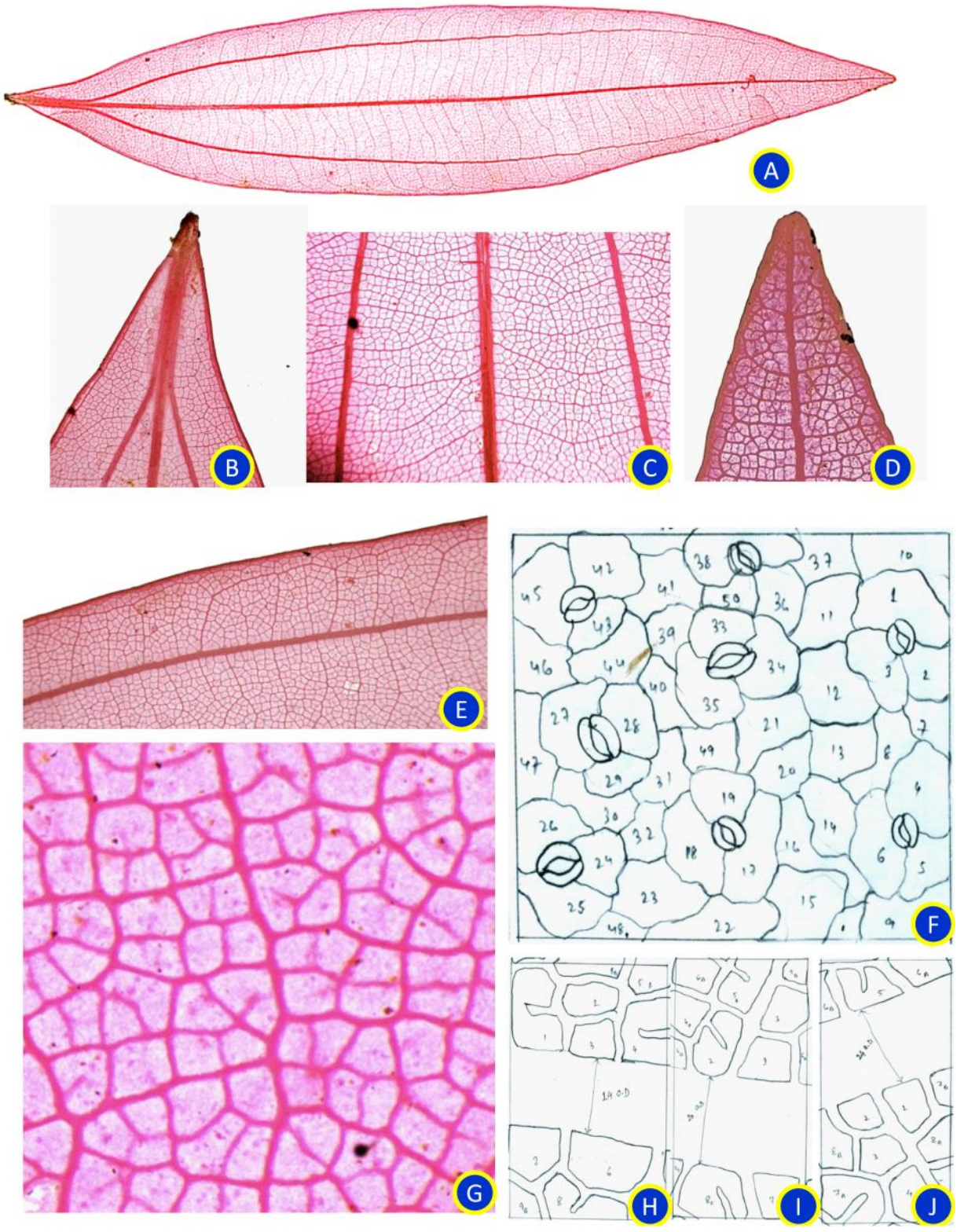


Figure 7.3. ***Cinnamomum tamala*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Camera lucida drawing of leaf stomata; **G**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **H**- Apex, **I**- Middle, **J**- Base

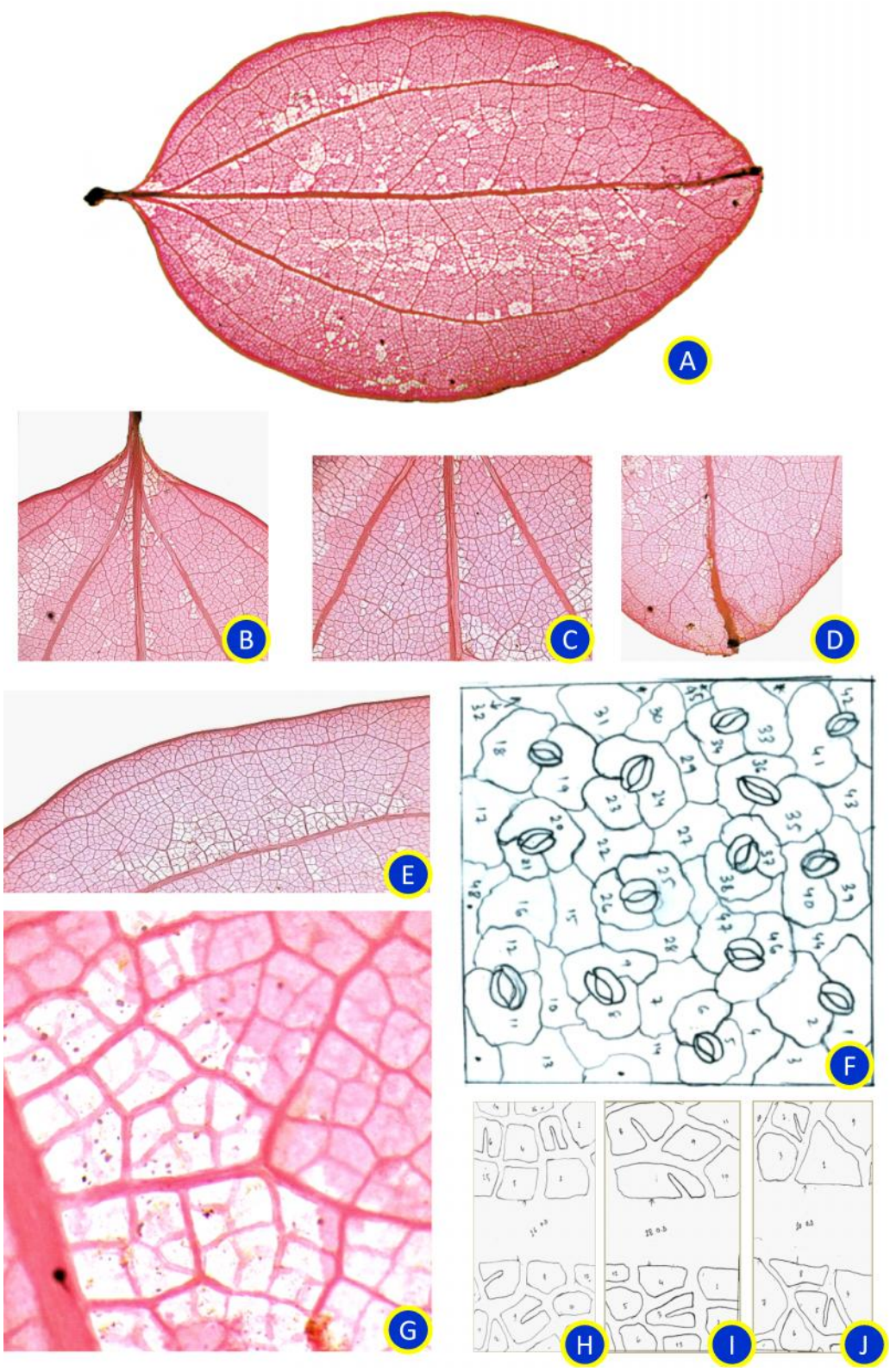


Figure 7.4. ***Cinnamomum verum*** **A-** Cleared whole leaf; **B-** Base; **C-** Middle; **D-** Apex; **E-** Untoothed margin; **F-** Camera lucida drawing of leaf stomata; **G-** Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **H-** Apex, **I-** Middle, **J-** Base

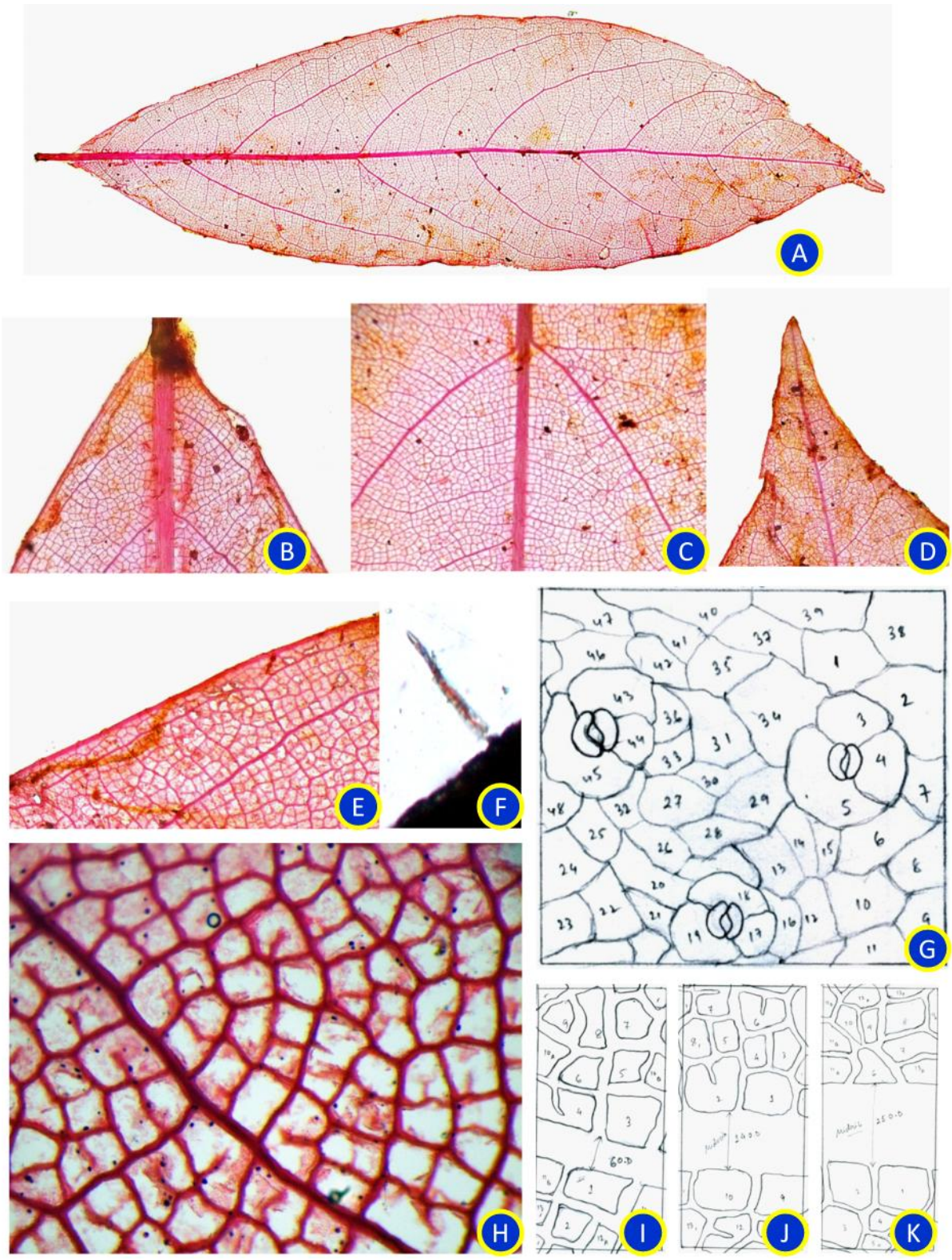


Figure 7.5. ***Litsea assamica*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Hair; **G**- Camera lucida drawing of leaf stomata; **H**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **I**- Apex, **J**- Middle, **K**- Base

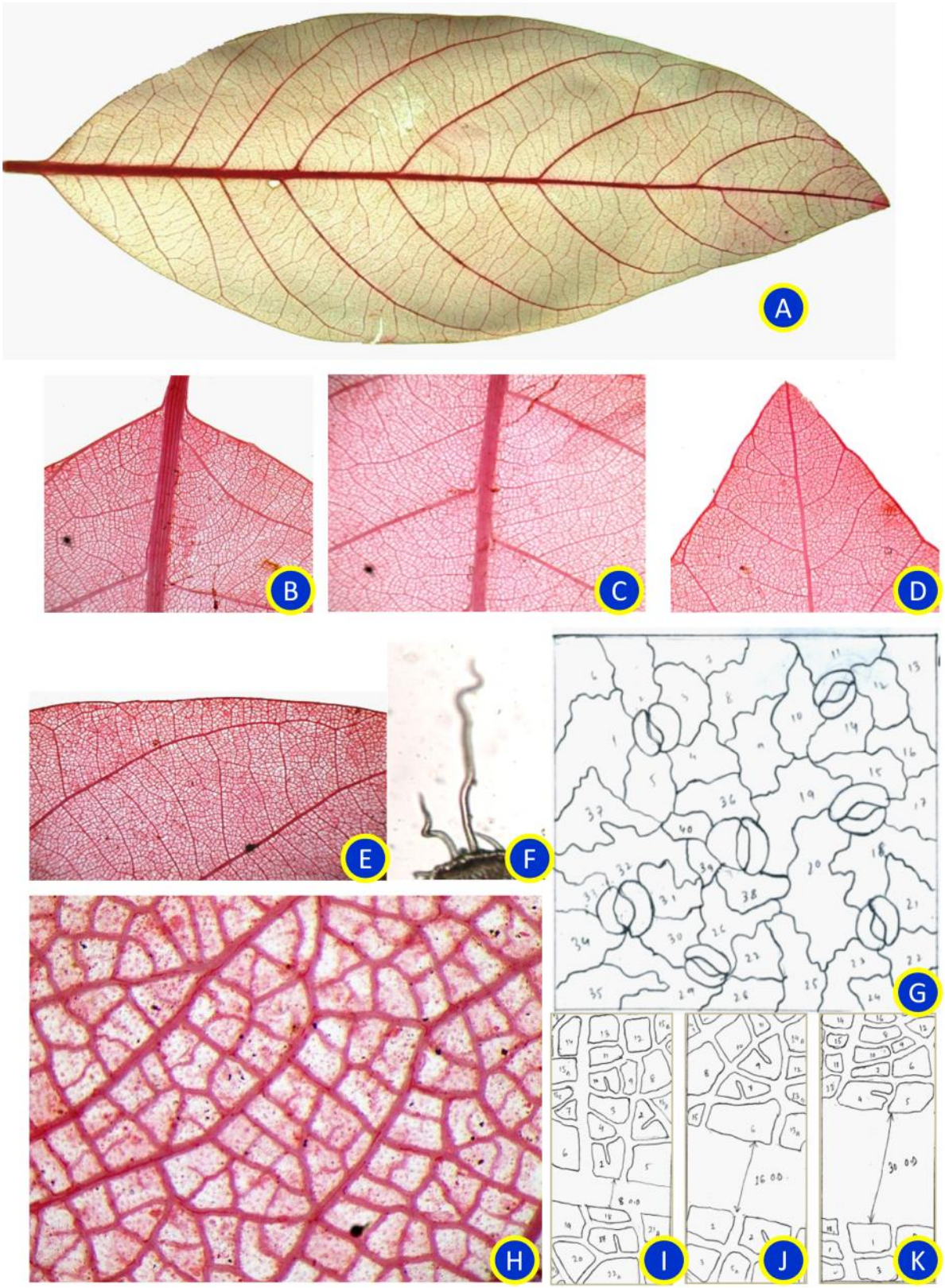


Figure 7.6. ***Litsea glutinosa*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Hair; **G**- Camera lucida drawing of leaf stomata; **H**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **I**- Apex, **J**- Middle, **K**- Base

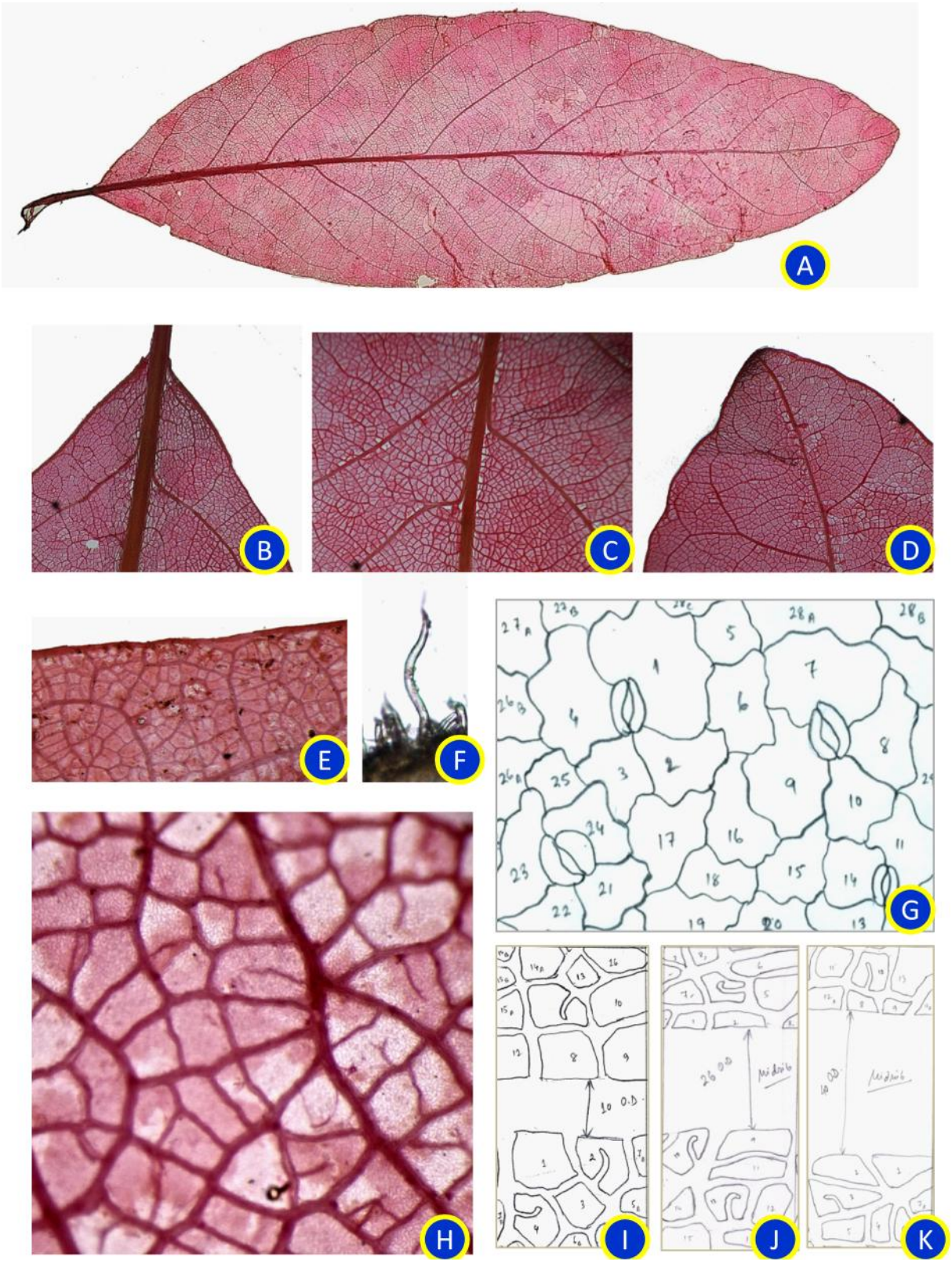


Figure 7.7. ***Litsea laeta*** **A**- Cleared whole leaf; **B**- Base; **C**- Middle; **D**- Apex; **E**- Untoothed margin; **F**- Hair; **G**- Camera lucida drawing of leaf stomata; **H**- Areolation & F.E.V.S.; Camera lucida drawing of areoles , F.E.V.S. & 1° vein: **I**- Apex, **J**- Middle, **K**- Base

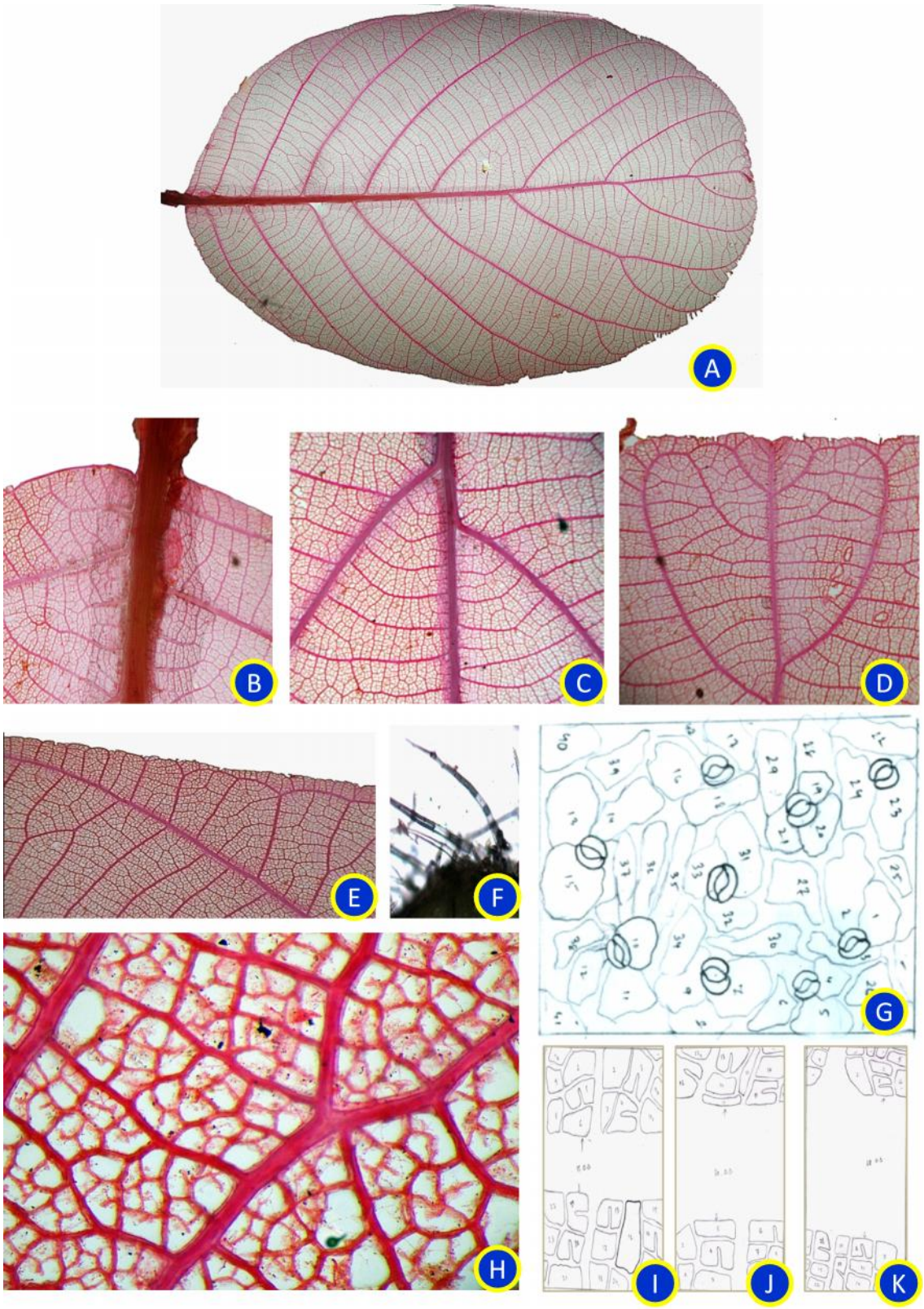


Figure 7.8. ***Litsea monopetala*** **A-** Cleared whole leaf; **B-** Base; **C-** Middle; **D-** Apex; **E-** Untoothed margin; **F-** Hair; **G-** Camera lucida drawing of leaf stomata; **H-** Areolation & F.E.V.S.; Camera lucida drawing of areoles, F.E.V.S. & 1° vein: **I-** Apex, **J-** Middle, **K-** Base

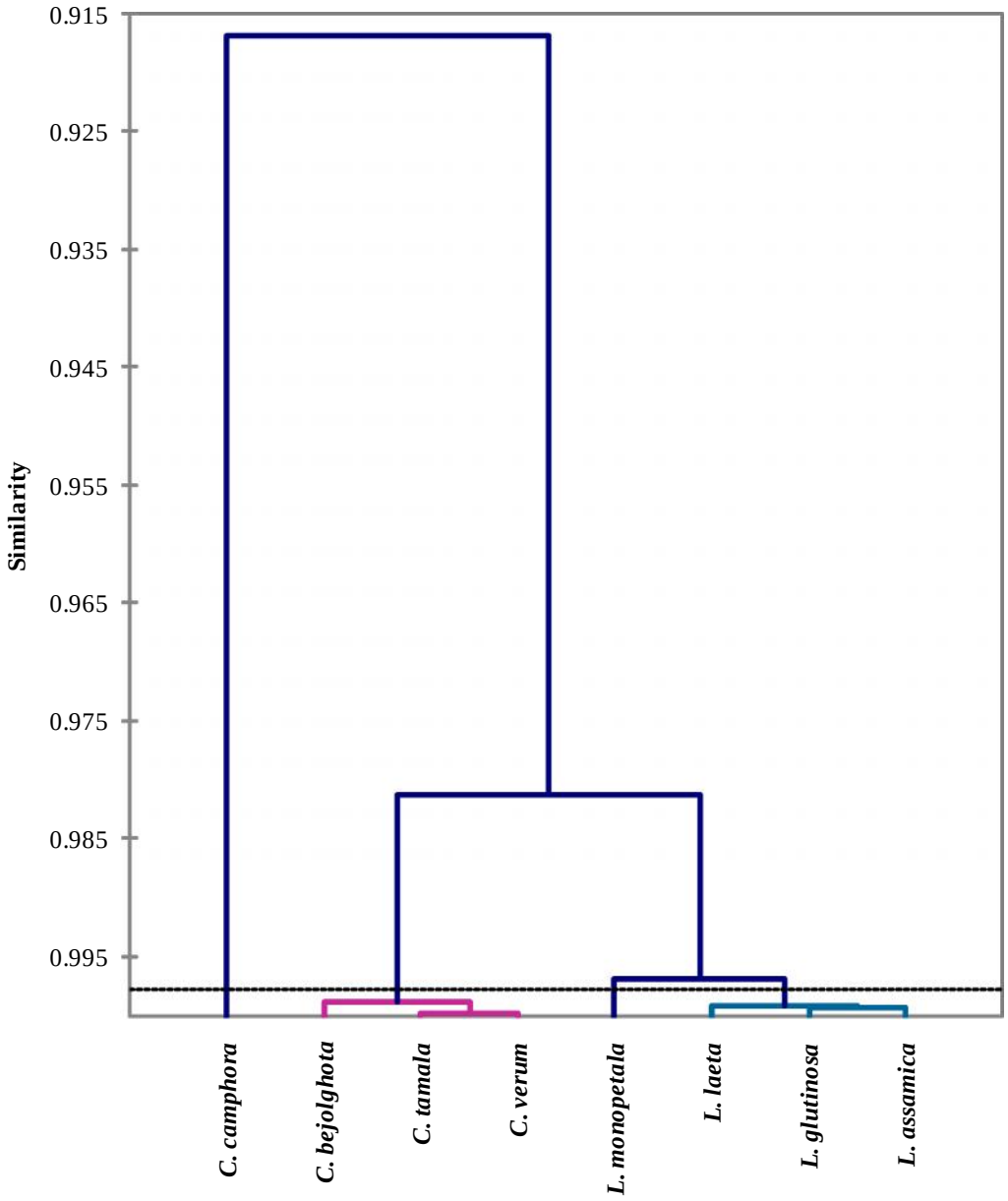


Figure 7.9. Cladistic approach of studied taxa on the basis of leaf architecture

7.3. DISCUSSION

It is well known that floral characters are the most common method to determine taxonomic identity of angiospermic plants. However, taxonomic significance of epidermal characters including epidermal cell architecture, such as the stomatal complex and surface ornamentation have been used in some cases as a substitute method to classify at the genus as well as species level (Sharma & Dunn 1969; Stace 1965). A number of works have been performed on leaf architecture to identify the species successfully and established the relationship between them (Merrill 1978; Inamdar *et al.*1983). In the present study, it is clear that leaves in all the selected species of Laurels are simple with week brochidodromous or acrodromous type of venation. In all cases of Laurels inter 2º veins were absent. Similarly inter 2º veins

are absent in many woody perennials (Loutfy *et al.* 2005). Based upon the size classes, the leaves of all the studied Laurels belong to the mesophyll or notophyll type.

An important characteristic of foliar architecture is the minor venation pattern. Several information are available regarding the taxonomic usefulness of minor venation pattern in different plant species. According to Levin (1929), Varghese (1969) and Gupta (1961), for a species, the vein islet number is more or less constant and possibly is suitable to use as diagnostic character even at the species level. On the other hand, different authors like Banerjee and Das (1972), Singh *et al.* (1976), Sehgal and Paliwal (1974) and Jain (1978) suggested that the size of areole and vein islet termination number are highly variable, therefore it cannot be used as reliable taxonomic criteria, especially in genera with large number of species.

In the present study, minor venation pattern are distinctly different in *Litsea* and *Cinnamomum*, notable differences in the size and number of areoles were observed. Similarly, the observation of F.E.Vs is also parallel to above results. Minor veins serve both mechanical as well as conducting functions of the leaf. Therefore, quantitative differences in minor venation pattern may carry on physiological or adaptive significance. As a taxonomic method, the use of architectural leaf characters described in the present study overcomes the requirement of reproductive structures to identify the taxon and provides a very much suitable and reliable method for systematic studies of Laurels.

Qualitative morphological data including leaf base, form, blade class, primary vein size, areole development, variation in angle of divergence of the eight species of Laurels were subjected to cluster analysis. The dendrogram produced with the use of such characters showed that *Litsea* can be easily distinguished from *Cinnamomum*. Cluster analysis is a statistical mechanism which produces a hierarchical classification of different taxa. The results of this study indicated that leaf architectural characters are essential and must be combined with floral characters of those species to support and make stronger their current status as eight distinct and separate species.

No doubt recent studies like bioinformatics, biotechnology, biochemistry, biomolecular etc. are necessary, but the classical disciplines should not be neglected. Now a days, like most other classical studies, leaf architecture is a neglected feature. However, importance of leaf architectural data, like many other parameters remains unquestionable. This study once again emphasizes the importance of leaf architectural data for systematic as well as taxonomic purposes. The present study also highlights the significance and requirements of leaf architectural data for comparative studies. As principal characteristics of the leaf venation pattern of a species are, in general, genetically fixed. So, this study can be used as pharmacognostics tool.

7.3.1. An artificial key of the studied taxa based on the investigated aspects is presented as below:

1a. Hairs absent; 2^o vein spacing absent or increasing toward base; 3^o vein angle to 1^o acute; 3r leaf rank ……. ……. **2**

1b. Hairs present; 2^o vein spacing irregular or decreasing toward base; 3^o vein angle to 1^o obtuse; 4r leaf rank ……. ……. **5**

2a. Alternate phyllotaxy;1^o vein category pinnateand 2^o vein weak brochidodromous; 2^o vein spacing increasing toward base;uniform 3^ovein angle variability ……. ……. ……. ***C. camphora***

2b. Opposite phyllotaxy; 1^{o} and 2^{o} vein type acrodromous; 2^{o} vein spacing absent; inconsistent3^{o}vein angle variability ……. **3**

3a. Blade class notophyll ……. ……. ……. …….. ***C. verum***

3b. Blade class mesophyll ……. ……. ……. …… **4**

4a. Freely ending ultimate veins are unbranched; stomata anisocytic type;petiole base not swollen ……. ……. ……. ***C. tamala***

4b. Freely ending ultimate veins are two or more branched; stomata cyclocytic type;petiole base slightly swollen ……. …….. ***C. bejolghota***

5a. Apex obtuse, base rounded; 2^{o}vein spacing irregular; straight 3^{o} vein course……. ……. ……. ……. ….. ***L. monopetala***

5b. Apex acute to acuminate, base cuneate; 2^{o}vein spacing decreasing toward base;sinuous 3^{o} vein course……. ……. ……. **6**

6a.2^{o} vein angle smoothly decreasing toward base; 3^{o} vein angle variability inconsistent; petiole base not swollen; marginal ultimatevenationlooped ……. ……. ……. …….. ***L. assamica***

6b. 2^{o} vein angle smoothly increasing toward base; 3^{o} vein angle variabilityincreasing basally; petiole base slightly swollen; marginal ultimatevenationfimbrial ……. ……. …… **7**

7a. Blade class mesophyll; Freely ending ultimate veins are linear; stomata anomocytic type ……. ……. ……. ***L. glutinosa***

7b. Blade class notophyll; Freely ending ultimate veins are curve; stomataanomotetracytic type ……. ……. ……. ***L. laeta***

CHAPTER - 8

Antioxidant Based Chemotaxonomic Approach

Antioxidant Based Chemotaxonomic Approach

8.1. INTRODUCTION

Plants are the main source of energy for the animal kingdom. In addition, plants can produce a large variety of chemical products which have several physiological importance (Kretovich 2005). Herbal, medicinal and aromatic plants form a large segment of the flora, which offer raw materials for use of pharmaceutical, cosmetic, fragrance and flavour industries. They have been used in India for a long time for their medicinal properties. Most of the plants contain antioxidant compounds which protect cells against the damaging effects of oxidative stress. The result of oxidative stress is production of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl, hydroxyl and peroxynitrite radicals (Dasgupta & De 2006).

Recently, there is a great interest in the study of antioxidants mainly due to the findings concerned with the property of free radicals in the organism. Polyphenolic compounds have attracted considerable attention for being main sources of antioxidant activity. Antioxidant activity of phenolics is due to their redox properties, which allow them to act as reducing agents as well as singlet oxygen quenchers. Side by side, they have a metal chelation potential. These phenolics play an important role in adsorption and neutralization of free radicals (Basile *et al*. 2005). Synthetic antioxidants are commercially accessible but most of them have been reported to be toxic (Madhavi & Salunkhe 1995). Plants have been documented to exhibit antioxidant activity due to the presence of natural antioxidant compounds such as phenolics, pro-anthocyanidins and flavonoids (Rice-Evans *et al*. 1995).

Polyphenols are secondary metabolites which often are diversely distributed among limited taxonomic groups within plant kingdom. Taxonomically linked species might exhibit considerable similarity in qualitative polyphenolic profile. But, quantity of individual polyphenols might differ widely in various species of the same family (Hossain *et al*. 2011). Both qualitative and quantitative phytochemical profile together with total antioxidant activity measured by different ways could be used to classify different species (Pennington & Fisher 2009). Agglomerative Hierarchical Clustering (AHC) is the mathematical tools which allow the visualization of underlying structure in experimental data and relationships between samples through data. The present study aims to use these chemometric tools to gain insights into variations in the complex antioxidant profiles among eight economically important species of Lauraceae and to classify them based on antioxidant capacity and the levels of total polyphenolic compounds.

8.2. RESULT AND DISCUSSION

Phenolic compounds are widely investigated and are naturally occurring antioxidant components of plants. These phenolic compounds are found in medicinal plants as well as fruits and vegetables and play important roles in preventing degenerative diseases, including inflammation, cancer, and arteriosclerosis (Sato *et al*. 1996; Li *et al*. 2008b). Figure 8.1-8.2 presents the extractable total phenol and flavonoid

contents of eight different Laurels of Terai and Duars region. The total phenolic contents of the bark extracts were much higher than those of the leaf extracts (except *Litsea assamica*). The contents of total extractable flavonoid compounds in the extracts were varied between 22.09 to 66.10 mg/100 g and showed almost similar trend like total phenolics. In 2008, Muhammad *et al*. had worked on *Litsea monopetala* bark and they found four different phenolic compounds from the methanolic extract. In several studies it was recommended that plant flavonoids, which showed antioxidant activity *in vitro,* also function as antioxidants *in vivo* (Lee *et al*. 2005; Shin *et al*. 2008). Naturally occurring polyphenols and flavonoids can prevent lipid peroxidation, low density lipoprotein oxidation, and the development of atherosclerosis and heart disease (Samak *et al*. 2009). According to Agrawal *et al*. (2011), the genus *Litsea* contained several secondary metabolites. The extractive yields of eight Laurels of Terai and Duars of West Bengal were presented in Figure 8.3. Relatively higher extractive yields were obtained from leaves, when compared with bark. These results showed that the methanolic extractive yields varied widely with plant specimens, indicating that each part (leaf and bark) of these plants consist of different components. Several studies reported the increase of extractive yield by the action of pectinases, cellulases and hemicellulases; reduction of particle size increases the polyphenols extraction rate and the extraction yield (Landbo & Meyer 2001). In an earlier study, many medicinal plants contained high amounts of phenolic compounds and there was a positive linear correlation between the total phenolic

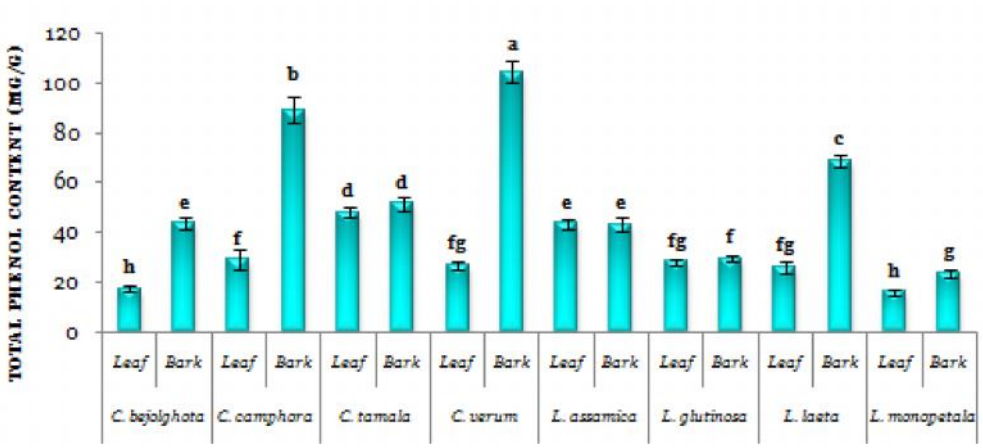


Figure 8.1. Total phenol content of leaf and bark of studied taxa

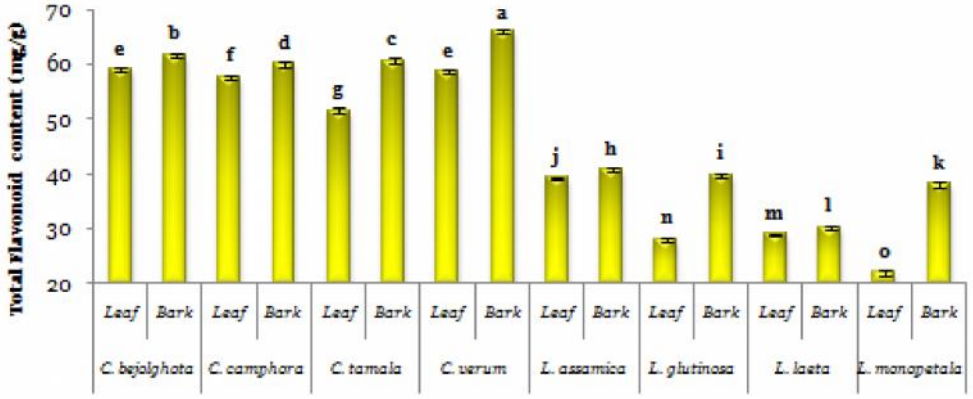


Figure 8.2. Total flavonoids content of leaf and bark of studied taxa

content and antioxidant activity of the plants (Li *et al*. 2008b; Ozsoy *et al*. 2008). These records suggest that the two genera of Lauraceae, which contained higher levels of polyphenols, might have high antioxidant properties. In 2009, Kshirsagar and Upadhyay found that the stem of *L. glutinosa* had high DPPH scavenging capacity than the twigs of this plant. It has been proved that *Cinammomum* spp. have capacity to prevent free radicals (Chen *et al*. 2012; Thong *et al*. 2008). In this present study the antioxidant activity of the methanolic extracts of the different parts (leaf and bark) of eight Laurels were investigated by using DPPH scavenging, reducing power, metal chelating, superoxide scavenging and

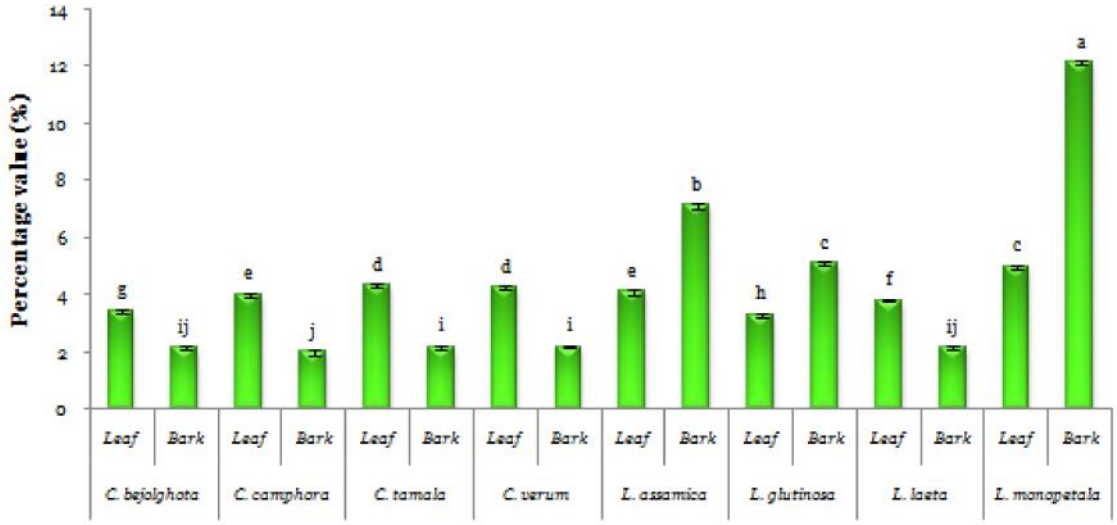


Figure 8.3. Extractive values of leaf and bark of studied taxa

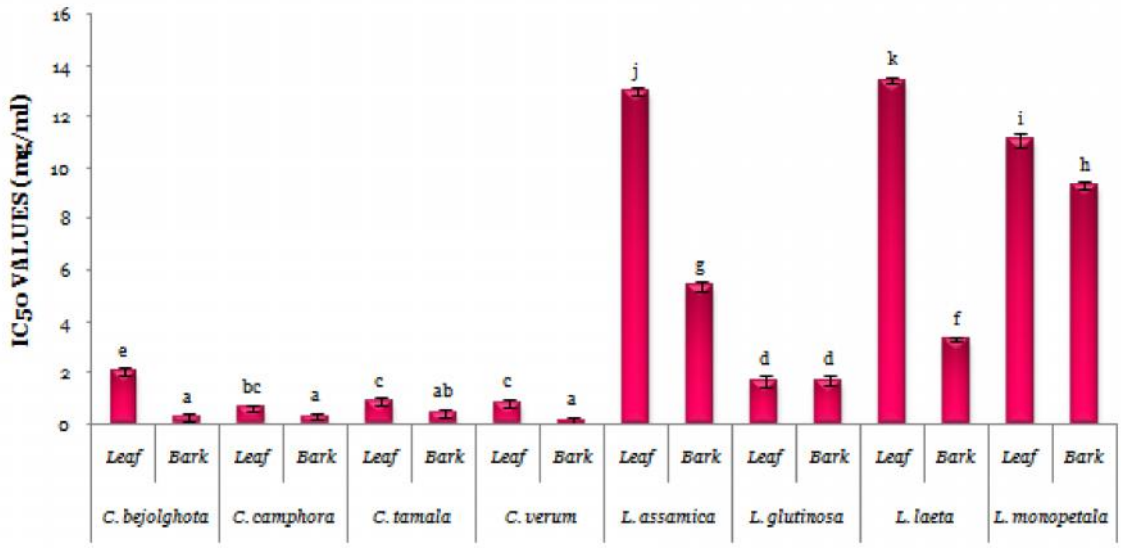


Figure 8.4. DPPH scavenging activity of leaf and bark of studied taxa

nitric oxide scavenging assay of the extracts. Methanolic extracts of two parts of these plants have exhibited excellent antioxidant activity. As shown in the Figure 8.4, extracts from bark had relatively strong DPPH scavenging activity (low IC_{50} value), thus exhibiting high antioxidant capacity compared to extracts from leaves (except *L. monopetala*). Possible mechanism of DPPH scavenging was suggested to be through reduction of this radical by antioxidant molecule to a more stable DPPH form. Because of its unpaired or free electron, DPPH has absorption maxima at 517 nm and as it gets reduced in the

presence of free radical scavengers, the absorbance decreases at this wavelength with respect to the number of electrons taken up. For the measurement of the reducing ability, Fe^{+3}-Fe^{+2} transformations in the presence of phenolic compounds of Laurels was found. The reducing ability of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 8.5 shows the reducing capability of the *Litsea* and *Cinnamomum* species. Bark of the genus of *Litsea* is more potent in reducing capacity than leaf. The bark of *C. camphora* has greatest reducing capacity (1.02 µg ascorbic acid eqv. /gm) than other extracts. Iron is known to generate free radicals through the Fenton and Haber–Weiss reaction. Metal ion chelating activity of an antioxidant compound prevents oxy-radical generation and subsequent oxidative damage. Metal ion chelating capacity acts as significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in lipid peroxidation (Duh *et al*.

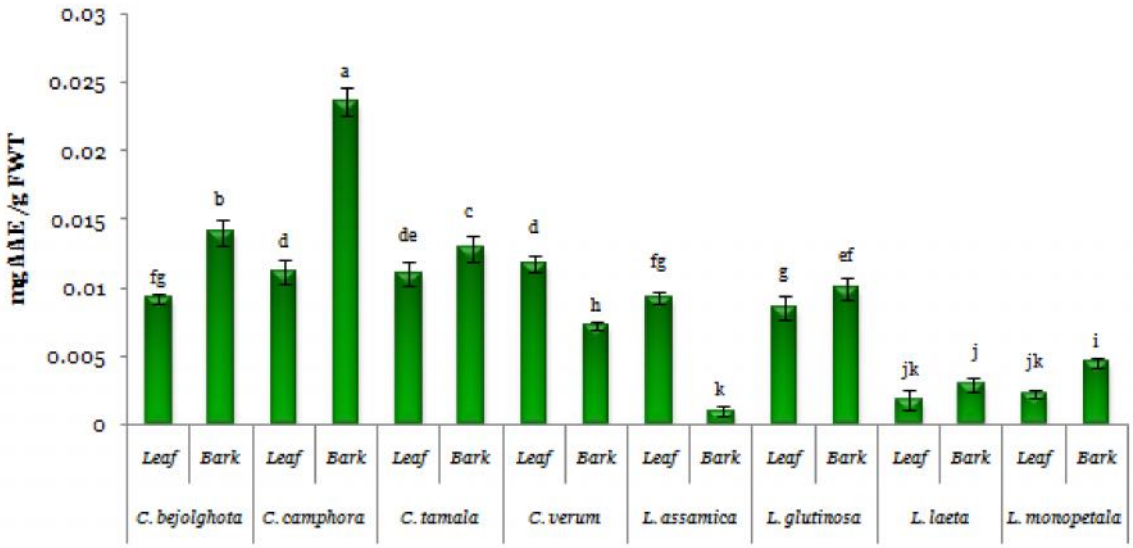


Figure 8.5. Reducing power of leaf and bark of studied taxa

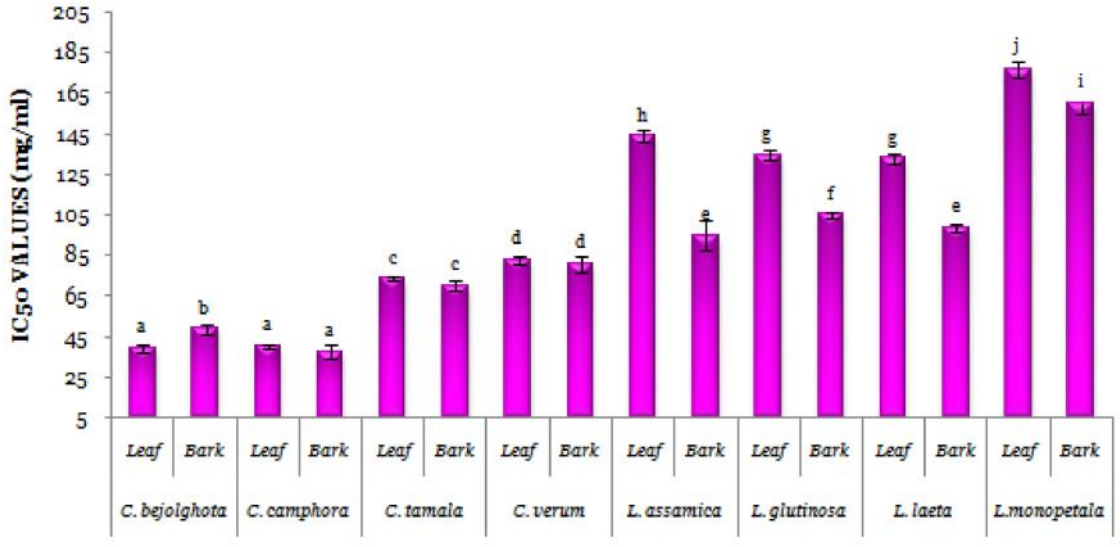


Figure 8.6. Metal chelating activity of leaf and bark of studied taxa

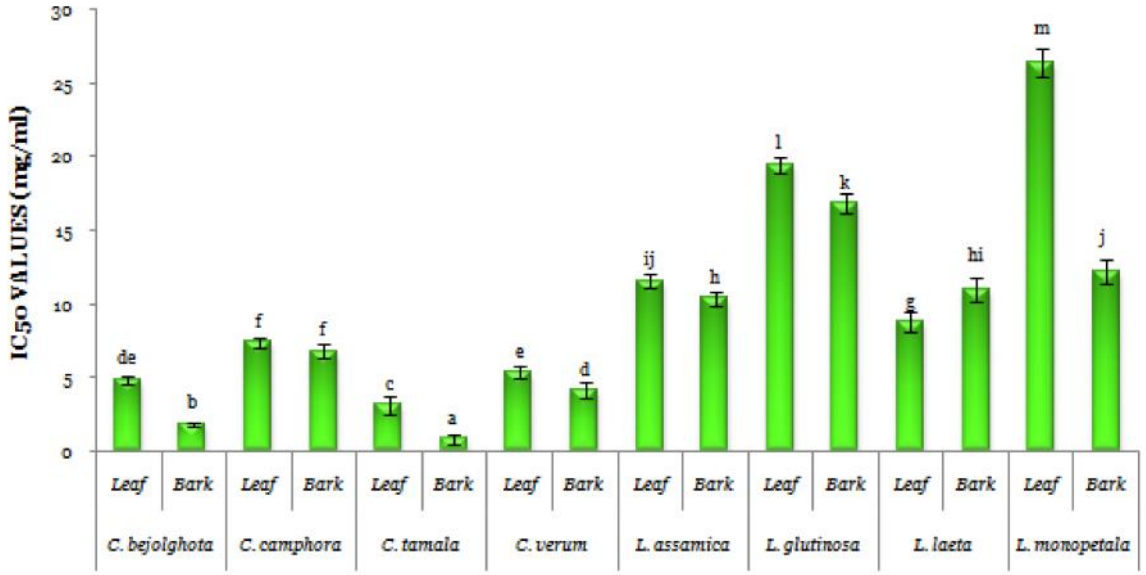


Figure 8.7. Nitric oxide scavenging activity of leaf and bark of studied taxa

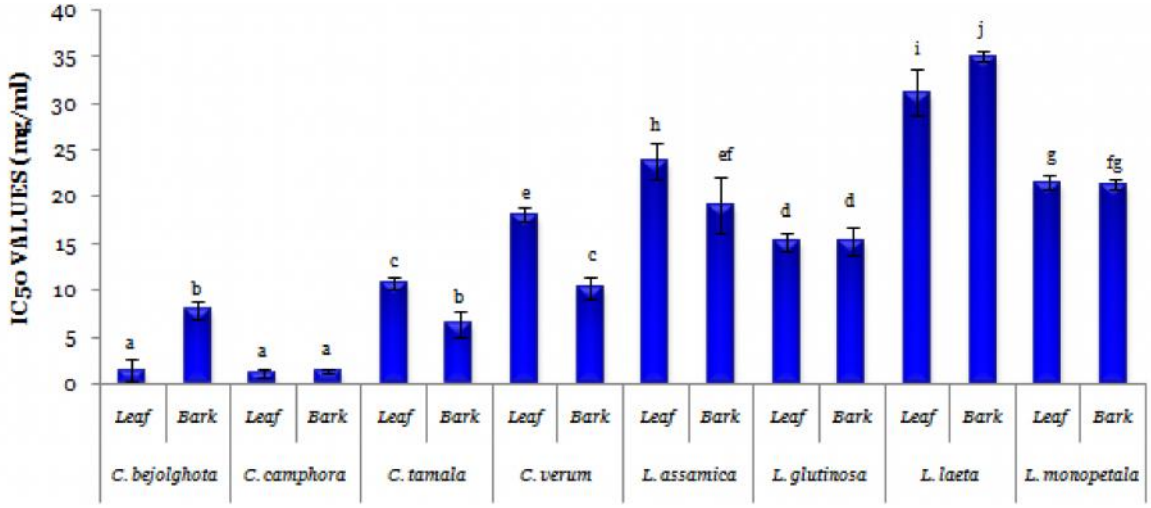


Figure 8.8. Superoxide radical scavenging of leaf and bark of studied taxa

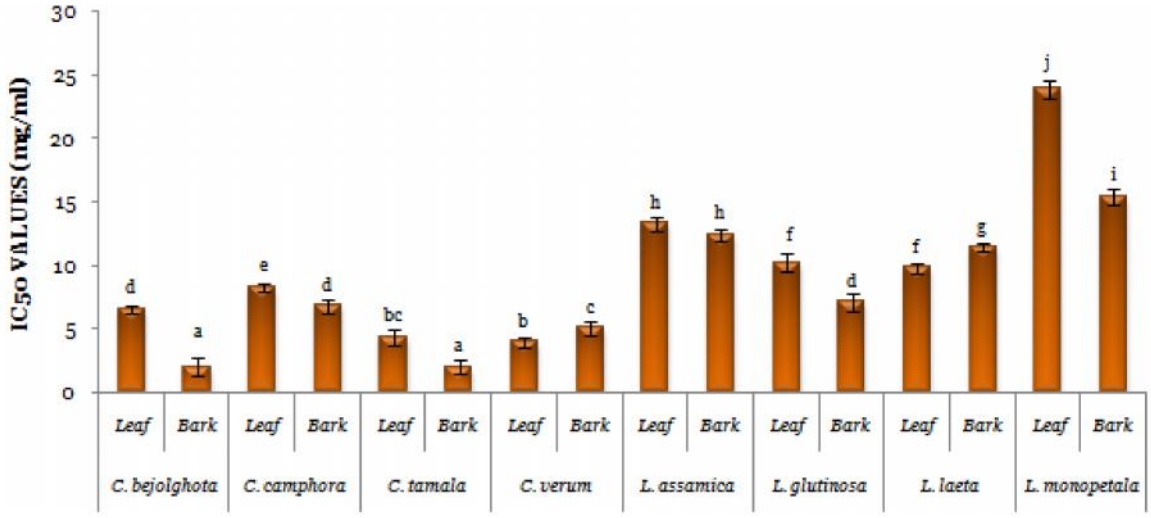


Figure 8.9. Anti-lipid peroxidation activity of leaf and bark of studied taxa

1999). In 1990, Gordon reported that chelating agents form *s*-bonds with a metal, are effective as secondary antioxidants since they reduce the redox potential by stabilizing the oxidized form of the metal ion. In the present study it was observed that all the extracts have the capacity to interfere with the ferrous-ferrozine complex formation, suggesting that they have chelating activity and captured ferrous ion before ferrozine. *Litsea* spp. have superior potency to chelate metals than *Cinnamomum* spp. Figure 8.6 shows that IC_{50} of the bark and leaf extract of *C. camphora* and *C. bejolghota* for metal chelating activity are 40.37 and 39.10 mg/ml respectively which is higher than the other plant extracts. An important messenger molecule involved in many physiological and pathological processes within the mammalian body is nitric oxide (Hou *et al.* 1999). The plant products may have the property to counteract the effect of NO• formation and in turn might generate considerable interest in preventing the ill effects of excessive NO• generation *in vivo*. *In vitro* prevention of nitric oxide radical is another measure of antioxidant activity of plant drugs. Figure 8.7 shows that *C. camphora and C. bejolghota* plant have better nitric oxide radical scavenging activity than other plant extracts that might compete with oxygen to react with nitric oxide and thus the inhibition of synthesis of anions. The toxicity of NO• increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-) (Huie & Padmaja 1993). This superoxide radical is also very harmful to cellular components (Korycka-Dahl & Richardson 1978). As shown in Figure 8.8, the plant extracts have significant amount of superoxide scavenging activity, as evidenced through reduced production of purple coloured chromogenic reagents by superoxide with nitroblue tetrazolium. In 2009, Ghosal and Mandal worked on *Litsea cubeba* of Darjeeling Himalaya and they proved that this plant has greater potency in anti-lipid peroxidation. Similarly, Smerq and Sharma (2011) proved that *C. tamala* had the potential to tolerate lipid peroxidation. In our study, eight different Laurels showed the capacity to reduce lipid peroxidation due to presence of high amount of antioxidants which is in agreement with the findings of earlier authors (Figure 8.9).

The analysis of methanolic extracts of these plants indicated the presence of glycosides, cardiac glycosides, phytosterol, triterpenoids, tannins, alkaloids and amino acids (Table 8.1). A variety of phytochemicals have been found to possess an extensive range of actions, which might help in protection against chronic disorders. It was reported that the phenolic components constitute a major group of compounds that generally act as primary antioxidants (Hatano *et al.* 1989). It was also stated that flavonoids showed anti-allergic, anti-inflammatory and antimicrobial activity. These compounds might reduce the risk of a variety of carcinogenesis and also prevent menopausal abnormalities (Hodek *et al*. 2002). Epidemiological studies suggest that the use of flavonoids is effective in preventing the risk of coronary heart diseases (Ferguson 2001). Table 8.2 showed that flavonoids are highly correlated with superoxide ion scavenging, metal chelating and anti-lipid peroxidation capacity. So, flavonoids are the main components for controlling these above mentioned disorders. Dharmananda (2003) claimed that plants containing tannins are astringent and the tannins are used for treating intestinal disorders like diarrhoea and dysentery. The presence of tannins in *Litsea and Cinnamomum* supports the traditional medicinal use of these plants in the treatment of different diseases. Motra *et al*. (1985) discovered that tannins are used for the treatment of inflamed or ulcerated tissues. Trease and Evans (1983) stated that tannins are potent antimicrobial, anti-cancer as well as antioxidants activities. The observations (Table 8.1) support the use of most of the Laurels available in the forests of Terai and Duars in herbal remedies as ethnomedicine. Another compound *i.e*. steroids, abundant in most of the plants have hypercholesterolemic effects (Kapil *et al*. 1994). Plant steroids are important for their cardiotonic activities; they possess anticancer, anti-viral, insecticidal agents and antimicrobial properties (Minocha & Tiwari 1981; Kokpol *et al*. 1984). They are also used as herbal medicine, nutraceuticals and cosmetics (Callow 1936). Table 8.1 also showed that almost all the selected

Table 8.1. Phytochemical profile of studied taxa

Plants	Plant part	Alkaloid	Steroids	Anthraquinones	Amino acid	Tannin	Triterpenoids	Resin	Cardiac glycoside	Glycosides
C. bejolghota	Leaf	++++	++	+	Nil	++	Nil	+++	Nil	Ni
	Bark	Nil	++	+++	Nil	+++	++	+++	Nil	Ni
C. camphora	Leaf	+++	++	+++	Nil	+++	-	+++	Nil	Ni
	Bark	++	++	+++	Nil	+++	+++	+++	Nil	Ni
C. tamala	Leaf	++	++++	++++	Nil	+++	+++	+++	Nil	Ni
	Bark	+	++	+++	Nil	+++	-	+++	Nil	Ni
C. verum	Leaf	++	+++	+	Nil	++	-	++	Nil	Ni
	Bark	++	++++	++++	Nil	++++	++	-	Nil	Ni
L. assamica	Leaf	+++	++	Nil	Nil	++	-	++	Nil	Ni
	Bark			Nil	Nil				Nil	Ni
L. glutinosa	Leaf	++	++++	Nil	Nil	-	Nil	+++	Nil	Ni
	Bark	+	++	Nil	Nil	++++	-	+++	Nil	Ni
L. laeta	Leaf	++++	+++	Nil	Nil	+++	-	+++	Nil	Ni
	Bark	+	++++	Nil	Nil	++	-	++	Nil	Ni
L. monopetala	Leaf	+++	+++	Nil	Nil	++	Nil	++	Nil	Ni
	Bark	+++	+++	Nil	+	+++	-	-	Nil	Ni

Table 8.2. Correlation matrix of antioxidant activity with phytochemicals

	EV	DPPH	SO	NO	RP	MC	ALP	TPC
DPPH	0.304							
SO	0.285	**0.800(*)**						
NO	0.298	0.425	0.368					
RP	-0.073	-0.409	-0.605	-0.233				
MC	0.366	**0.754(*)**	**0.827(*)**	**0.802(*)**	-0.557			
ALP	0.515	0.660	0.406	**0.894(**)**	-0.188	**0.774(*)**		
TPC	-0.103	-0.015	0.079	-0.455	-0.359	-0.070	-0.416	
TFC	-0.172	-0.689	**-0.708(*)**	**-0.832(*)**	0.456	**-0.924(**)**	**-0.763(*)**	0.191

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

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

plant species contain steroids. Therefore, therapeutic effects of the Laurels can be attributed to the antioxidant properties of their constituents. The extracts of leaves and barks of only *Cinnamomum* plants might be used as dye due to the presence of anthraquinones (Table 8.1). Tri-terpenoids decreased blood sugar level (Cherian & Augusti 1995; Luo *et al.* 1999). Alkaloid is another type of antidiabetic agent (Oliver 1980) with wide diversity which is present in almost all plants (Table 8.1). Due to this characteristic feature, it might be predicted that these plants have potency to prevent hyperglycaemia.

Besides the pharmacological values, the secondary metabolite based chemotaxonomic technique can be used as an important device for identifying and classifying these eight economically important plants if one can recognize different species-specific metabolites as selection marker. In 2011, Hossain *et al.* clearly explained that antioxidant activity as well as secondary metabolites were assisted for clustering different plants by Higher Archival Cluster analysis. They proved that this data analysis technique provided powerful insights into variations in the antioxidant profiles between different species. Similarly, five locally available plants of Lahore taxa of *Solanum nigrum* complex were investigated to determine the International taxonomic controversy about those plants by analyzing their flavonoid profiles (Mohy-Ud-Din 2009). Emerenciano *et al.* (2001) also showed that flavonoids are the good taxonomic markers for Asteraceae. The chemotaxonomic importance and the potential bioactivity of secondary metabolites i.e. antioxidants in Lauraceae family were confirmed by this study. Differences in antioxidant patterns of leaves and barks illustrate some chemotaxonomic relationships between the members of the family Lauraceae studied in Terai and Duars region. Figure 8.10 and Figure 8.11 showed that *Litsea* and *Cinnamomum* were divided in two different branches. Though the leaves and barks were collected from same plants but the dendrogram was slight dissimilar in some cases, because the deposition pattern of the secondary metabolites in barks are stable than leaves (Ahmad *et al.* 2009); thus for further study we have selected barks of these plants in next chapters.

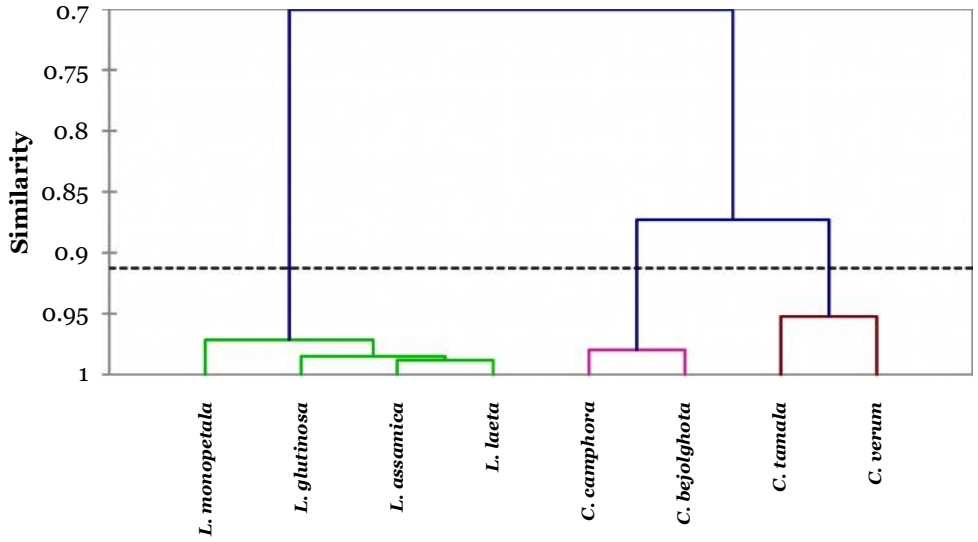


Figure 8.10. Cladistic approach of eight Laurels on the basis of antioxidant activity of their leaves

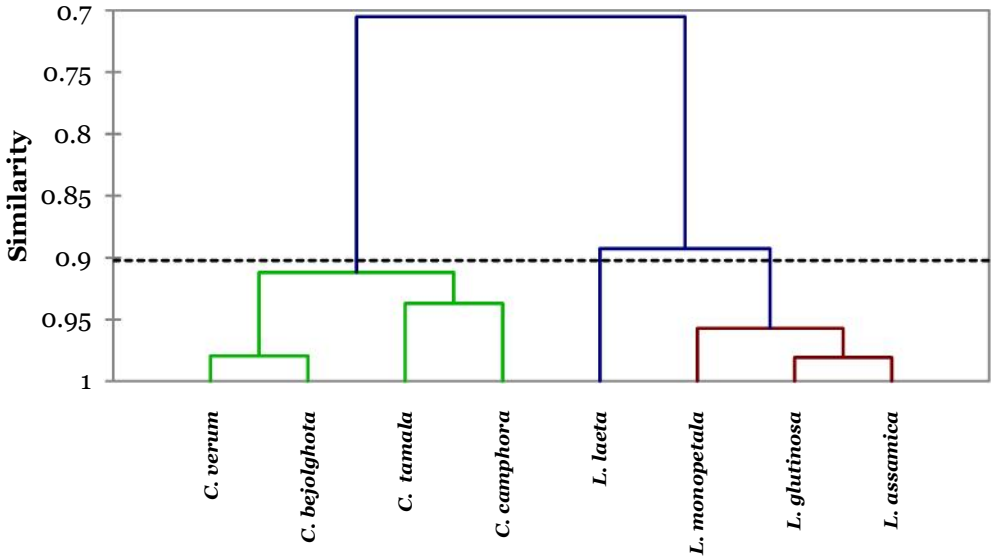


Figure 8.11. Cladistic approach of eight Laurels on the basis of antioxidant activity of their barks

With various pharmacological and biological activities, secondary metabolites are the principal components in assessing the quality as well as taxonomy of various taxa of Laurels (Ho & Hung 2011). Although there are some controversies on usage of secondary metabolites to solve taxonomic problems, we have explained chemotaxonomic significances in this study. Besides demonstrating that antioxidants data exhibit certain coherence with the classifications of Lauraceae, we have shown that it acts as a powerful tool. Because of the taxonomic misunderstanding surrounding the position of the species due to very restricted seasonal availability of reproductive parts and vegetative parts with striking resemblance, it is advisable that the information noticed in literature should be reinterpreted and any medicinal or commercial use of the taxa should be carried out in light of above chemotaxonomic suggestion. The commercial plant materials are frequently adulterated. Therefore *in vitro* quantitative and qualitative determination of secondary metabolites and antioxidant evaluation is the easy process to detect these

adulterants. In 1960, it was predicted by Willis that the time has come when every student of natural product should have a handbook on the plant taxonomy on his desk. The word is becoming true.

In conclusion, we can say that, human nutrition and health are still one of the most interesting topics. Thorough investigations of the natural compounds which are coming from plants are studied for their potentially beneficial effects. In this study, we aimed at relationships of various Laurels of West Bengal. The Laurels were analyzed from different analytical points of view including antioxidant capacities, total polyphenols content and also different phytochemical components. To find similarities among the species, cluster analysis of data obtained was used. It can be concluded that most of the species of same genus are similar from the biochemical point of view. The information is interesting, not only from the nutritional point of view, but also from the point of view of phylogeny of the plants.

CHAPTER - 9

Chemotaxonomy through Antioxidant activity of Essential oil

Chemotaxonomy through Antioxidant activity of Essential oil

9.1. INTRODUCTION

An essential oil is an aromatic product and usually has complex composition. In connection with their multifarious composition, the range of biological functions of this oil is wide (Burger & Wachter 1998). For external application, they are therapeutically used as spasmolytic, anti-inflammatory, anodyne, antiviral, antibacterial and anti-mycotic activities (Chaudhry & Tariq 2006). Internally they are usually handled as capsule; and also as tablet, sugar-coated tablet, suppository, spirituous extract, unguent or even pure oils themselves (Hamid *et al.* 2011). These oils are not only used medicinally, but also as perfumery agent and frequently applicable as fragrance for aromatherapy (Edris & Abd El-Galeel 2010).

Lauraceae is an oil yielding family (Li *et al.* 2008b), and the examination of the essential oil content of different species was continued. The essential oil of two genera viz. *Cinnamomum* and *Litsea*, which are commonly available plants of Terai and Duars region of West Bengal were used as food preservatives, folk remedy to treat several diseases, disorders and ailments (Geiger 2005; Senhaji *et al.* 2007). Since long time, essential oil of *Cinnamomum* has been used to treat gastritis, dyspepsia, blood circulation disturbance and inflammatory disorders in many countries (Wang *et al.* 2009). Also, they showed potential antipyretic, analgesic, anti-allergenic, antitussive (Gurdip *et al.* 2008) and chemo-preventive activities (Sabulal *et al.* 2007). Therefore, the aim of this study was to isolate the essential oil from the bark of eight available species of Laurels and evaluate antioxidant properties of these essential oils obtained from two genera, *viz. Cinnamomum and Litsea*. The antioxidant activity can also be examined as a tool for chemotaxonomy for clustering these plants. For the said purpose, the free-radical scavenging data obtained from mature barks of these two genera were used for Hierarchical Clustering through correlation co-efficient matrix for determining the relationships among these plants.

9.2. RESULT AND DISCUSSIONS

Natural products are in growing demand from the manufacturers of pharmaceuticals, cosmetics and food additives to consumers using these products. Thus the importance of conducting works on essential oils lies not only in the chemical characterization but also in the possibility of making meaningful relation among different species of economically important families through particular bioactive functional properties.

Figure 9.1- 9.6 showed the capacity of antioxidant activity of the eight Laurels of Terai and Duars region. DPPH is a compound that has been generally used to test the free radical-scavenging capacity of various samples. Figure 9.1 depicts the free scavenging activity of eight essential oils on DPPH radicals at various concentrations. Essential oils of *Litsea assamica* demonstrated the highest inhibitory activity compared to other antioxidants studied. But in case of superoxide anion scavenging, nitric oxide scavenging, metal chelating capacity of those species *Cinnamomum tamala*,

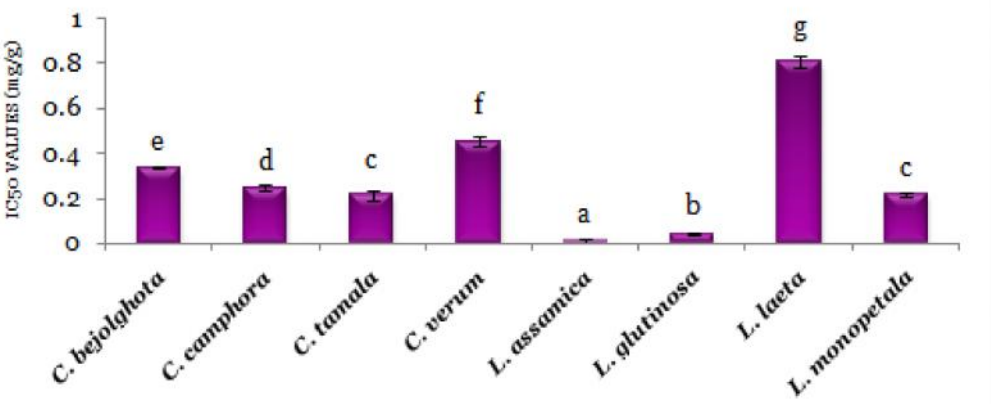


Figure 9.1. DPPH scavenging activity of essential oils of studied taxa

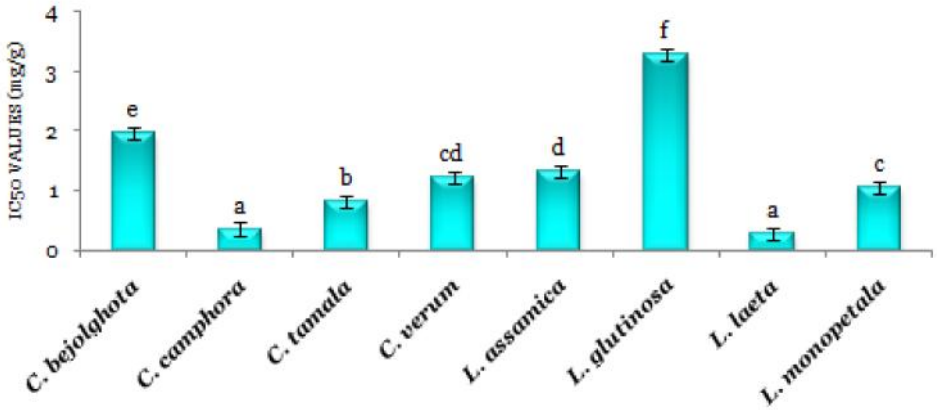


Figure 9.2. Superoxide radical scavenging activity of essential oils of studied taxa

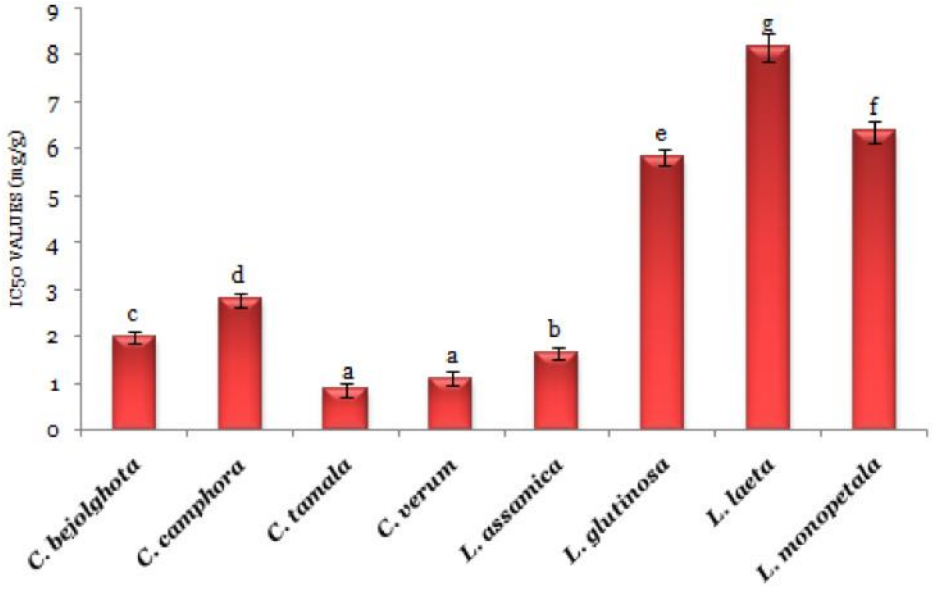


Figure 9.3. Nitric oxide scavenging activity of essential oils of studied taxa

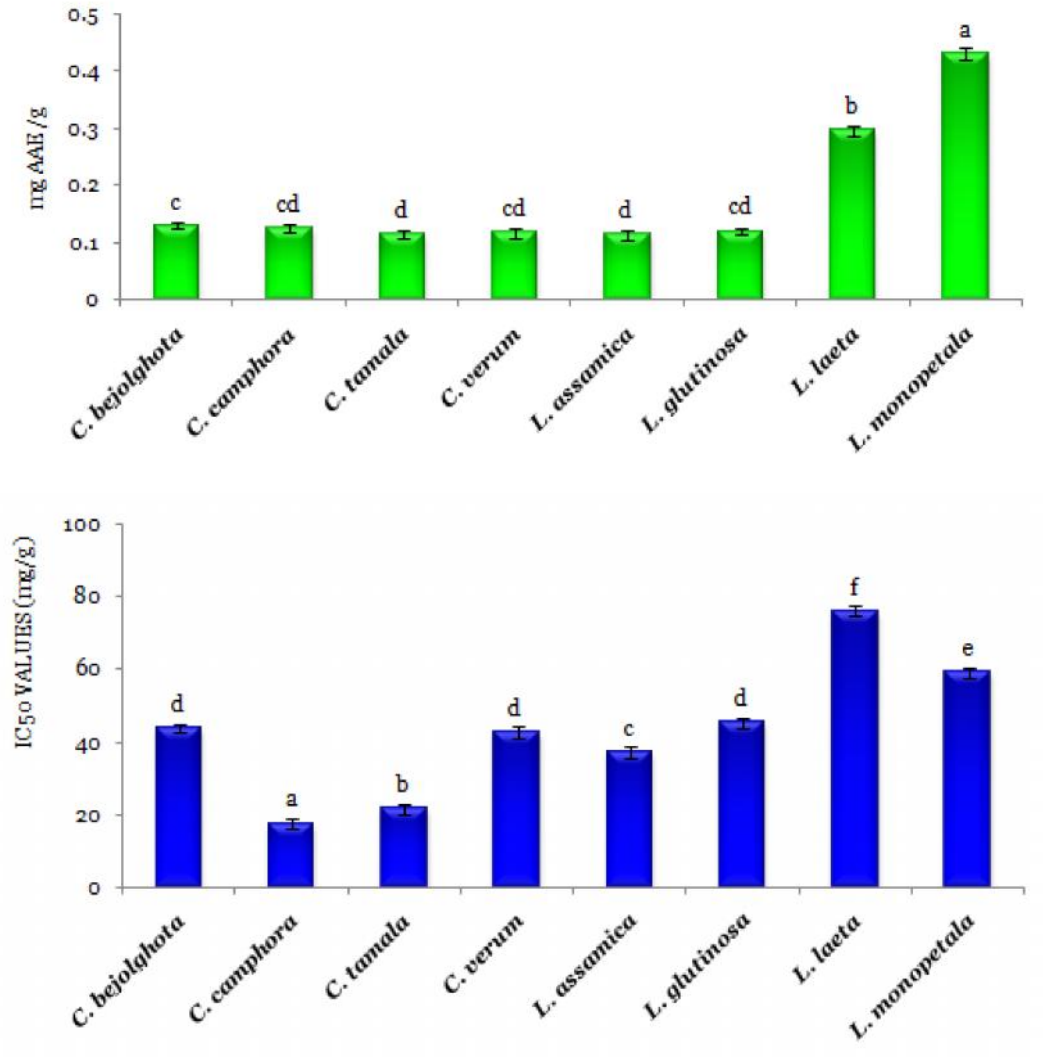


Figure 9.5. Metal chelating activity of essential oils of studied taxa

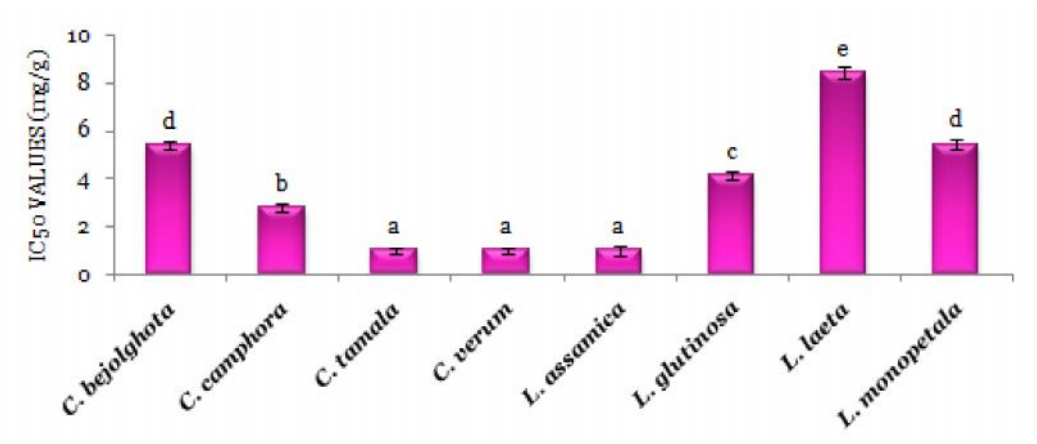


Figure 9.6. Anti-lipid peroxidation activity of essential oils of studied taxa

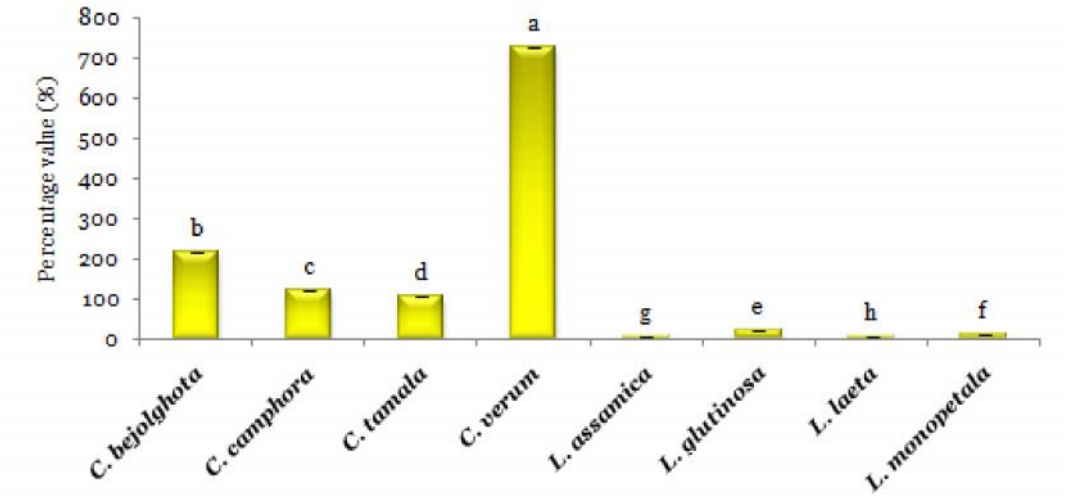


Figure 9.7. Extractive values of essential oils of studied taxa

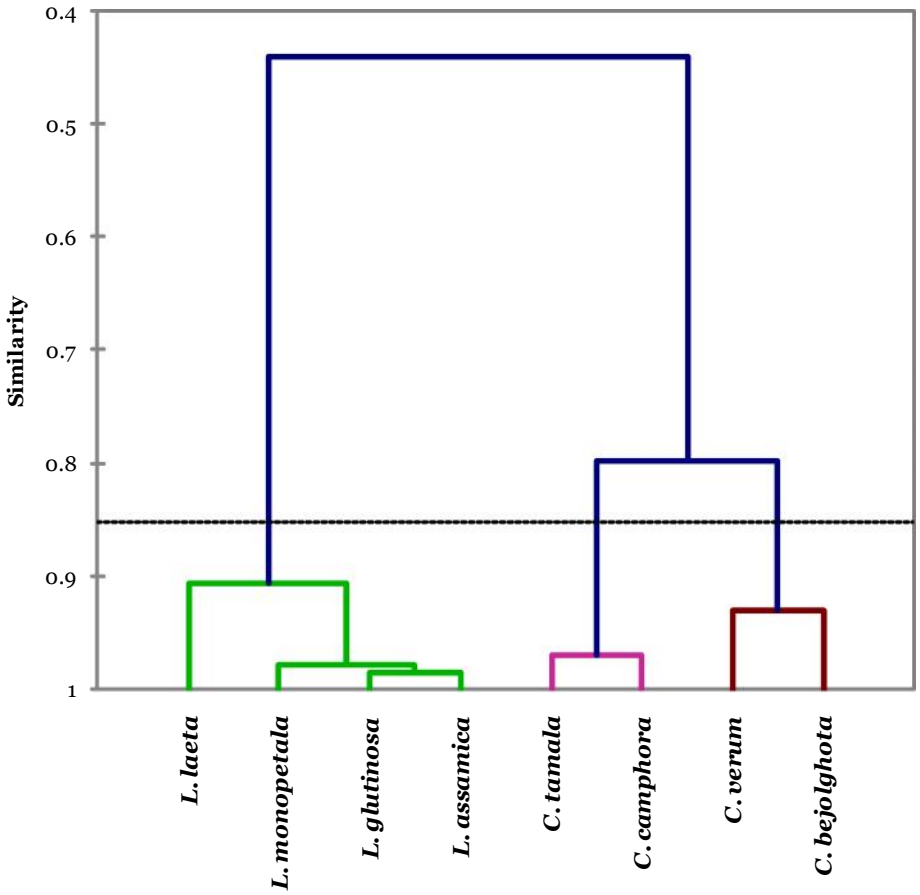


Figure 9.8. Cladistic approach of eight Laurels on the basis of antioxidant activity of their essential oils extracted from bark

C. verum and *C. camphora* showed higher activity than *Litsea* species (Figure 9.2-9.4). Another activity like reducing power is highest in *L. monopetala,* whereas antilipid peroxidation is just like DPPH scavenging capacity in Laurels.

Table 9.1. Correlation matrix analysis of different free-radical scavenging potential of essential oil obtained from studied taxa

	EV	DPPH	SO	NO	RP	MC
DPPH	0.250					
SO	-0.019	-0.530				
NO	-0.518	0.390	0.010			
RP	-0.343	0.334	-0.303	**0.728***		
MC	-0.152	0.591	0.011	**0.789***	0.698	
ALP	-0.409	0.595	-0.051	**0.849****	0.646	**0.793***

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

The extractive yields of different essential oils of eight Laurels were presented in Figure 9.7. Relatively higher extraction yields were obtained from *Cinnamomum* than *Litsea.* The highest extraction yield was found in the dichloromethane extract from the bark of *C. verum* with 725.9%, while the oil of *L. laeta* (4.2%) had the lowest extraction yield. *Cinnamomum* oil has also noticeable higher antioxidant activity towards the free radicals confirmed by the lowest IC_{50} value. The results were parallel to the outcome of the Schmidt *et al.* (2006) works. They worked with *C. verum* and claimed the tremendous effective antioxidant capacity of the oil of that plant. The essential oil of all plants in our work demonstrated high chelation activity with respect to Fe^{3+}, resulting in a prevention of hydroxyl radicals' initiation. The oil inhibited effectively conjugated diene formation as well as the formation of secondary products from lipid peroxidation. In 2010, Joshi *et al.* investigated the antioxidant activity of seven Laurels of Himalaya. They also confirmed the significant lipid peroxidation capacity of those plants of that region.

The table 9.1 showed the correlation matrix of essential oils of different Laurels based on antioxidant activities. The table represented that nitric oxide scavenging capacity is highly correlated with reducing power, metal chelating capacity and antilipid peroxidation activity. On the other hand, metal chelating capacity is well correlated with antilipid peroxidation.

When comparing the eight Laurels with the cladogram based on the antioxidant profile (Figure 9.8), it was found that *Litsea* genus is separated from the genus *Cinnamomum*, which corresponds the similar grouping developed by the morphological characteristics (Chapter-5). In the cladogram the two species of *Litsea* like *L. assamica* and *L. glutinosa* are present in same clade and appear to be much related with *L. monopetala* than *L. laeta*. Next in the cladogram, *C. verum* and *C. bejolghota* was branched from same clade and these are the representatives with higher free radical scavenging property. Finally, we see another clade with *C. camphora* and *C. tamala*

which are not phytochemically very similar but when the functional attributes of essential oils are considered, they exhibited similar trend like morphological clustering. Overall it might be considered that different species of *Cinnamomum* differ markedly from the representatives of *Litsea* in terms of antioxidant properties and quantitative existence of bioactive phytochemicals in their essential oils as reflected in present dendrogram (Figure 9.8).

From numerous other studies, it is evident that the chemotaxonomic studies based on the average or absolute concentration of functional phytochemicals present in numerous predefined taxa would not yield reliable results might be due to differential accumulation of secondary metabolites and functional alteration of related genes under changing environmental perspectives. Moreover, the predefined compounds cannot signify the overall chemical information, and some predefined compounds also may not be ideal chemotaxonomic markers. Thus, the fingerprint-based data were used in the next chapter.

CHAPTER - 10

TLC Based Chemotaxonomic Approach

TLC Based Chemotaxonomic Approach

10.1. INTRODUCTION

Nature has donated us a very rich botanical wealth as well as a large number of diverse kinds of plants. Aromatic plants and medicinal constitute are the major segment of the flora and they provides raw materials in pharmaceuticals, cosmetics and drug industries (Bhattacharjee 2008). During the 20th century, in spite of the tremendous developments in the field of allelopathy, it has proved that plants are still one of the major resources of drugs in modern as well as traditional system of medicine all over the world (Choudary *et al.* 2009). The separation and purification of plant components are generally carried out through chromatographic techniques based on their size, shape, or charge (Helftmann 1992). Thin layer chromatography (TLC) is generally considered as reproducible and authentic methods for analysis of different drugs. It is extensively adopted for the rapid analysis of drugs and drug preparations. This technique provides a chromatographic drug fingerprint in very short time. It is therefore suitable for observing the identity and purity of drugs, and for detecting adulterations as well as substitutions (Wagner *et al.* 1984). Not only that, this method can help in chemotaxonomic clustering for solving phylogenic problems. Through chemotaxonomy, many authors compared different category of phytochemicals, present in genera, and classify different groups of by preparing cladogram on the basis of TLC spot appeared after processing (Zafar *et al.* 2011; Mohy-Ud-Din *et al.* 2010).

Cinnamomum and *Litsea* (specially the oil yielding plants) have some similar morphological characters. Blooming season is very restricted for this Lauraceae family. In view of the diversity of different phytochemicals and essential oil known for this family, a detailed chemotaxonomic study of these secondary metabolites of different economically important medicinal and aromatic plants of these two genera may contribute valuable information towards a comparison of the genera. Since no recorded information was previously available on the essential oil and phytochemicals of these plants grown in Terai and Duars region of North Bengal, in this chapter, the study was carried out to evaluate qualitative phytochemical constituents of various secondary metabolites present in the bark of eight Laurels by using TLC and compared them through Agglomerative Higherarchical Clustering (AHC) by Ward's method (Crisosto 2007).

10.2. RESULT AND DISCUSSION

There have always been debates regarding the taxonomic complexity of the species associated with the family Lauraceae. The species related to this family have shown variations and similarities in different morphological aspects (Chapter III). There are many morphological limits of the species of Lauraceae regarding identification due to the short flowering and fruiting time. For this reason, this family has been re-classified many times but no satisfactory revision of this whole section has

yet been worked out. The boundaries between many of the species of this family are still ill-defined, with several of the separately described 'new' taxa have established themselves with insignificant morphological variants. Many authors suggested that the presence or absence of certain compounds in plants supplied several taxonomic markers with valuable information regarding the systematic position of this species within a family (Hillig & Mahlberg 2004; Zafar *et al.* 2011; Demetzos & Perdetzogloul 1999; Mohy-Ud-Din *et al.* 2010).

Twenty six species belonging to Lauraceae have been reported from Terai and Duars region of North Bengal, India (Chapter 5). Among them, eight economically important Laurels are commonly available in this region. The qualitative analysis of essential oil, anthraquinones, bitter principle, flavonoids and phenolics of these eight Laurels was done by Thin Layer Chromatography. In TLC, the qualitative analysis of these components was done on aluminium based silica gel plates using specific solvent systems for each secondary metabolite's group (Figure 10.1– 10.6).

When the methanolic extracts of these eight species were subjected to the solvent system ethyl acetate: methanol: water (100:13.5:10), which is specific for anthraquinones (Wagner *et al.* 1993; Nandhasri *et al.* 2005), *Cinnamomum* spp showed many red coloured bands on preparative silica gel plates under UV-365nm indicating the presence of various anthraquinone derivatives (Figure 10.1). Similar solvent system was used for the experiment of bitter principles. After the spraying of vanillin-sulphuric acid reagent many coloured bands like red-violet, brown-red, blue-green and blue bands were appeared which were identified and shown in the Table 10.2 and Figure 10.2. On the other hand, these plants were able to scavenge free radicals for the presence of different phytochemicals (already discussed in Chapter 8). For that purpose, the methanolic extracts of all plants were applied to silica gel plates and developed in ethyl acetate: formic acid: acetic acid: water (100:11:11:27) (Nandhasri *et al.* 2005) for detection of flavonoids, phenolics and DPPH fingerprinting respectively. The samples showed orange, orange-yellow and yellow-green bands on flavonoids' specific solvent system (Figure 10.3), while pinkish orange bands on phenolics' specific solvent system indicating their existence in tested samples (Figure 10.4). After separation on TLC plates, the antioxidant compounds of Laurels were determined *in situ* with DPPH reagent, in which the TLC plate was observed through visible light (Figure 10.5). As shown in Figure 10.5, the separated components producing yellowish bands on the purple background were considered as antioxidants. Many authors examined that the purple background colour was visualized with distinct yellow bands after spraying the plate with DPPH reagent when crude herbal drugs were separated through TLC (Ruiz-Terán *et al.* 2008; Rumzhum *et al.* 2012). TLC was also used for identification of essential oils of these eight Laurels. After separation, the TLC plates were pulverized with anisaldehyde, warmed for spots colouring and studied in visible range (Figure 10.6).

In 1972, Gottlieb carried out extensive survey on most of the genera in Lauraceae, but insufficient records are available on the chemo-taxonomical aspects on this family. The results of the cluster analysis (Figure 10.7) provide useful chemotaxonomic correlations of two genera of this family. Dendrogram clustering analysis based on thin layer chromatographic data sorted out *C. camphora* as independent entities. The result is much similar with the result of Chapter-7. A qualitative study of phytochemicals itself through TLC-based separation profile and fingerprint thus seems to be much useful in the taxonomic study of the genus *Cinnamomum* and *Litsea*.

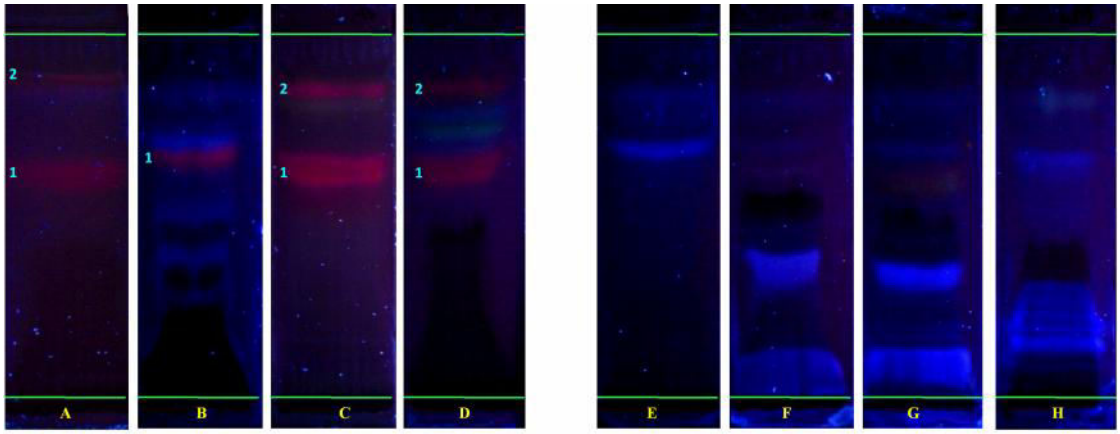


Figure 10.1. Detection of anthraquinones in bark of studied taxa

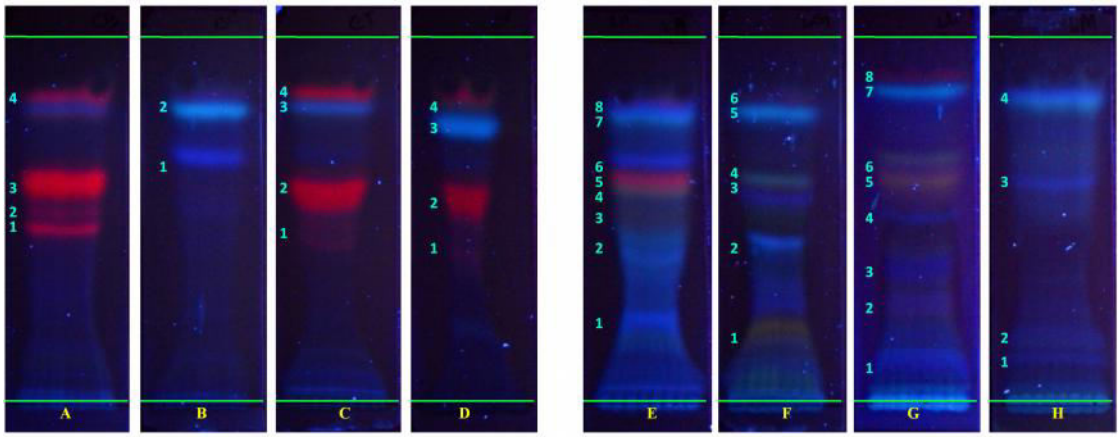


Figure 10.2. Detection of bitter principles in bark of studied taxa

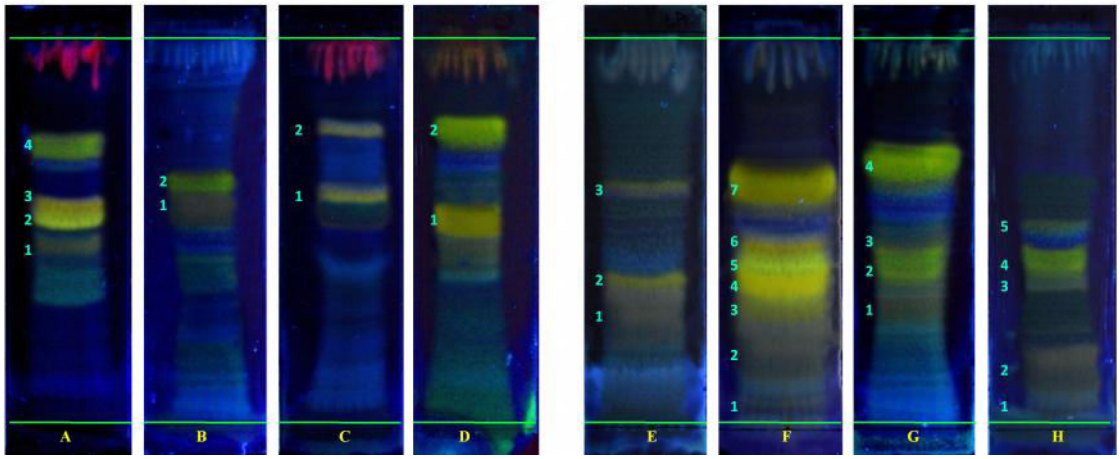


Figure 10.3. Detection of flavonoids in bark of studied taxa

[A] *Cinnamomum bejolghota,* **[B]** *C. camphora,* **[C]** *C. tamala,* **[D]** *C. verum,* **[E]** *L. assamica,* **[F]** *L. glutinosa,* **[G]** *L. laeta,* **[H]** *L. monopetala*
1, 2, 3, 4…….. are the bands of different compounds

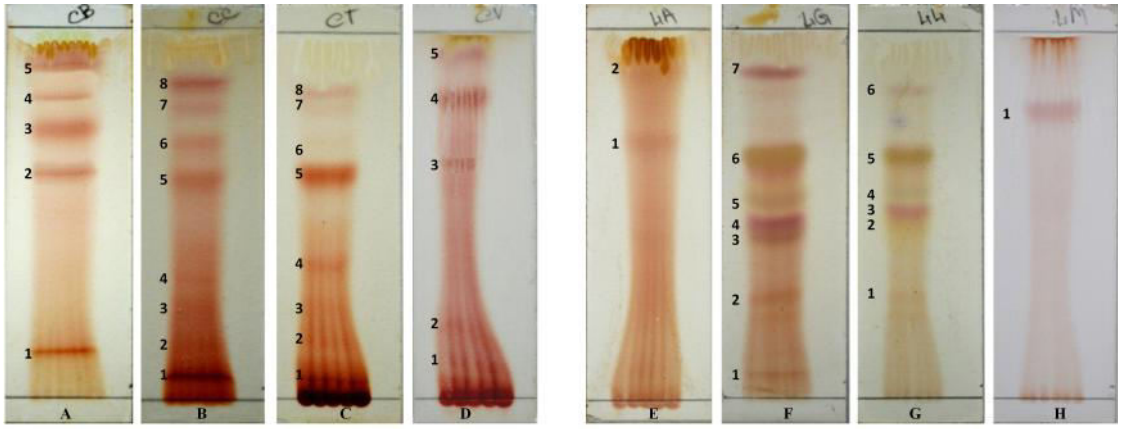


Figure 10.4. Detection of phenolics in bark of studied taxa

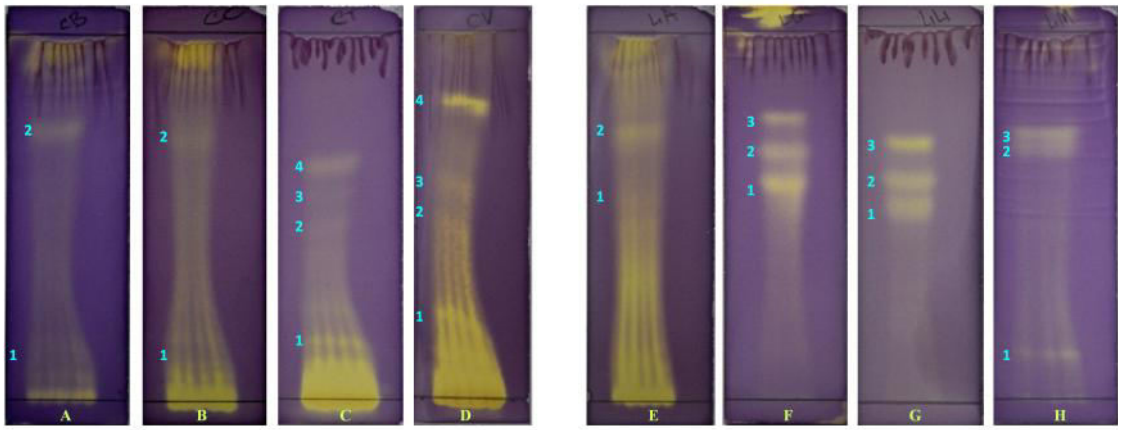


Figure 10.5. Detection of DPPH free radical scavenging potency in bark of studied taxa

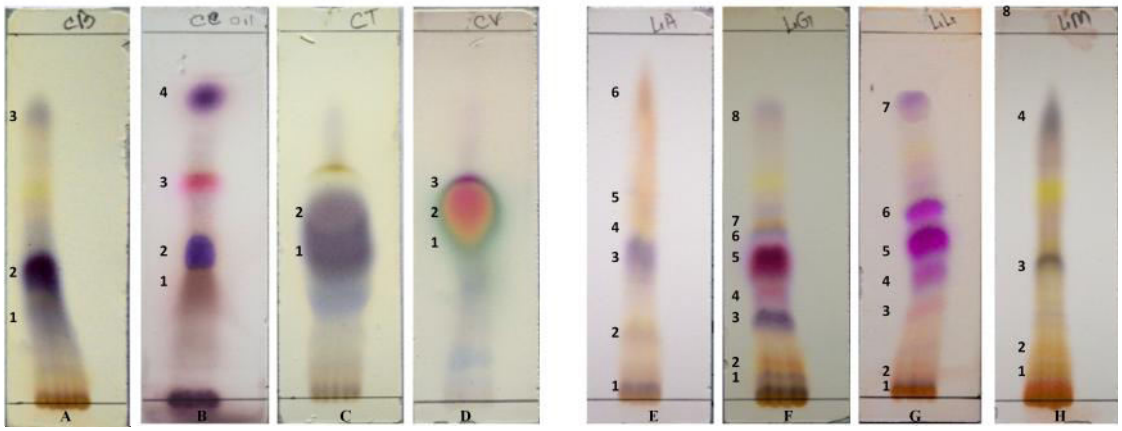


Figure 10.6. Detection of essential oils in bark of studied taxa

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[A] *Cinnamomum bejolghota,* [B] *C. camphora,* [C] *C. tamala,* [D] *C. verum,* [E] *L. assamica,* [F] *L. glutinosa,* [G] *L. laeta,* [H] *L. monopetala*

1,2,3,4…….. are the bands of different compounds

Table 10.1. Detection of anthraquinones of studied taxa

Plant	Spot ID	hR_f Value	Colour of band	Expected compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum bejolghota	1	60.65	Red	Frangulin A, B; Glucofrangulin A, B; Emodin; Rhein	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	10% ethanolic KOH reagent	UV-365
	2	86.88						
Cinnamomum camphora	1	63.95						
Cinnamomum tamala	1	60.65						
	2	85.25						
Cinnamomum verum	1	60.65						
	2	85.25						
Litsea assamica	Absent							
Litsea glutinosa								
Litsea laeta								
Litsea monopetala								

Table 10.2. Detection of bitter principle of studied taxa

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum bejolghota	1	47.54	Red violet	Neohesperidin, Naringin, Harpagoside	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Vanillin sulphuric acid reagent 1% ethanolic vanillin (solution I). 10% ethanolic sulphuric acid (solution II). The plate is sprayed with 10 ml sclution I, followed immediately by 10 ml solution II. After heating at 110°C for 5-10 min under observation	UV-365
	2	52.45						
	3	59.01						
	4	81.96	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	5	85.24	Red violet	Neohesperidin, Naringin, Harpagoside				
Cinnamomum camphora	1	59.01	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	2	68.85						
	3	80.32						
Cinnamomum tamala	1	45.9	Red violet	Neohesperidin, Naringin, Harpagoside				
	2	57.37						
	3	68.85	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	4	80.32						
	5	85.24	Red violet	Neohesperidin, Naringin, Harpagoside				

Table 10.2. Detection of bitter principle of studied taxa (Cont.....)

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum verum	1	44.26	Red violet	Neohesperidin, Naringin, Harpagoside	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Vanillin sulphuric acid reagent 1% ethanolic vanillin (solution I). 10% ethanolic sulphuric acid (solution 11). The plate is sprayed with 10 ml solution I, followed immediately by 10 ml solution II. After heating at 110ºC for 5-10 min under observation	UV-365
	2	54.09						
	3	68.85	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	4	77.04						
	5	85.24	Red violet	Neohesperidin, Naringin, Harpagoside				
Litsea assamica	1	21.31	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	2	40.98						
	3	50.81						
	4	57.37	Red violet	Neohesperidin, Naringin, Harpagoside				
	5	60.65						
	6	65.57	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	7	80.32						
	8	85.24						
Litsea glutinosa	1	19.67	Brown red	Gentiopicroside, Swertiamarin				
	2	42.62	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	3	55.73						
	4	60.65						
	5	80.32						
	6	85.24						

Table 10.2. Detection of bitter principle of studied taxa (Cont.....)

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Litsea laeta	1	9.83	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Vanillin sulphuric acid reagent 1% ethanolic vanillin (solution I). 10% ethanolic sulphuric acid (solution II). The plate is sprayed with 10 ml solution I, followed immediately by 10 ml solution II. After heating at 110°C for 5-10 min under observation	UV-365
	2	22.95						
	3	34.42						
	4	50.81						
	5	59.01	Red violet	Neohesperidin, Naringin, Harpagoside				
	6	66.21	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	7	85.24						
	8	90.16	Red violet	Neohesperidin, Naringin, Harpagoside				
Litsea monopetala	1	9.83	Blue	Foliamenthin, menthiafolin, Quassin, Marrubiin, Absinthin, Cnicin				
	2	18.63						
	3	60.65						
	4	85.24						

Table 10.3. Detection of flavonoids of studied taxa

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum bejolghota	1	42.62	Orange	Leutedin	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Natural products-polyethylene glycol reagent The plate is sprayed with 1% methanolic diphenylboric acid-β-ethylamino ester (=diphenylboryloxyethylamine, NP), followed by 5% ethanolic polyethylene glycol-4000(PEG) (10 ml and 8 ml, respectively)	UV-365
	2	52.45	Yellow green	Kaempferol, isohamnetin, apigenin				
	3	55.73	Orange yellow	Quercetin, myricetin				
	4	72.13	Yellow green	Kaempferol, isohamnetin, apigenin				
Cinnamomum camphora	1	55.73	Orange	Leutedin				
	2	60.65	Yellow green	Kaempferol, isohamnetin, apigenin				
Cinnamomum tamala	1	59.01	Orange yellow	Quercetin, myricetin				
	2	77.01						

Table 10.3. Detection of flavonoids of studied taxa (Cont....)

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum verum	1	44.26	Orange	Leutedin	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Natural products-polyethylene glycol reagent The plate is sprayed with 1% methanolic diphenylboric acid-β-ethylamino ester (=diphenylboryloxyethylamine, NP), followed by 5% ethanolic polyethylene glycol-4000(PEG) (10 ml and 8 ml, respectively)	UV-365
	2	52.45	Orange yellow	Quercetin, myricetin				
	3	75.4	Yellow green	Kaempferol, isohamnetin, apigenin				
Litsea assamica	1	27.86	Orange	Leutedin				
	2	36.06	Orange yellow	Quercetin, myricetin				
	3	59.01						
Litsea glutinosa	1	4.91	Orange	Leutedin				
	2	19.67						
	3	29.5	Orange yellow	Quercetin, myricetin				
	4	36.06						
	5	40.98	Yellow green	Kaempferol, isohamnetin, apigenin				
	6	45.9	Orange yellow	Quercetin, myricetin				
	7	60.65						

Table 10.3. Detection of flavonoids of studied taxa (Cont....)

Plant	Spot ID	hRf Value	Colour of band	Compounds	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Litsea laeta	1	31.14	Orange	Leutedin	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Natural products-polyethylene glycol reagent The plate is sprayed with 1% methanolic diphenylboric acid-β-ethylamino ester (=diphenylboryloxyethylamine, NP), followed by 5% ethanolic polyethylene glycol-4000(PEG) (10 ml and 8 ml, respectively)	UV-365
	2	40.98	Yellow green	Kaempferol, isohamnetin, apigenin				
	3	47.54	Orange	Leutedin				
	4	68.85	Yellow green	Kaempferol, isohamnetin, apigenin				
Litsea monopetala	1	3.27	Orange	Leutedin				
	2	14.75						
	3	36.06						
	4	40.98	Yellow green	Kaempferol, isohamnetin, apigenin				
	5	52.45	Orange	Leutedin				

Table 10.4. Detection of phenolics of studied taxa

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis	Compounds
Cinnamomum bejolghota	1	12	Pinkish orange	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Fast blue reagent 0.5 g fast blue salt B is dissolved in 100 ml water. (Fast blue B = 3,3' -dirnethoxybiphenyl-4,4' -bist diazonium) -dichloride)	Visible	Phenolics
	2	61.33						
	3	73.33						
	4	81.33						
	5	89.33						
Cinnamomum camphora	1	6.66						
	2	14.66						
	3	25.33						
	4	38.66						
	5	60						
	6	69.33						
	7	78.66						
	8	85.33						

Table 10.4. Detection of phenolics of studied taxa (Cont....)

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis	Compounds
Cinnemomum tamala	1	6.66	Pinkish orange	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Fast blue reagent 0.5 g fast blue salt B is dissolved in 100 ml water. (Fast blue B = 3,3' -dirnethoxybiphenyl-4,4' -bist diazonium) -dichloride)	Visible	Phenolics
	2	16						
	3	25.33						
	4	37.33						
	5	62.66						
	6	70.66						
	7	80						
	8	84						
Cinnemomum verum	1	8						
	2	18.66						
	3	64						
	4	81.33						
	5	93.33						
Litsec assamica	1	70.66						
	2	92						

Table 10.4. Detection of phenolics of studied taxa (Cont....)

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis	Compounds
Litsea glutinosa	1	6.66	Pinkish orange	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	Fast blue reagent 0.5 g fast blue salt B is dissolved in 100 ml water. (Fast blue B = 3,3' -dimethoxybiphenyl-4,4' -bist diazonium) -dichloride)	Visible	Phenolics
	2	26.66						
	3	44						
	4	48						
	5	53.33						
	6	65.33						
	7	81.33						
Litsea laeta	1	26.66						
	2	48						
	3	30.66						
	4	56						
	5	66.66						
	6	84						
Litsea monopetala	1	77.33						

Table 10.5. Detection of DPPH scavenging capacity of studied taxa

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis
Cinnamomum bejolghota	1	9.85	Yellow	Methanol	Ethyl acetate: Methanol: Water 100: 13.5: 10	0.2% DPPH Solution	Visible
	2	73.23					
Cinnamomum camphora	1	9.85					
	2	71.83					
Cinnamomum tamala	1	14.08					
	2	47.88					
	3	56.33					
	4	64.78					
Cinnamomum verum	1	18.3					
	2	49.29					
	3	59.15					
	4	71.83					
Litsea assamica	1	55.55					
	2	72.22					
Litsea glutirosa	1	58.33					
	2	68.05					
	3	76.38					
Litsea laeta	1	50.68					
	2	58.9					
	3	68.49					
Litsea monopetala	1	14.08					
	2	67.6					
	3	73.23					

Table 10.6. Detection of essential oil of studied taxa

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis	Compounds
Cinnamomum bejolghota	1	24.32	Strong blue	Dichloromethane	Toluene: Ethyl acetate 93:7	Anisaldehyde sulphuric acid (0.5 ml anisaldehyde is mixed with 10ml glacial acetic acid. The plate is sprayed with 5-10ml and then heated at 120°C for 7-10min.)	Visible	Essential oils
	2	36.48						
	3	79.72						
Cinnamomum camphora	1	31.5	Brown					
	2	41.09	Strong blue					
	3	58.9	Red					
	4	82.19	Strong blue					
Cinnamomum tamala	1	41.09	Strong blue					
	2	52.05						
Cinnamomum verum	1	45.94	Green					
	2	52.7	Red					
	3	59.45						
Litsea assamica	1	4.1	Strong blue					
	2	17.8	Brown					
	3	39.72	Strong blue					
	4	46.57	Brown					
	5	82.19						

Table 10.6. Detection of essential oil of studied taxa (Cont....)

Plant	Spot ID	hRf Value	Colour of band	Solvents used for extraction	Running solvent	Spraying reagents	UV/Vis	Compounds
Litsea glutinosa	1	4.05	Strong blue	Dichloromethane	Toluene: Ethyl acetate 93: 7	Anisaldehyde sulphuric acid (0.5 ml anisaldehyde is mixed with 10ml glacial acetic acid. The plate is sprayed with 5-10ml and then heated at 120°C for 7-10min.)	Visible	Essential oils
	2	9.45	Brown					
	3	21.62	Strong blue					
	4	29.72	Red					
	5	37.83						
	6	43.92	Strong blue					
	7	48.64	Brown					
	8	77.02	Strong blue					
Litsea laeta	1	4.1	Strong blue					
	2	8.21	Brown					
	3	24.65	Red					
	4	34.24						
	5	42.46						
	6	50.68						
	7	79.45	Strong blue					
Litsea monopetala	1	5.47	Strong blue					
	2	12.32	Brown					
	3	38.35	Strong blue					
	4	76.71						

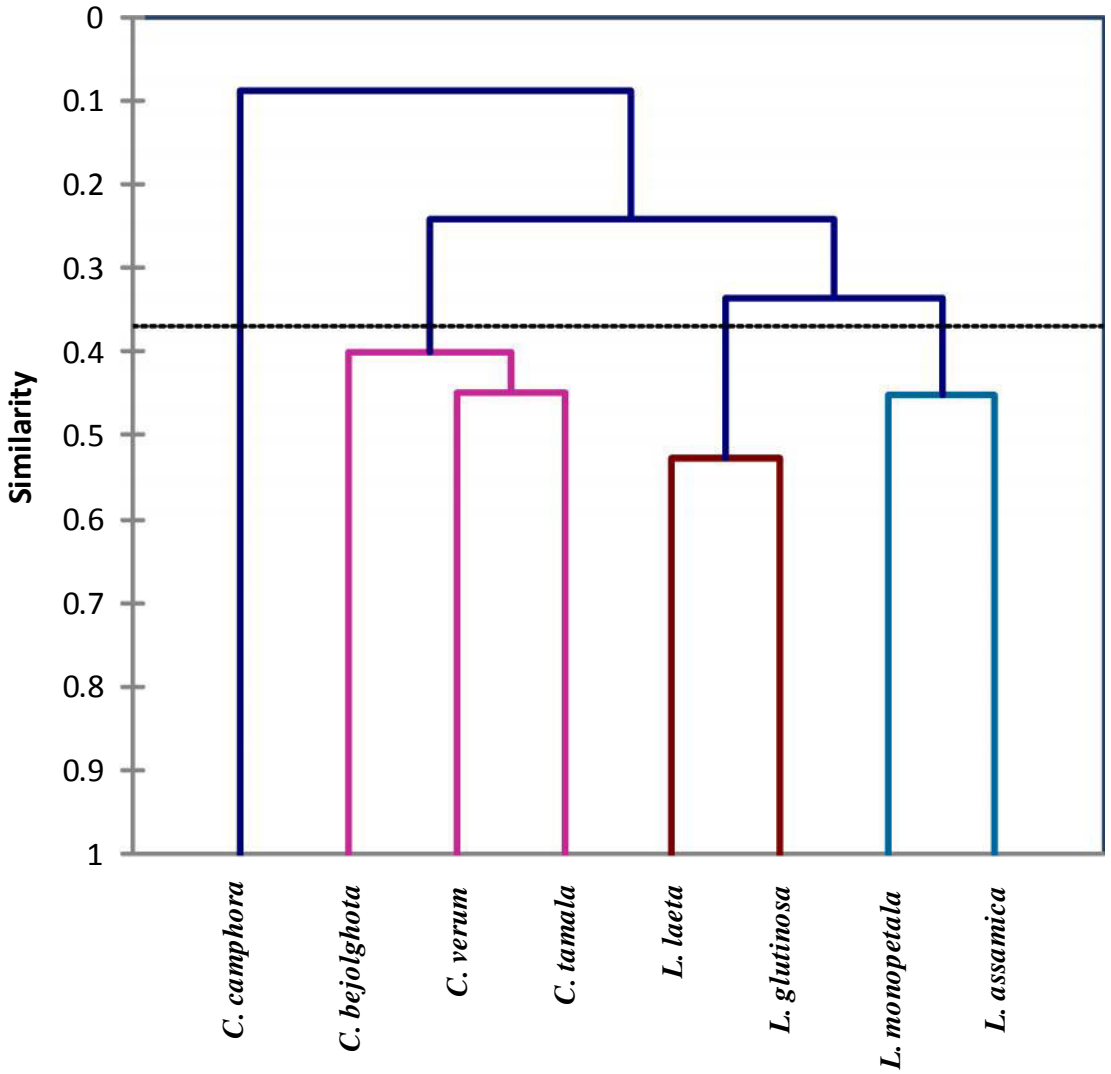

Figure 10. 7. Dendrogram based on phytochemical constituents present in eight Laurels

Considerable variations were thus observed between different spices of Lauraceae in terms of antioxidant activity and phytochemical attributes. Unsupervised pattern recognition techniques facilitated visualization of the complex dataset and clustered dendrogram was established on the basis of distribution of phytochemical attributes. The combination of chemical characters and multivariate data analysis reinforce easy interpretation of similarities and differences among eight Laurels on their antioxidant activity and content of secondary metabolites. Information from this study might be useful for choosing the specific type of spices for consumption in order to prevent lifestyle-related disorders among consumers. On the other hand, the work was also helpful in taxonomic study and cladogram analysis of the family Lauraceae.

CHAPTER - 11

General Discussion

General Discussion

Natural resources provide the basic needs of life. Human civilization has already exploited enormous reserve of biological resources of this planet for fulfilling their various purposes of daily requirement. The hills of Darjeeling and Sikkim forms an integral part of the Eastern Himalayan region and is the storehouse of biological resources, representing of a large number of important, endemic, rare and endangered plants (Das 1995, 2004; Bhujel 1996; Bhujel & Das 2002; Ghosh & Das 2009). Even today, this region attracts a large number of botanists, naturalists and tourists from different parts of the world. Terai and Duars area of West Bengal is situated under Eastern sub-Himalayan region. Due to suitable environmental set-up, the Terai-Duars region becomes one of the richest botanical diversity in India (Das *et al*. 2010). This region equally represents all the habit group of plants such as herbs, shrubs, small to large climbers and trees. Many wild species of this region are found to be of great economic significance for the local people including food and ornamental potentiality, and also having therapeutic, religious and socio-cultural values (Ghosh 2006; Sarkar 2011). On the other hand, a large number of trees belonging to the diverse families are known for their high quality timber, which are quite costly, durable and are much demanding in global market. However, many plant species of this region were found to be threatened and endangered (Das *et al*. 2010).

11.1. LAUREL FLORA

The forests of Terai and Duars region are also the ideal home for oil yielding plants, among those Laurels are predominant. The present work clearly exhibited that Lauraceae is one of the well represented families in Terai-Duars region of West Bengal, represented by the recorded 9 genera and 26 species. Among the 26 species, *Litsea* Lamarck is the highest representative with its nine species. From several protected areas of Terai-Duars region, Mahananda Wildlife Sanctuary showed highest species diversity of Laurels (Table 11.1).

Table 11.1. Present record of Laurels from different Protected Areas and other places of Terai-Duars **[BTR =** Buxa Tiger Reserve; **GMP =** Garden of Medicinal Plant, University of North Bengal; **GNP =** Gorumara National Park; **JNP =** Jaldapara National Park; **MWLS =** Mahananda Wildlife Sanctuary**]**

Species	Floristic region				Other places
	MWLS	GNP	BTR	JNP	
Actinodaphne longipes	+	-	-	-	-
Actinodaphne obovata	+	+	+	-	-
Actinodaphne sikkimensis	+	+	-	+	-
Beilschmiedia assamica	-	+	-	-	-
Cinnamomum bejolghota	+	+	+	-	GMP
Cinnamomum camphora	-	-	-	-	GMP
Cinnamomum glaucescens	-	+	-	-	-
Cinnamomum impressinervium	+	-	-	-	-

Species	Floristic region				Other places
	MWLS	GNP	BTR	JNP	
Cinnamomum tamala	-	-	+	+	GMP, commonly cultivated
Cinnamomum verum	-	-	-	-	GMP, commonly cultivated
Cryptocarya amygdalina	-	+	-	-	-
Lindera assamica	+	+	-	-	-
Litsea assamica	-	-	-	+	Falakata near JNP
Litsea cubeba	+	+	-	-	Sal Bagan, NBU Campus
Litseae longata	+	-	-	-	-
Litsea glutinosa	+	+	+	+	GMP, also widely distributed throughout Terai & Duars
Litsea hookeri	+	+	-	-	-
Litsea laeta	+	-	-	-	GMP
Litsea monopetala	+	+	+	+	GMP, widely distributed throughout Terai & Duars
Litsea panamanja	+	+	+	-	-
Litsea salicifolia	+	+	+	+	-
Machilus duthiei	+	-	-	-	-
Machilus gamblei	+	-	-	-	-
Machilus glaucescens	+	-	-	-	-
Parsea odoratissima	-	+	+	-	-
Phoebe hainesiana	-	-	+	-	-

11.1.1. New records of distribution

The present work exposed the presence of quite a few species as new record of distribution for this region. As much as 16 species *viz*., *Actinodaphne longipes, A. sikkimensis, Beilschmiedia assamica, Cinnamomum camphora, C. impressinervium, C. tamala, C. verum, Lindera assamica, Litsea assamica, L. elongata, L. hookeri, L. laeta, L. panamanja, Machilus duthiei, Parsea odoratissima* and *Phoebe hainesiana* were not recorded in earlier accounts for this region (Cowan & Cowan 1929; Long 1984) are now reported here for the first time to occur in the Terai and Duars belt of West Bengal. However, apart from *Litsea assamica*, all other species were known to grow from different other localities of the state. The distribution of *Litsea assamica* was earlier known only from North–East India (Kanjilal *et al.* 1940; Bhuinya *et al.* 2009); therefore, the present collection of the species from Terai and Duars is a new record of its occurrence in West Bengal.

11.1.2. Endemics

A species which is only found growing naturally in a given region and nowhere else in the world is known as endemic. It must be make prominent that the concept of the endemism is very much dependent on the knowledge of geographical range of a species. From the recorded Laurels of Terai and Duars regions, three species, namely *Actinodaphne longipes*, *Cinnamomum impressinervium*, *Phoebe hainesiana* are endemic to Eastern Himalaya and three more species, *viz*. *Beilschmiedia assamica, Cinnamomum glaucescens* and *Lindera assamica* are endemic to north-eastern region of the Indian subcontinent. Also, another economically important species i.e. *Cinnamomum tamala* is basically a tropical Himalayan plant.

11.1.3. Exotics

Migration of plant species from one part to other distant part is a continuous process and is assisted by various geographical, climatic and ethnic factors. Various connecting links like land-bridge and vast marine carriages might be the probable route for migration of exotics among several countries. Migration appears to be a long process for many of the plants. Exotics, once migrated need to acclimatise itself, establish, propagate and naturalise to a new habitat. Exotic species forms important component of Eastern Himalayan flora, many of them being already naturalized and some is being in the process of naturalization. Some of these exotic species are desirably introduced for food, fibre, fruits, flowers and drug values (Cowan & Cowan 1929).

Three exotic species *viz*. *Cinnamomum camphora*, *Cinnamomum verum* and *Litsea cubeba* were recorded from study area. Among these, *C. camphora* is native to China as well as in Japan, *L. cubeba* is from China and Indonesia (Ara *et al.* 2007), and *C. verum* is native to Sri Lanka (Li *et al.* 2008a). *L. cubeba* has become truly naturalized in this zone and now represented with good population structure. On the other hand, *C. verum* is commonly planted in this region for the spice *Daruchini* (i.e. Cinnamon-bark) and cinnamon oil.

11.2. IMPORTANCE

The Laurels of Terai-Duars are of high value assets from the economic point of view. Some of these species have been found to be economically very important and are precious medicinal plant resources (Das *et al.* 2010). Besides, many others have high economic value like aromatic, edible, spice, timber and more being used for various domestic purposes as well as industrial uses (Choudhury *et al.* 2013b). The importances of these species have shown in the Table 11.2.

11.3. PHYLOGENETIC RELATIONSHIP OF STUDIED LAURELS

Among the 26 recorded economically important species of Lauraceae, 4 species of *Litsea* and 4 species of *Cinnamomum* are commonly available in the belt of Terai and Duars. Morphologically these eight species are quite overlapping. From the numerical data of morphology, a relationship was drawn through the production of a dendrogram in the Chapter 5. But it is not always possible to depend on morphological characteristics as for the substantial numbers of species, the fruits or flowers are difficult to procure. This makes generic placement of many species uncertain, since most taxa are defined mainly by floral characters. A second problem lies with the difficulties associated with timely collection of flowers and fruits required for identification of specimen as flowering and fruiting period of Laurels are very short and most of the characters for arrangement of generic key are taken from reproductive parts of Lauraceous specimen.

Table 11.2. Importance of different Laurels of Terai and Duars

Species	Uses
Actinodaphne longipes	Timber used for light construction and interior furnishing
Actinodaphne obovata	Bark used to treat fractured bones
Actinodaphne sikkimensis	Timber used for light construction
Beilschmiedia assamica	Wood used for making boats and boxes
Cinnamomum bejolghota	Leaf and bark used as condiment
Cinnamomum camphora	Camphor oil used in perfumery and treatment of nervous depression, acne, inflammation, arthritis, cold and fever
Cinnamomum glaucescens	Produce essential oils to use in perfumery and cosmetics; locally used against various skin diseases
Cinnamomum impressinervium	Bark used as substitute for or as an adulterant of *Cinnamomum verum*
Cinnamomum tamala	Leaves used as spice; bark and leaves also used to treat several disease e.g. diarrhoea, colic, vomiting, cardiac disorder, etc.
Cinnamomum verum	Produce important spice 'cinnamon-bark' of commerce; used medicinally to treat stomach-ache; bark and leafy branch lets contain volatile oil
Cryptocarya amygdalina	Timber used for light construction
Lindera assamica	Wood used for house construction
Litsea assamica	Wood used for making match boxes
Litsea cubeba	Fruit oil is added to food for flavouring and also used as bio-pesticide
Litsea elongata	A good fodder for cattle; wood used for construction works, making furniture, etc.
Litsea glutinosa	Bark used to treat diarrhoea, dysentery, rheumatic joint pain etc.; bark powder used as an adhesive paste in incense stick production
Litsea hookeri	Timber used for house construction and for making furniture
Litsea laeta	Seed oil is with high antioxidant activity
Litsea monopetala	Leaves used to treat arthritis and for rearing muga-silk moth larvae
Litsea panamanja	Wood used for house construction, making furniture and as fire wood
Litsea salicifolia	Seed oil used as bio-pesticide; leaves as good food for muga-silk moth larvae
Machilus duthiei	Roots used to treat inflammation, asthma, pain, bronchitis, vomiting and blood diseases
Machilus gamblei	Produce good quality firewood
Machilus glaucescens	Used as firewood
Persea odoratissima	A red dye is prepared from its bark
Phoebe hainesiana	Wood used for making furniture and plywood

A drastic remedy for this problem would be to use the different other attributes like anatomy, leaf architecture and chemotaxonomy.

11.3.1. Anatomical characterization

Anatomical attributes have long been used for solving many taxonomic disputes (Agbagwa & Ndukwu 2004; Kharazian 2007). Like morphological characters, anatomical features also can provide distinguishable characters for the preliminary identification of different species. For this purpose stem, lamina and petiole anatomy played crucial role (Metcalfe & Chalk 1950). Lersten & Curtis (2001) indicated that stem anatomical studies support to solve many systematic problems. In 2012 Rao *et al.* applied anatomical characteristics for the identification of economically important *Litsea glutinosa*. Baruah and Nath in 2006 also investigated the leaf anatomy of *Cinnamomum pauciflorum* Nees and in 2012, Bhatt and Pundya examined the leaf anatomy of *Litsea chinensis* Lamarck. They noticed that uniseriate epidermal layer, anisocytic stomata and presence of abundant mucilage in developed vascular bundle might eventually help in recognition as well as standardization of the circumscription for a species. Therefore, the valuable anatomical characters are helpful in designated taxonomical structures of some Laurels. In Chapter 6, it was established that the anatomical characters of leaf, petiole and stems of eight Laurels of Terai-Duars region can also provide very significant data, if recognized from taxonomic view point.

11.3.2. Characterization through Leaf Architecture

Similarly, Leaf-architecture is also frequently used as important aspects for solving many taxonomic disputes (Moore 2008; Todzia 1991). Leaf-architecture was initially used by Hickey to represent the placement of plant species by leaf-structure with leaf shape, gland position, venation pattern and marginal configuration (Hichey 1973). After that many authors created categories, groups and subgroups on the basis of lamina venation. Todzia and Keating (1991) have showed the link between Lauraceae and Chloranthaceae by using the leaf-architecture data. Beside these characters, stomata also provide many taxonomically important diagnostic characters, like stomatal index, stomata type and the occurrence of stomata on the adaxial or abaxial leaf surface. (Tripathi & Mondal 2012).

So, in Chapter 7 a study was undertaken to produce a comprehensive account of the leaf-architecture in eight species under two genera, *Cinnamomum* Schaeffer and *Litsea* Lamarck of the Lauraceae of Terai-Duars region. It was noticed that the minor venation pattern and F.E.Vs are distinctly different in *Litsea* and *Cinnamomum*, also notable differences are observed in the size and numbers of areoles. Therefore, quantitative differences in minor venation pattern might embrace physiological or adaptive significance. As principal characteristics of the leaf venation pattern of a species are genetically fixed. So, this study can also be used as important pharmacognostic tool.

11.3.3. Phytochemical Characterization

Similar to the structural characteristics of different plant parts the chemical constitutions in plants are also constant.

11.3.3.1. Studies on antioxidants and polyphenols

As we know, polyphenols are secondary metabolites and are differentially distributed in the plant kingdom. Taxonomically related species might show considerable similarity in qualitative polyphenol profile. Though, quantity of individual polyphenols could differ widely in different species of the same family or same

group, both qualitative and quantitative polyphenol profiling together with total antioxidant capacity measured by various methods could be used to classify plants. The application of chemometric tools for characterization and determination of geographic origin of species has recently become a very active research area (Arvanitoyannis *et al.* 1999; Downey *et al.* 2003; Woodcock *et al*. 2007). Wang*et et al.* (2009) presented an overview of the similarities and differences among ten algal species and also investigated the relationships between total phenolic content and different antioxidant activity assays by chemical properties. Agglomerative Hierarchical Clustering (AHC) is a mathematical tool which can represent relationships between data and samples. In Chapter-8, a study was performed to make relationship among the selected species based on antioxidant activity and levels of total polyphenols and flavonoids. Polyphenols are naturally occurring antioxidant components of plants. These phenolic compounds are found in almost all plants and play important roles in preventing aging diseases like inflammation, cancer, and arteriosclerosis (Sato *et al*. 1996; Li *et al*. 2008b). In Chapter 8, it was noticed that the total phenolic contents of the bark extracts was much higher than those of the leaf extracts (except *L. assamica*) of eight different Laurels of Terai and Duars region. In different studies it was suggested that plant polyphenols, which showed antioxidant activity *in vitro*, also function as antioxidants *in vivo* (Lee *et al*. 2005; Shin *et al*. 2008). Li *et al.* (2008b) and Ozsoy *et al.* (2008) showed a positive linear correlation between the total phenolic content and antioxidant activity of the plants. The results suggest that these two genera of Lauraceae, which contained higher levels of polyphenols might have high antioxidant properties. It was already established that *L. glutinosa and Cinammomum verum* and *C. tamala* had high DPPH scavenging capacity (Kshirsagar & Upadhyay 2009; Chen *et al*. 2012). As we have already discussed that, the secondary metabolite-based chemotaxonomic technique can be used as an important tool for identifying and classifying these eight economically important plants according to species-specific metabolites, the chemotaxonomic importance and potential of secondary metabolites i.e. antioxidants in this family were confirmed by this study. Though the leaves and barks of eight plants were collected from same individual plants, the dendrogram was slightly dissimilar (Showed in Chapter 8) because the deposition pattern of the secondary metabolites in barks are more stable than leaves (Ahmad *et al.* 2009). Thus we selected barks of these plants in further chapters.

11.3.3.2. Antioxidant Activities of Essential oils

The plants of Lauraceae are directly related with the cosmetics and food additives industries for the presence of essential oils (Wang 2009). So in Chapter-9, the antioxidant activities of the essential oil of eight Laurels were determined and a relationship was established among these species. Several authors worked on antioxidant capacity of the oils of different lauraceous plants (Schmidt *et al.* 2006). The results of present investigation were similar to other contributors. When comparing the eight Laurels, with the cladogram based on the antioxidant profile of oil of Lauraceae, it was found that the genus *Litsea* is separated from the genus of *Cinnamomum*, which is parallel to the grouping developed by the morphological characteristics.

11.3.3.3. Chromatographic profiling data

It is already known that thin layer chromatography is another widely applied chemotaxonomic tool to constitute one of the most important methods of determining the taxonomic positions of taxa. So, it is now possible to study secondary metabolite profiles of low or high taxonomic levels, even of individual genotypes (Zafar *et al.* 2011). In 2010, Mohy-Ud-Din *et al.* stated that TLC is a major tool for

investigating accession structures, species, taxonomic problems and phylogenetic relationships at the generic level.

In addition, different plant compounds like flavonoids have been used as a chemotaxonomic marker (Yang 1998). With flavonoids, other secondary metabolites like anthraquinone, bitter principles, phenolics, essential oils and DPPH based free radical screening with TLC fingerprint of eight Laurels were performed which is showed in Chapter 10. Like Chapters 8 and 9, free radical scavenging screen through TLC was showed a beautiful yellow band against DPPH solvent. Like DPPH Screening, different secondary metabolites produced various coloured bands against different solvents with different spraying reagents. By calculating the h*Rf* values, a dendrogram was constructed through Agglomerative Hierarchical Clustering (AHC) method.

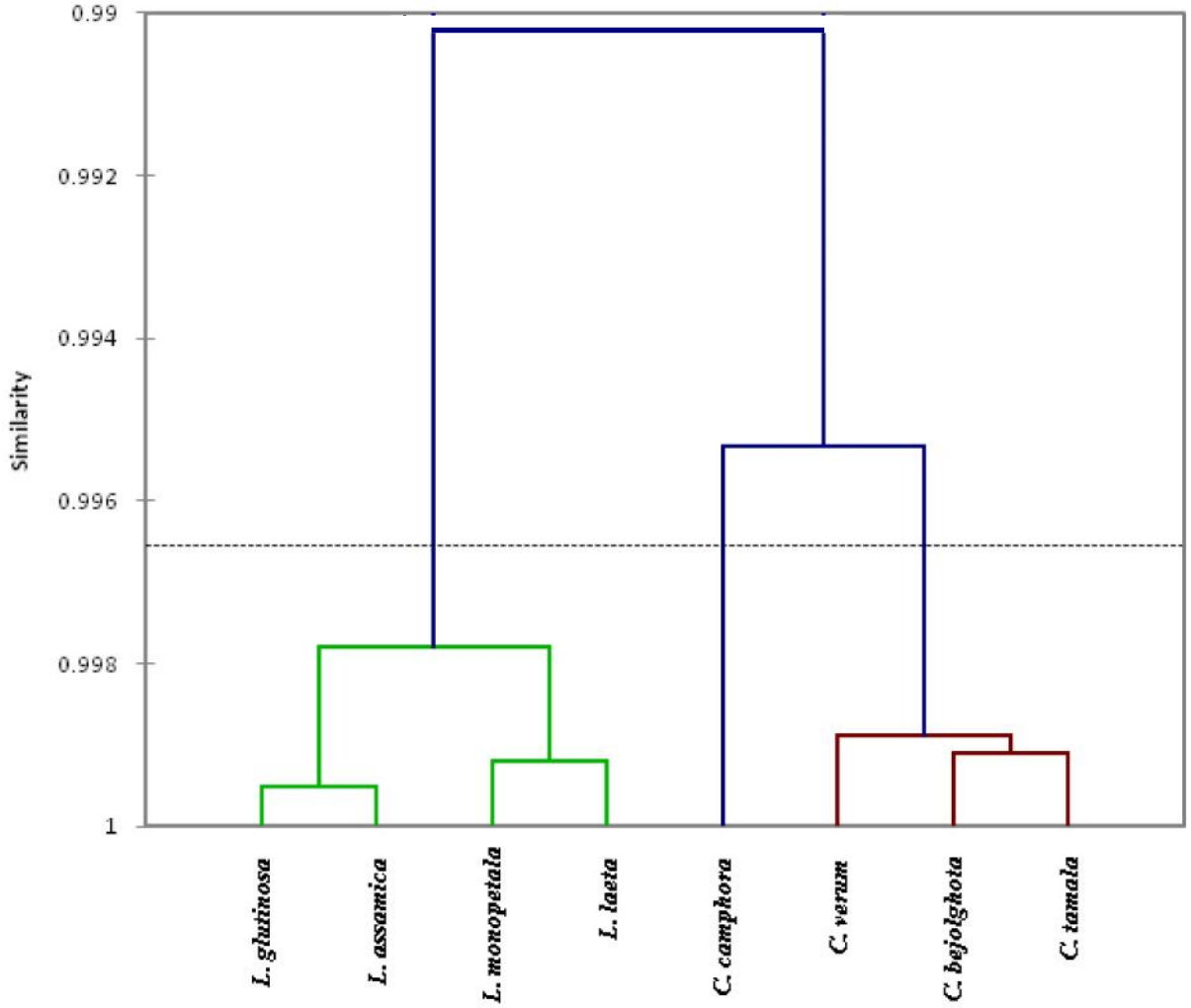

Figure 11.1. Dendrogram of eight species of Laurels from two genera from the Terai-Duars region with the combination of morphological, anatomical and chemical records

11.4. DETERMINED RELATIONSHIP

Although all chapters represent a standard relationship among the eight species of Lauraceae under intensive study but the representation of dendrogram was somewhat different from each other.

Considerable variations were thus observed between different spices of Lauraceae in terms of morphological, anatomical, leaf-architecture as well as different chemical constitutions. So, for obtaining more reliable results all data like morphological, anatomical, chemical and numerical data were applied. After the application, a dendrogram was found where two genera i.e. *Litsea* and *Cinnamomum* were separated (Figure 11.1). In case of *Litsea* two branches were produced first and each branch was further divided into two sub-groups. Total four sub-groupings were formed where *L. glutinosa* and *L. assamica* were more related, and in other branch it was noticed that *L. monopetala* and *L. laeta* were closely associated. On the other hand, for genus *Cinnamomum*, single node was divided into two branches. One branch was represented by *C. camphora* and the other branch split into three lines where the *C. bejolghota* and *C. tamala* exhibited more proximity than *C. verum.*

11.5. THREATS

It is now clear that the plant diversity of Laurels is significant and the ecosystem of Terai-Duars region is somewhat dependent on the wide range of Laurels. Along with other floristic elements, the Laurels of the region at present are under severe threat of losing their habitat mostly due to anthropogenic reasons. Such threats can be perceived only after detailed scientific investigations at different corners of diversified areas of Terai-Duars. The reasons of threats may be -

- The rapid extension of human settlement grabbing the natural habitat areas
- Too much of cattle grazing is destroying seedling and sapling of different plants including Laurels
- Establishment of large tea gardens replacing the natural habitat
- Continued extension of metalled roads and rail-links cris-crossing the forests of Terai and Duars
- Rampant legal and illegal extraction of timber and other plants and plant products
- High fragmentation of ecosystems of the area that has developed there due to their existence in the area for millions of years
- Monoculture plantations (mostly with fast growing exotic species) replacing the local natural forests over wide areas; the dense plants keep extremely limited scope for local species to enter and establish there
- Insect and fungi readily attack the seeds of Laurels
- Extensive socio-economic developmental activities and eco-tourism are adversely affecting the rich diversity of pristine vegetation structure of the entire area where most of the presently recorded species of Lauraceae are surviving
- The pressure for removal or death or extinction of many of these species, along with numerous other important and interesting non-laurel species, is increasing at every moment threatening the existence of the basic vegetation itself.

11.6. CONSERVATION STRATEGIES

As the species of Lauraceae are very important in different manner, so the existence of these species becomes in threat. Human beings are the main culprit for this situation. In one hand the availability of these plants is desirable but, on the other hand, their existence is now highly threatened. So, Laurels deserve special attention for their conservation. The conservation strategies of these species can be as follows-

- The strict regulation need to be strictly imposed to prohibit the entry of unauthorised people into protected areas
- The cattle grazing in the protected areas should be banned
- Illegal and legal extraction of timber and trees for any purpose should not be allowed at any cost
- The entry into the forests for the poachers, hunters and plant collectors should be prevented
- Collection of NTFP (including wild edibles fruits and medicinal plants) need to be controlled efficiently
- Tourism activities should be either minimised or efficiently managed. Eco-friendly procedures or initiatives should be built up in each and every tourism activity;
- Tourists need to be trained and given proper awareness about their activities while they are within the natural vegetation
- Any short of cultivation inside the protected areas should not be allowed
- Artificial forests should be developed only with the local species of different habit groups and not only with very few economically important species of trees
- Rare species need to be propagated through *in vivo* and *in vitro* methods and their populations need to be increased in the vegetation very carefully with extremely careful planning
- Natural habitat need to be saved at any cost not only to save some rare species of plants and animals but also the entire biosphere.

CONCLUSION

Conclusion

The present study on the different elements of Lauraceae in the flora of Terai and Duars, led to a conclusion that, the family has been studied worldwide from the botanical and chemical standpoints especially due to their usefulness to the human society. Terai-Duars is under the sub-Himalayan region and Laurels (wild and cultivated) are playing important role in the plant diversity and ecosystem of this part of West Bengal. However, with the rapid extension of human settlement areas, establishment of more and more tea gardens, extension of the net-work of metalled roads, too much of legal and illegal timber extraction, monoculture plantations (mostly with fast growing exotic species), intensive tourism related activities and other socio-economic developmental activities are adversely affecting the rich diversity of the pristine vegetation of the entire area where most of the presently recorded species of Lauraceae are surviving. The pressure for removal or death or extinction of many of these species, along with numerous other important and interesting non-laurel species, is increasing at every moment threatening the existence of the basic vegetation itself. The activities in the name of 'eco-tourism' are creating havoc in many places especially in the Lataguri – Gorumara region. Active steps for the conservation under proper surveillance are deemed essential since a thorough scientific research is certain to reveal their benevolent aspects as well as positive ecological functioning. Everyone needs to remember that conservation is best when a species is permitted to grow undisturbed in its own habitat, i.e. in its natural home! If the desired amount of seedlings and saplings can be produced then these plants can be saved through plantation programs. But the periods of flowering and fruiting condition is very restricted in Laurels, so the production of seeds is also very limited and difficult to procure. Few seeds which are produced with much difficulty are much disturbed and degraded in their habitat. Insect and fungi in their habitat readily attack the seeds and young seedlings. In addition, forestry related and other anthropogenic activities are creating extreme pressure on the maintenance of safe population structure for many of these species. Such habitats fail to protect the seedling in natural conditions and therefore the rate of seedling survival is also very low. Keeping this view experiments were carried out to try vegetative propagation through cutting and air-layering in some species can be tried for quick and low-cost propagation.

The Lauraceae has the reputation of being one of the most difficult families of angiosperms to identify; the problem mainly arise from the ambiguity in morphological characters. So, the several methods of systematics, which were used in present study can help in identification as well as resolve numerous phylogenic problems. Any species which is difficult to identify on the basis of morphological character like Laurels where specimens almost never bear both flowers and fruits, can be easily identify and classify using different anatomical and chemotaxonomic characters. As in the present study, a species can be recognized from any plant parts and not only with floral characters. So, these methods appears much easier than morphology especially when flowers and fruits are not properly available. Thus, different anatomical and chemotaxonomic methods appear as quite reliable, which are not so expensive in identification and determining the phyletic relations specially in taxonomically difficult of plant kingdom.

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APPENDICES

Appendix - A

Abbreviation and symbols used

°C	Degree centigrade
AHC	Agglomerative Hierarchical Clustering
ALP	Antilipid peroxidation
APG	Angiosperm Phylogeny Group
BHT	Butylated hydroxytoluene
BR-	Biosphere Reserve
BTR	Buxa Tiger Reserve
CAL	Central National Herbariyum
conc.	Concentrated
DMRT	Duncan's Multiple Range Test
DNA	De-oxyribonucleic acid
DPPH	2,2-diphenyl-1- picrylhydrazyl
DPX	Dibutyl Phathalate Xylene
F.E.Vs	freely ending ultimate veins
FAA	Formaldehyde: Acetic Acid: Alcohol
Fe^{2+}	Ferrous ions
FWT	Fresh weight tissue
GMP	Garden of Medicinal Plant, University of North Bengal
GNP	Gorumara National Park
h	Hour
H_2SO_4	Sulphuric acid
hR_f	Retention factor
IC_{50}	50% Inhibitory Concentration
IUCN	The International Union for Conservation of Nature and Natural Resources
JNP	Jaldapara National Park
mg	Milligram
MWLS	Mahananda Wildlife Sanctuary
NADPH	Nicotinamide-adanine dinucleotide phosphate
NBT	Nitroblue tetrazolium
NBU	North Bengal University
$NO^{\bullet}$	Nitric oxide
NP	National Parks
$ONOO^-$	Peroxynitrite
PAs	Protected Areas
PMN	Phenazine methosulphate
ROS	reactive oxygen species
SEE	standard error of estimates
SI	Stomatal index
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
WLS	Wildlife Sanctuaries

Appendix-B

List of publications

I. Based on Thesis works

Das, A.P.; Ghosh, C.; Sarkar, A.; Biswas, R.; Biswas, K.; **Choudhury, D.**; Lama, A.; Moktan, S. & Choudhury, A. 2010. Preliminary report on the Medicinal Plants from three MPCAs in Terai and Duars of West Bengal, India. *Pleione.* 4(1): 90 - 101.

Choudhury, D.; Ghosal, M.; Das, A.P. & Mandal, P. 2013. *In vitro* antioxidant activity of methanolic leaves and barks extracts of four *Litsea* plants. *Asian Journal of Plant Science and Research.* 3(1): 99 - 107.

Choudhury, D.; Biswas, R.; Mandal, P. & Das, A.P. 2013. Diversity of *Cinnamomum* Schaeffer (Lauraceae) in Terai and Duars region of West Bengal, India. *Pleione.* 7(2): 441 - 448.

Choudhury, D.; Biswas, R.; Mandal, P. & Das, A.P. 2014. Diversity of *Litsea* Lamarck [Lauraceae] in Terai and Duars regions of West Bengal, India. *Pleione.* 8(1): 68 - 78.

II. Other than Thesis works

Mitra, P.K.; Mitra, P.; Das, A.P.; Ghosh, C.; Sarkar, A. & **Choudhury, D.** 2010. Screening the efficacy of some East Himalayan medicinal plants on ethanol induced gastric ulcer in albino rats. *Pleione.* 4(1): 69 - 75.

Mitra, P.K.; Maitra, T.; Mitra, P.; Paul, B.; Ghosh, D.; Guria, M.; **Choudhury, D.** & Das, A.P. 2011. Antiulcer activity of an isolated compound (MK-1) from Murraya koenigii (Linnaeus) Sprengel leaf in rats. *Pleione.* 5(1): 49 - 55.

Choudhury, D.; Ghosal, M.; Das, A.P. & Mandal, P. 2013. Development of single node cutting propagation techniques and evaluation of antioxidant activity of *curcuma aeruginosa* roxburgh rhizome. *International Journal of Pharmacy and Pharmaceutical Sciences*. 5(2): 227 - 234.

Chowdhury, A.; Chowdhury, M.; **Choudhury, D.** & Das, A. P. 2013 *Ludwigia peruviana* (Linnaeus) H. Hara [Onagraceae]: a new record for West Bengal, India. *Pleione.* 7(1): 286 - 289.

Gupta, S. K.; Ghosal, M.; **Choudhury, D**. & Mandal, P. 2014. Dynamic changes in antioxidant activity during floral development of *Couroupita guianensis*. *British Journal of Pharmaceutical Research.* 4(6): 676 - 694.

Gupta, S. K.; Ghosal, M.; **Choudhury, D**. & Mandal, P. 2014. Assessment of antioxidant activity and polyphenolic content of *Couroupita guianensis* during flower and fruit maturation. *International Journal of Recent Scientific Research*. 5(5):940 – 947.

Choudhury, D. & Das, A.P. 2014. Propagation of *Ginkgo biloba* Linnaeus through air-layering in tropical conditions of West Bengal, India. *NBU Journal of Plant Science.* 8(1): 1 - 4.

Mukhia, S.; Mandal, P.; Singh, D.K.; Singh, D. & **Choudhury, D.** 2014. *In-vitro* free-radical scavenging potential of three liverworts of Darjeeling Himalaya. *International Journal of Pharmaceutical Science and Research.* 5(10): 4552 - 4561.

III. *Book Chapter*

Choudhury, D.; Ghosal, M.; Das, A.P. & Mandal, P. 2011. Improvement of propagation techniques and evaluation *in vitro* antioxidant activity of *Curcuma aeruginosa* Roxburgh. *Recent Studies in Biodiversity and Traditional Knowledge in India*. Ed. Ghosh, C. & Das, A.P. 287 - 293.

IV. *Book*

Das, A.P.; Ghosh, C.; Sarkar, A. & **Chouydhury, D.** 2010. *Hundred Medicinal Plants from North Bengal.* University of North Bengal, India.

Appendix-C

Seminars and Workshops Participated

- Propagation & Cultivation of Medicinal Plants, NBU, West Bengal
- Biodiversity Showcasing Northern West Bengal, Siliguri, West Bengal
- Sustainable Utilization Of Plant & Microbial Resources, NBU, West Bengal
- Access to E-resources under UGC INFONET Digital Library Consortium, NBU, West Bengal
- Microbial Wealth- Plant Health, NBU, West Bengal
- Diversity conservation and Sustainable Utilization of Plant and Traditional Knowledge in Eastern Himalaya, Department of Botany, NBU, West Bengal
- The Exploration, Protection and Conservation of Biodiversity and Traditional Knowledge, Gour Mahavidyalaya, Malda, West Bengal
- UGC sponsored Research Scholars' training programme. Academic Staff College, NBU, West Bengal
- Workshop on Bioinformatics, Department of Botany, NBU, West Bengal
- Advances in Abiotic and Biotic Stress Management of Plants, DRS- Department of Botany, NBU, West Bengal
- User Awareness Programme on Access to E-resources under N-LIST Programme, NBU, West Bengal
- Biology and Bioinformatics of Economically Important Plants and Microbes. Department of Botany, NBU, West Bengal
- Recent trends in plant diversity study and conservation strategies, Department of Botany, Nagaland University, Lumami
- National seminar on Biotechnology for people: Applications and Awareness. Department of Botany, Prasannadeb Women's College Jalpaiguri, West Bengal
- National Symposium on Recent Trends in Plants and Microbial Research. Department of Botany, NBU, West Bengal
- National Symposium on Advances in Plant and Microbial Research. Department of Botany, NBU, West Bengal

Appendix-D

Published Articles

[Based on Thesis Works]

Pleione 4(1): 90 - 101. 2010. ISSN 0973 - 9467

Preliminary report on the Medicinal Plants from three MPCAs in Terai and Duars of West Bengal, India

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Abstract

FRLHT in collaboration with the West Bengal Forest Department and scientists from Universities, Research Institutes and other organizations in a meeting at Kolkata during December 4 – 7, 2007 selected three Medicinal Plants Coservation Areas (MPCA) in Terai and Duars region of West Bengal [1. Rajavatkhaoa PMCA, 2. Lataguri MPCA and 3. North Sevoke Forest MPCA]. Four season floristic survey in these MPCAs resulted in the record of 309 species of potential Medicinal Plants. Out of these, 25 species are representing the list of 46 threatened species prepared in the meeting referred above.

Key words: Medicinal Plants, Conservation, Terai, Duars

INTRODUCTION

The northernmost part of West Bengal touching the feet of Eastern Himalaya is generally referred as Terai and Duars (west and east of the river Tista, respectively). This forest clad region is quite rich in biodiversity and its vegetations are contiguous with the Eastern Himalaya. Also, this area is covered by the IUCN recognised Himalaya Hotspot for conservation (Conservation International 2005). Numerous rare and threatened species of plants are the inhabitants of these forests. The wide diversity in habitat structure helped numerous species of plants to settle in the area (Rai & Das 2008). The forests are mostly mixed-deciduous types and other prominent vegetations include riverine scrubs and forests, herblands, shrubby-scrubs, savannah type tall grasslands etc. In addition, wide areas in Terai and Duars are also covered with mono- or mixed-cultured plantation forests using both local and exotic species. However, with the rapid extension of civilisation, the exploitation and damage to the natural habitat by anthropogenic reasons is beyond any limit. This is certainly adversely affecting the biodiversity of this region.

The collection of wide variety of medicinal and aromatic plants from different types of vegetation of this area is in practice since long and the control over such exploitation is negligible. There are regular collectors and vendors for this trade and huge quantity of plant materials, legally or illegally, are exported to long distant markets.

The recent realisation about the need of conservation has forced the concerned agencies to take up some steps in this regard but there are numerous constrains. Whatever may be the situation, the biodiversity is being affected and the population of useful plants is now dwindling rapidly. Man is now realising the benefits of using plant-based medicines over the synthetic chemicals for the remedy of their various diseases. This has led to the unimaginable expansion of the market of medicinal plants round the world. Being a megadiversity country, India's participation in this market should bein a big way. Exploitation of naturally growing plants for this market can not help us to do so. For this we need to do following two things:

1. *In situ* and *ex situ* conservation of medicinal plants over wide areas; and
2. The cultivation of medicinal plants for marketing.

Realising the situation, different projects at the Government and NGO level are being undertaken for conservation and cultivation of medicinal plants.

Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore is in big way coming out in such activities taking the help of different state Forest Department, Universities, NGOs and other scientists. Accordingly, a meeting was held at Kolkata during December 4 – 7, 2007 and has recognised a number of Medicinal Plants Conservation Areas (MPCA) in West Bengal. For the plains of North Bengal i.e. Duars and Terai region three such MPCAs has been recognised as follows:

1. **Rajavatkhaoa Forests** (NRVK – 8; NRVK – 9): 400 hectares
2. **Lataguri Forest** (Sursuti – 4): 100 hectares
3. **North Sevok Forest**: 100 hectares

These forests are quite rich in biodiversity (both plants and animals) with appreciably low anthropogenic activities. The selected Forest Compartments are mainly natural forests with small amount of plantations and are very well connected or contiguous with other protected areas like

(i) Buxa Tiger Reserve for Rajavatkhaoa MPCA

(ii) Gorumara National Park for Lataguri MPCA, and

(iii) Mahananda Wildlife Sanctuary for North Sevoke MPCA.

Present paper deals with the records of different medicinal plants growing in these 3 *in-situ* conservatories in Duars and Terai of West Bengal.

The said meeting also prepared a list of 46 species of medicinal plants those are to be treated as threatened in West Bengal.

MATERIALS AND METHODS

The methodogoly followed for the entire work is quite big, but for the present report preparation of a detailed flora for each of the three MPCAs was the basic requirement. Sampling was done through two methods, (i) random collection from all places covering all types of habitat in different seasons of the year; and (ii) collection through nested quadrates.

For quadrate sampling, each MPCA was properly demarcated. Then, a number of longitudinal and transverse grids were marked in the forest. On such grids, in one particular corner one nested quadrate was selected. This selection was completely random as no other criteria were considered for this purpose. All the quadrates were marked properly using wooden pegs and also markings on nearest trees in all the four corners of the large (20 m x 20 m) quadrate. Three sizes of quadrates were demarcated: 20m x 20m for canopy, 5m x 5m for shrub layer and 1m x 1m for the ground covering vegetation (Misra 1966; Rai 2006; Ghosh 2006). Surveys were conducted during 2008 – 2010 in four different seasons (i) Premonsoon, (ii) Post-monsoon, (iii) Winter, and (iv) Summer.

Specimens of all types of plants were collected and processed into mounted herbarium sheets following Jain & Rao (1977) and were identified in the Taxonomy and Environmental Biology Laboratory of the Department of Botany, University of North Bengal using different literature (Hooker 1872 - 1897; Prain 1903; Hara 1966, 1971, Ohashi 1975; Hara *et al* 1978, 1979, 1982; Grierson & Long 1983, 1984, 1987,1991, 1999, 1001; Noltie 1994, 2000; Pearce & Cribb 2002). Finally, the specimens were matched at CAL and NBU Herbaria.

One set of specimens has been stored at NBU-Herbarium and the remaining sets were submitted to the Forest Department for their onward transport to the FRLHT.

Medicinal and aromatic plants of the recorded flora have been recognised using a number of references including Kirtikar & Basu (1935), Biswas & Chopra (1956), Chopra *et al* (1956), Jain

(1991), Gurung (2002), Das & Mandal (2003), Khare (2004). In addition to medicinal plants used for the production of medicines commercially, ethnomedicinal plants are also recorded.

Uses of plants if any or any other observations of interest made during the fieldwork.

RESULT AND DISCUSSION

Quite a large number of plants of different major taxonomic groups including Pteridophytes and Angiosperms have been recorded from these three MPCAs (unpublished data). However, plants with known aromatic and/or medicinal values were recognized and presented in APPENDIX I.

The record of the occurrence of 309 species of medicinal plants in three MPCAs located in the Terai and Duars of West Bengal is an expression of the importance of the vegetation and flora of this region. Not only that, out of the 46 species of threatened medicinal plants recognized in December 2007 meeting 25 species has been recorded from these three MPCAs (Table 1). Names of these 25 species are *Abelmoschus moschatus, Alpinia calcarata, Ampelocissus barbata, Aphanamixis polystachya, Aristolochia indica, Asparagus racemosus, Celastrus paniculatus, Cinnamomum bejolghota, Ciannamomum cecidodaphne, Dioscorea prazeri, Drosera burmanii, Gloriosa superba, Gynocardia odorata, Helminthostachys zeylanica, Litsea glutinosa, Lycopodiella cernua, Mesua ferrea, Mucuna pruriens, Ophioglossum reticulatum, Pericampylus glaucus, Persea glaucescens, Pterocarpus marsupium, Rauvolfia serpentina, Stereospermum colais* and *Toona ciliata.*

Table 1. Numerical distribution of recorded threatened species of medicinal plants from three MPCAs under study.

Sl. No.	MPCAs	No. of listed species	Total no. of listed species recorded
1.	Rajavatkhawa MPCA	24	20
2.	Lataguri MPCA	24	
3.	North Sevoke MPCA	20	

The distribution of remaining 11 species [*Aconitum bisma, Aconitum ferox, Aconitum spicatum, Berberis aristata, Desmodium motorium, Gymnema sylvestre, Ipomoea mauritiana, Lumnitzera racemosa, Morinda citrifolia, Nypa fruticans, Olax nana, Panax pseudoginseng, Picrorhiza kurroa, Podophyllum hexandrum, Sonnertia caseolaris, Swertia chirayita, Taxus wallichiana, Thalictrum foliosum, Tylophora indica, Viscum articulatum* and *Xylocarpus granatum*], are restricted mostly to othe regions of the state. It is interesting to note that all the three MPCAs of North Bengal plains support the occurrence of a large number of (20 or more species) Medicinal Plants.

However, the occurrence of some other species like *Persea glausescens, Gloriosa superba, Drosera burmanii, Alpinia calcarata* and *Cinnamomum cecidodaphne* are also not very rare as it was discussed in December 2007 meeting. The population of all these plants is increasing and plants are with good health. The occurrence of *Pterospermum mersupium* is quite low in this region.

In spite of this, the result of the survey in three different MPCAs shows the occurrence of huge number of medicinal plant species which are only available in these three conservation plots. So, it is expected that along with the conservation of these 25 threatened Medicinal Plants, these MPCAs will also conserve all other medicinal plants now known to grow there. However, proper

conservation strategies are to be framed and to be implemented in its strictest form to protect these important green wealth of the country.

Acknowledgements

Authors are thankful to W.B. Forest Department Officials and FRLHT authorities for their constant support and hospitality.

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APPENDIX I

List of medicinal and aromatic plants recorded from three MPCAs in Terai and Duars of West Bengal

Name of Plants	Family	Field No.	Local Name	In MPCA	Used as
Abelmoschus moshatus Medikus	Malvaceae	3888	*Lata kasturi*	R/ L	A
Abrus pulchellus Wallich *ex* Thwaites	Papilionaceae	257	*Badami Kunch*	R/ L/ S	M
Acacia catechu (L.f.) Willdenow	Mimosaceae	3783	*Khayer*	R/ L/ S	M
Acacia pennata (Linnaeus) Willdenow	Mimosaceae	3514		R/ L/ S	M
Achyranthus bidentata Blume	Amaranthaceae	28	*Ankhlay Jhar*	R/ L/ S	M
Acmella calva (DC.) Jansen	Asteraceae	3900	*Kalijhar*	R/ L/ S	M
Acmella uliginosa (Swartz) Cassini	Asteraceae	3580	*Kalijhar*	R/ L/ S	M
Acmella uliginosa (Swartz) Cassini	Asteraceae	4098		R/ L/ S	M
Actinodaphne obovata (Nees) Blume	Lauraceae	2708		R/ L/ S	M
Aerva sanguinolenta (Linnaeus) Blume	Amaranthaceae	3673	*Lopang*	R/ L/ S	M
Aesculus assamica Griffith	Hippocastanaceae	452	*Satpate*	R/ L	M
Ageratum conyzoides Linnaeus	Asteraceae	10	*Elame jhar*	R/ L/ S	M
Alocasia fallax Schott	Araceae	44	*Kalo kachu*	R/ L/ S	
Alpinia calcarata Roscoe	Zingiberaceae	3554		L	M
Alpinia nigra (Gaertner) Burtt	Zingiberaceae	2478	*Purundi*	R/ L	M
Alstonia scholaris (Linnaeus) R. Brown	Apocynaceae	3538	*Chhatian,Chhatim*	R/ L/ S	M
Alternanthera sessilis (Linnaeus) DC.	Amaranthaceae	485	*Nunia Saag*	R/ L/ S	M
Amorphophallus napalensis (Wallich) Bogner & Mayo	Araceae	3865	*Bon Ol*	R/ L/ S	M
Ampelocissus barbata (Wallich) Planchon	Vitaceae	4099	*Jangli angur*	R/ L/ S	M
Anisomeles indica (Linnaeus) Kuntze	Lamiaceae	130	*Gopali*	R/ L/ S	A
Antidesma acidum Retzius	Euphorbiaceae	491	*Archal*	R/ L/ S	M
Antidesma acuminatum Wight	Euphorbiaceae	220	*Archal*	R/ L	M
Antidesma bunius (L.) Sprengel	Euphorbiaceae	2511	*Archal*	R/ L	M
Aphanamixis polystachya (Wallich) Parker	Meliaceae	505	*Rasune Lali*	R/ L/ S	M
Argyreia roxburghii Choisy	Convolvulaceae	3791		R/ L/ S	M
Aristolochia indica Linnaeus	Aristolochiaceae	4158	*Iswarmul*	R/ L	M
Aristolochia saccata Wallich	Aristolochiaceae	4334		R/ L/ S	M
Artemesia indica Willdenow	Asteraceae	244	*Titeypati*	S	M
Artocarpus chama Buch.-Ham.	Moraceae	4296	*Lathar, Chaplash*	R/ L/ S	M
Asparagus officinalis Linnaeus	Asparagaceae	387	*Asparagus*	R/ L/ S	M
Asparagus racemosus Willdenow	Asparagaceae	2723	*Satabari, Satamuli*	R/ L	M
Baccaurea ramiflora Loureiro	Euphorbiaceae	379	*Kusum, Latka*	R/ L/ S	M
Barleria strigosa Willdnew	Acanthaceae	3704	*Nil Jati*	R/ L/ S	M

Name of Plants	Family	Field No.	Local Name	In MPCA	Used as
Bauhinia malabarica Roxburgh	Caesalpinaceae	4455	*Kanchan*	R/ L	M
Bauhinia purpurea Linnaeus	Caesalpinaceae	3797	*Rakta Kanchan*	R/ L/ S	M
Bauhinia variegata Linnaeus	Caesalpinaceae	4150	*Swet Kanchan*	R/ L/ S	M
Biophytum reinwardtii (Zuccarini) Klotzsch	Oxalidaceae	3551	*Rani Lajjabati*	R/ L/ S	M
Biophytum sensitivum DC.	Oxalidaceae	151	*Rani Lajjabati*	R/ L/ S	M
Bischofia javanica Blume	Bischofiaceae	4408	*Kainjal*	R/ L/ S	M
Bombax ceiba Linnaeus	Bombacaceae	3611	*Simul*	R/ L/ S	M
Bridelia retusa (Linnaeus) Sprengel	Euphorbiaceae	3774	*Datan, Gayo*	R/ L/ S	M
Bridelia sikkimensis Gehrmann	Euphorbiaceae	2250	*Kasai Datan*	R/ L/ S	M
Bridelia tomentosa Blume	Euphorbiaceae	269	*Kasai Datan*	S	M
Buddleja asiatica Loureiro	Buddlejaceae	3757	*Bhimsen pati*	S	M
Caesalpina cucullata Roxburgh	Caesalpiniaceae	3732	*Ultey kate*	R/ L/ S	M
Callicarpa arborea Roxburgh	Verbenaceae	2178	*Gwelo*	R/ L/ S	M
Careya arborea Roxburgh	Barringtoniaceae	4195	*Kumbhi*	R/ L/ S	M
Caryota urens Linnaeus	Arecaceae	3571	*Rambhang*	R/ L/ S	M
Cassia fistula Linnaeus	Caesalpiniaceae	330	*Bandarlathi*	R/ L/ S	M
Cassia tora Linnaeus	Caesalpiniaceae	2411	*Tapre*	R/ L/ S	M
Celastrus paniculatus Willdenow	Celastraceae	3525	*Malkaguni*	R/ L/ S	M
Centella asiatica (Linnaeus) Urban	Apiaceae	4130	*Thankuni*	R/ L/ S	M
Chlorophytum arundinaceum Baker	Antheriaceae	3853	*Makai phul*	S	M
Chromolaena odoratum (Linnaeus) King & Robinson	Asteraceae	3553	*Bonmara*	R/ L/ S	M
Chukrasia tabularis A. Jussieu	Meliaceae	2074	*Chikrasi*	R/ L/ S	M
Ciannamomum cecidodaphne Meisner	Lauraceae	4433	*Malagiri*	R/ L	M
Cinnamomum bejolghota (Hamilton) Sweet	Lauraceae	373	*Janglee tejpat, Sin Kaule*	R/ L/ S	M
Cissampelos pareira Linnaeus	Menispermaceae	4213	*Batulepati*	R/ L/ S	M
Citrus medica Linnaeus	Rutaceae	3785	*Lebu*	R/ L/ S	M
Clausena excavata Burm.f.	Rutaceae	3700	*Junglee Karipata*	R/ L	A
Clerodendrum indicum (L.) Kuntze	Verbenaceae	4142	*Bamanhati*	R/ L/ S	M
Clerodendrum viscosum Ventenat	Verbenaceae	117	*Bhant, Ghentu*	R/ L/ S	M
Cocculus laurifolius DC.	Menispermaceae	4164	*Dai gachh*	R/ L/ S	
Colocasia esculenta (Linnaeus) Schott	Araceae	60	*Man kachhu*	R/ L/ S	M
Combretum decandrum Roxburgh	Combretaceae	63	*Kali Lahara*	R/ L/ S	M
Commelina suffruticosa Blume	Commelinaceae	3818	*Kane jhar*	R/ L/ S	M
Costus speciosus (Koenig *ex* Retzius) Smith	Costaceae	3629	*Betlaure*	R/ L/ S	M
Crateva religiosa Forst.f.	Capparaceae	419	*Chipley, Barun*	R/ L/ S	M
Crinum amoenum Roxburgh	Amaryllidaceae	4520	*Nagdan*	R/ L/ S	M
Crotalaria alata Buch-Ham *ex* D. Don	Fabaceae	4204		R/ L/ S	M
Cryptolepis buchanani R. Br. *ex* Roemer & Schultes	Asclepiadaceae	3579	*Kankrashringi*	R/ L/ S	M
Cryptolepis sinensis (Loureiro) Merrill	Asclepiadaceae	4278	*Kankrashringi*	R/ L/ S	M

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Curculigo capitulata (Loureiro) O. Kuntze	Hypoxidaceae	3657	*Dhoti sara*	R/ L/ S	M
Curculigo orchioides Gaertner	Hypoxidaceae	3854	*Talmuli*	R/ L/ S	M
Curcuma aromatica Salisbury	Zingiberaceae	3856	*Jangli halud*	S	A, M
Curcuma ceaesia Roxburgh	Zingiberaceae	4117	*Kala Haldi*	R/ L/ S	M
Curcuma zedoaria (Chirstmann) Roscoe	Zingiberaceae	129	*Kala haldi*	R/ L/ S	M
Cuscuta reflexa Roxburgh	Cuscutaceae	4477	*Swarnalata*	L/ S	M
Cyanotis axillaris (Linnaeus) Sweet	Commelinaceae	4111		R/ L/ S	M
Cymbopogon jwarancusa (Jones) Schultes	Poaceae	4542		R	M
Cynodon dactylon (Linnaeus) Persoon	Poaceae	4097	*Dubo*	R/ L/ S	M
Cyperus rotundus Linnaeus	Cyperaceae	4105	*Mutha ghas*	R/ L/ S	M
Dactyloctenium aegypticum (Linnaeus) P. Beauvois	Poaceae	4545		R	M
Dalbergia pinnata (Loureiro) Prain	Fabaceae	469		R/ L/ S	M
Dalbergia stipulacea Roxburgh	Fabaceae	4331	*Lahara Sirish*	R/ L/ S	M
Daphne involucrata Wallich	Thymeleaceae	4500		R/ L	A
Deeringia amaranthoides (Lamarck) Merrill	Amaranthaceae	3782	*Chhorachhurisag*	R/ L/ S	M
Dentella repens J. & G. Forster	Rubiaceae	4177		R/ L/ S	M
Dicliptera bupleuroides Nees	Acanthaceae	3681		R/ L/ S	M
Dillenia indica Linnaeus	Dilleniaceae	2316	*Chalta, Panchphol*	R/ L/ S	M
Dillenia indica Linnaeus	Dilleniaceae	4431	*Paanch Phal*	R/ L/ S	M
Dioscorea belophylla Voigt *ex* Haines	Dioscoreaceae	272	*Ban Tarul*	R/ L/ S	M
Dioscorea bulbifera Linnaeus	Dioscoreaceae	4447	*Gittha Tarul*	R/ L/ S	M
Dioscorea hispida Dennstedt	Dioscoreaceae	4123		R/ L/ S	M
Dioscorea pentaphylla Linnaeus	Dioscoreaceae	2453	*Ban Tarul, Bhegur*	R/ L/ S	M
Dioscorea prazeri Prain & Burkill	Dioscoreaceae	3508	*Kukur tarul*	R/ L/ S	M
Diplazium esculentum (Koenig *ex* Retzius) Swartz	Athyriaceae	3563	*Dhekia saag*	R/ L/ S	M
Drosera burmanii Vahl	Droseraceae	4109		R/ L/ S	M
Drosera burmannii Vahl	Droseraceae	4575	*Surjasisir*	R	M
Drymaria diandra (Blume) Duke	Caryophyllaceae	2498	*Avijal*	S	M
Drynaria quercifolia (Linnaeus) J. Smith	Polypodiaceae	4467		R/ L/ S	M
Dysoxylum mellisimum Blume	Meliaceae	4143	*Chhalegach*	L	M
Echinochloa crussgalli (Linnaeus) P. Beauvois	Poaceae	4205	*Sama*	R/ L/ S	M
Eclipta prostrata (Linnaeus) Linnaeus	Asteraceae	4505	*Keshud*	R/ L/ S	M
Elephantopus scaber Linnaeus	Asteraceae	3686	*Gajialata*	R/ L/ S	M
Elusine indica (Linnaeus) Gaertner	Poaceae	4473	*Kodho jhar*	R/ L/ S	M
Entada rheedii Sprengel	Mimosaceae	2720	*Gila*	R/ L	M
Equisetum diffusum D. Don	Equisetaceae	3761	*Kurkure Jhar*	S	M
Erigeron canadensis (Linnaeus) Cronquist	Asteraceae	4113		R/ L/ S	M
Erythrina stricta Roxb.	Fabaceae	4462	*Madar*	R/ L/ S	M
Eupatorium adenophorum Sprengel	Asteraceae	4182	*Kalo Banmara*	S	M
Euphorbia heyneana Sprengel	Euphorbiaceae	4277		R/ L	M

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Euphorbia hirta Linnaeus	Euphorbiaceae	4332		R	M
Euphorbia hypericifolia Linnaeus	Euphorbiaceae	4525		R/ L	M
Evolvulus alsinoides (Linnaeus) Linnaeus	Convolvulaceae	4336		R/ L/ S	M
Ficus benghalensis Linnaeus	Moraceae	4115	*Bot, Bor*	R/ S	M
Ficus hispida L.f.	Moraceae	3533	*Kak Dumur,Koksa*	R/ L/ S	M
Ficus racemosa Linnaeus	Moraceae	4237	*Dumri*	R	M
Ficus religiosa Linnaeus	Moraceae	4559	*Ashathwa*	R/ L/ S	M
Ficus semicordata J.E.Smith	Moraceae	4146	*Khaniun*	R/ L/ S	M
Flacourtia jangomas (Loureiro)Raeuschel	Flacourtiaceae	4347	*Panial*	R/ L/ S	M
Flemingia strobilifera (Linnaeus) Aiton	Fabaceae	4215	*Ghora chabuk*	R/ L/ S	M
Flueggea virosa (Willdenow) Voigt	Euphorbiaceae	4166	*Darim pate*	R/ L/ S	M
Garuga gamblei King *ex* Smith	Burseraceae	185	*Dobdabe*	R/ L/ S	M
Girardinia diversifolia (Link) Friis	Urticaceae	36	*Bhyangrey Shishnu*	R/ L/ S	M
Glinus oppositifolius (L.) A. DC.	Aizoaceae	4378	*Gimasaag*	R/ L/ S	M
Glinus oppositifolius (Linnaeus) A. DC.	Aizoaceae	4108	*Gima Saag*	R/ L/ S	M
Gloriosa superba Linnaeus	Liliaceae	2412	*Ulatchandal*	L/ S	M
Glycosmis cymosa (Kurz) Narayanaswami	Rutaceae	181	*Ban jamir, Ashseora*	L	M
Glycosmis pentaphylla (Retzius) DC.	Rutaceae	348	*Ban jamir, Ashseora*	R/ L/ S	M
Grangea maderaspatana (Linnaeus) Poiret	Asteraceae	4314		L/ S	M
Grewia asiatica Linnaeus	Tiliaceae	3790	*Falsa*	R/ L/ S	M
Gynocardia odorata R. Brown	Flacourtiaceae	217	*Chalmogra, Ramphal*	R/ L/ S	M
Hedyotis scandens Roxburgh	Rubiaceae	3651	*Kanchiru Lahara*	R/ L/ S	M
Helminthostachys zeylanica (Linnaeus) Hook.	Helminthost-achyaceae	3863		R/ L/ S	M
Holarrhena pubescens (Buchanon -Hamilton) G. Don	Apocynaceae	3833	*Kurchi, Khirra*	R/ L/ S	M
Homalomena rubescens (Roxburgh) Kunth	Araceae	399		L	M
Hoya parasitica (Roxburgh) Wight	Asclepiadaceae	4106		R/ L/ S	A, M
Hydrocotyle sibthorpioides Lamarck	Apiaceae	4356	*Gande jhar*	R/ L/ S	M
Hygrophila auriculata (Schumacher) Heine	Acanthaceae	4244	*Kulekhara*	R/ L/ S	M
Hypericum japonicum Murray	Hypericaceae	4533		R/ L/ S	M
Hyptis suaveolens (Linnaeus) Poiteau	Lamiaceae	4125	*Bon tulsi*	R/ L/ S	M
Ichnocarpus frutescens (Linnaeus) Aiton	Apocynaceae	3708	*Dudhe Lahara*	R/ L/ S	M
Imperata cylindrica (Linnaeus) Rauschel	Poaceae	4140	*Siru*	R/ L/ S	M
Jasminum glandiflorum Linnaeus	Oleaceae	4429		R/ L/ S	A
Jasminum multiflorum (Burm.f.) Andrews	Oleaceae	4174		R/ L/ S	A
Jasminum pubescens (Retzius) Willdenow	Oleaceae	4540		R/ L/ S	A
Jasminum scandens Vahl	Oleaceae	4224	*Hara Lahara*	R/ L/ S	A
Kaempferia rotunda Linnaeus	Zingiberaceae	3874	*Bhuichampa*	S	M

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Kyllinga nemoralis (J.R. & G. Forster) Dandy *ex* Hutchinson & Dalziel	Cyperaceae	4569		R/ L/ S	M
Lagerstroemia hirta (Lamarck) Willdenow	Lythraceae	205	*Jarul*	R/ L/ S	M
Lagerstroemia parviflora Roxburgh	Lythraceae	263	*Sidha*	R/ L/ S	M
Lannea coromandelica (Houttuyn) Merrill	Anacardiaceae	4461	*Jiol*	R/ L/ S	M
Lantana camara Linnaeus	Verbenaceae	3778	*Saibani lata*	R/ L/ S	M
Lasia spinosa (Linnaeus) Thwaites	Araceae	4202	*Kanta kachhu*	R/ L/ S	M
Leea aequata Linnaeus	Leeaceae	4325		R/ L/ S	M
Leea asiatica (Linnaeus) Ridsdale	Leeaceae	4510		R/ L/ S	M
Leea indica (Burman) Merrill	Leeaceae	4187		R/ L/ S	M
Leucus indica (Linnaeus) R. Brown *ex* Vatke	Lamiaceae	4121	*Swetodrone*	R/ L/ S	M
Lindenbergia indica (Linnaeus) O. Kuntze	Scrophulariaceae	4579		R/ L/ S	M
Litsea cubeba (Loureiro) Persoon	Lauraceae	2509		R/ L/ S	M
Litsea glutinosa (Loureiro) Robinson	Lauraceae	99	*Kawala*	R/ L/ S	A, M
Litsea monopetala (Roxburgh) Persoon	Lauraceae	4301	*Bonsum*	R/ L/ S	M
Litsea salicifolia (Nees) Hook.f.	Lauraceae	474		R/ L/ S	A
Luffa aegyptiaca Miller	Cucurbitaceae	4246	*Dhundhul*	R/ L/ S	M
Lycopodium cernuum Linnaeus	Lycopodiaceae	2439	*Nagbeli*	L/ S	M
Lygodium flexuosum (Linnaeus) Swartz	Lygodiaceae	359	*Bhutraj*	R/ L/ S	M
Maesa indica (Roxburgh) A. DC.	Myrsinaceae	4548	*Bilauney*	R/ L/ S	M
Mallotus philippensis (Lamarck) Mueller	Euphorbiaceae	210	*Sindure*	R/ L/ S	M
Maranta arundinacea Linnaeus	Marantaceae	4156	*Ararut*	L	M
Melastoma melabathricum Linnaeus	Melastomataceae	3766	*Datrangi, Futki*	R/ L/ S	M
Melilotus indica (Linnaeus) Allioni	Fabaceae	4562		R	M
Merremia hirta (Linnaeus) Merrill	Convolvulaceae	4311		R/ L/ S	M
Merremia vitifolia (Burm.f.) Hallier f.	Convolvulaceae	4571		R/ L/ S	M
Mesua ferrea Linnaeus	Clusiaceae	4236	*Nagkesar*	R/ L/ S	M
Meyna spinosa Link	Rubiaceae	408	*Moyna kata*	R/ L/ S	M
Michelia champaca Linnaeus	Magnoliaceae	4552	*Champ*	R/ L/ S	A
Michelia velutina DC.	Magnoliaceae	426	*Champ*	R/ L	A
Micromelum integerrimum (Roxburgh) Roemer	Rutaceae	2003	*Ban-kunch*	R/ L	M
Mikania micrantha Kunth	Asteraceae	317	*Assami lata*	R/ L/ S	M
Mimosa himalayana Gamble	Mimosaceae	4419	*Arare*	R/ L/ S	M
Mimosa pudica Linnaeus	Mimosaceae	3861	*Lajjabati*	R/ L/ S	M
Momordica charantia Linnaeus	Cucurbitaceae	472	*Karela*	R/ L/ S	M
Momordica cochincninensis Sprengel	Cucurbitaceae	4152	*Kakrol*	R/ L/ S	M
Monochoria vaginalis (Burman f.) Kunth	Pontederiaceae	4235	*Piralay*	R/ L/ S	M
Morinda angustifolia Roxbergh	Rubiaceae	4572	*Haldi kath*	R/ L/ S	M
Morinda angustifolia Roxburgh	Rubiaceae	52	*Haldikath*	R/ L/ S	M
Morus laevigata Brandis	Moraceae	31	*Jangli tunt*	R/ L/ S	M
Mucuna pruriens (Linnaeus) DC.	Fabaceae	4463	*Alkushi*	R/ L/ S	M

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Murraya koenigii (Linnaeus) Sprengel	Rutaceae	404	*Karipatta*	R/ L/ S	A
Murraya paniculata (Linnaeus) Jack	Rutaceae	422	*Kamini*	R/ L/ S	M
Mussaenda roxburghii Hook.f.	Rubiaceae	2438	*Katmatia Saag*	R/ L/ S	M
Naravelia zeylanica (Linnaeus) DC.	Ranunculaceae	3602	*Chhagalbati*	R/ L/ S	M
Natsiatum herpeticum Arnott	Icacinaceae	3747		R/ L/ S	M
Nyctanthes arbor-tristis Linnaeus	Verbenaceae	4471	*Sephali*	R/ L/ S	M
Oesbeckia nepalensis Hooker	Melastomataceae	4582		R/ L/ S	M
Oldenlandia corymbosa Linnaeus	Rubiaceae	4112	*Khetpapra*	R/ L/ S	M
Oldenlandia diffusa (Wildenow)Roxburgh	Rubiaceae	4288		R/ L/ S	M
Ophioglossum reticulatum Linnaeus	Ophioglossaceae	4459	*Gibre*	R/ L	M
Oroxylum indicum (Linnaeus) Ventenat	Bignoniaceae	2144	*Totola*	R/ L/ S	M
Oxalis corniculata Linnaeus	Oxalidaceae	4238	*Amruli Saag*	R/ L/ S	M
Paederia foetida Linnaeus	Rubiaceae	3701	*Gandhavadale*	R/ L/ S	M
Pandanus unguifer Hook.f.	Pandanaceae	4577	*Keya*	R/ L	M
Paspalum scrobiculatum Linnaeus	Poaceae	4119		R/ L/ S	M
Pavetta polyantha Bremekamp	Rubiaceae	4443	*Kanjol Phul*	R/ L/ S	M
Pericampylus glaucus (Lam.) Merrill	Menispermaceaea	3648	*Pipal-pati Lahara*	R/ L/ S	M
Persea glaucescens (Nees) Long	Lauraceae	4110	*Bhale Kawlo, Kawala*	R/ L/ S	A
Persicaria barbata (Linnaeus) Hara	Polygonaceae	4357		R/ L/ S	M
Persicaria chinensis (Linnaeus) H. Gross	Polygonaceae	143		R/ L/ S	M
Persicaria hydropiper (Linnaeus) Spach	Polygonaceae	4410	*Biskuthuli*	R/ L/ S	M
Persicaria orientalis (Linnaeus) Spach	Polygonaceae	4103		L	M
Phlogacanthus thyrsiformis (Hardwicke) Mabberley	Acanthaceae	4343	*Ram Basak, Chua*	R/ L/ S	M
Phoebe lanceolata (Nees) Nees	Lauraceae	4517	*Angare*	R/ L/ S	A
Phyllanthus emblica Linnaeus	Euphorbiaceae	281	*Amloki, Amla*	R/ L/ S	M
Phyllanthus reticulatus Poiret	Euphorbiaceae	4153	*Bhui amla*	R/ L/ S	M
Phyllanthus urinaria Linnaeus	Euphorbiaceae	2471	*Bhui amla, Hazarmani*	R/ L/ S	M
Phyllanthus virgatus Forster	Euphorbiaceae	4253		R/ L/ S	M
Physalis divaricata D. Don	Solanaceae	466	*Bon Tepari*	R/ L/ S	M
Physalis peruviana Linnaeus	Solanaceae	3879	*Bon Tepari*	R/ L/ S	M
Piper betle Linnaeus	Piperaceae	4515	*Pan*	R/ L/ S	M
Piper chuvya (Miquel) C. DC.	Piperaceae	4328	*Chaba*	R/ L/ S	M
Piper longum Linnaeus	Piperaceae	4116	*Pipal, Pipla*	R/ L/ S	M
Piper mullesua D. Don	Piperaceae	4242	*Pipla, Dala-chabo*	R/ L/ S	M
Piper peepuloides Roxburgh	Piperaceae	4491	*Ruk Pipla*	R/ L/ S	M
Piper retrofractum Vahl	Piperaceae	4159	*Choi*	R/ L/ S	M
Piper sylvaticum Roxburgh	Piperaceae	3765		R/ L/ S	M
Polyalthia simiarum (Hook.f. & Thomson) Hook. f. & Thomson	Annonaceae	4327	*Lapche Kath*	R/ L/ S	M
Polygonum hydropiper Linnaeus	Polygonaceae	4564	*Bis-kutuli*	R/ L/ S	M
Polygonum plebeium R. Brown	Polygonaceae	4104		L	M
Portulaca oleracea Linnaeus	Portulacaceae	4199		R	M

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Pothos cathcarti Schott	Araceae	4423		R/ L/ S	M
Pothos scandens Linnaeus	Araceae	4188		R/ L/ S	M
Pothos scandens Linnaeus	Araceae	4107		R/ L/ S	M
Pouzolzia hirta (Blume) Hasskarl	Urticaceae	4469		R/ L/ S	M
Pouzolzia zeylanica (Linnaeus) Bennet & Brown	Urticaceae	4536		R/ L/ S	M
Premna barbata Wallich	Verbenaceae	2427	*Gineri*	R/ L/ S	M
Premna bengalensis C.B. Clarke	Verbenaceae	4567	*Gineri*	R/ L/ S	M
Premna mucronata Roxburgh	Verbenaceae	4192	*Gineri*	R/ L/ S	M
Premna mucronata Roxburgh	Verbenaceae	4233	*Gineri*	R/ L/ S	M
Pseudognaphalium affine (D. Don) Anderberg	Asteraceae	4101		R/ L/ S	M
Psilanthus bengalensis (Schultes) Leroy	Rubiaceae	4285	*Chaitiful*	R/ L/ S	M
Pterocarpus marsupium Roxburgh	Fabaceae	4172		R/ L	M
Pterospermum acerifolium (Linnaeus) Willdenow	Sterculiaceae	2044	*Hantipahele*	R/ L/ S	M
Pterygota alata (Roxburgh) R. Brown	Sterculiaceae	3675	*Labshi, Narkeli, Phirphire*	R/ L/ S	M
Randia sikkimensis Hook.f.	Rubiaceae	4555		R/ L/ S	M
Rauvolfia serpentina (Linnaeus) Bentham ex Kurtz	Apocynaceae	4184	*Sarpagandha*	R/ L/ S	M
Rumex trisetifer Strokes	Polygonaceae	4460		S	M
Saccharum spontanum Linnaeus	Poaceae	4114	*Kush*	R/ L/ S	M
Sapindus rarak DC.	Sapindaceae	442	*Ritha*	R/ L/ S	M
Saurauja roxburghii Wallich	Actinidiaceae	4550	*Gogun*	L	M
Sauropus androgynus (Linnaeus) Merrill	Euphorbiaceae	74	*Multivitamine*	R/ L	M
Sauropus compressus Mueller	Euphorbiaceae	3542		R/ L/ S	M
Sauropus quadrangularis (Willdenow) Mueller	Euphorbiaceae	4179		S	M
Schima wallichii (DC.) Korthals	Theaceae	2232	*Chilauney*	R/ L	M
Scindapsus officinalis (Roxburgh) Schott	Araceae	4585		R/ L/ S	M
Scoparia dulcis Linnaeus	Scrophulariaceae	4313	*Ghuma, Mithapata*	R/ L/ S	M
Shorea robusta Gaertner f.	Dipterocarpaceae	4145	*Saal*	R/ L/ S	A
Sida acuta Burm.f.	Malvaceae	3526	*Berela*	R/ L/ S	M
Sida rhombifolia Linnaeus	Malvaceae	68	*Peet Berela*	R/ L/ S	M
Smilax lanceaefolia Roxburgh	Smilacaceae	4305	*Kukurdainey*	R/ L/ S	M
Smilax ovalifolia Roxb.	Smilacaceae	433	*Kukurdainey*	R/ L/ S	M
Solanum aculeatissimum Jacquin	Solanaceae	3860	*Kalobehi*	R/ L/ S	M
Solanum nigrum Linnaeus	Solanaceae	4458	*kakmachhi*	R/ L/ S	M
Solanum torvum Swartz	Solanaceae	3750	*Gothbegun*	R/ L/ S	M
Solanum viarum Dunal	Solanaceae	69	*Boksi Kanra*	R/ L/ S	M
Sonchus oleraceus Linnaeus	Asteraceae	4372		R/ L/ S	M
Spermacoce hispida Linnaeus	Rubiaceae	4440		R	M
Stephania glabra (Roxburgh) Miers	Menispermaceae	102	*Tamarke Lahara*	R/ L/ S	M
Stephania japonica (Thunberg) Miers	Menispermaceaea	3652	*Tamarki*	R/ L/ S	M
Sterculia villosa Roxburgh	Sterculiaceae	4466	*Odal*	R/ L/ S	M

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Stereospermum colais (Dillwyn) Mabberley	Bignoniaceae	3641	*Parari*	R/ L/ S	M
Streblus asper Loureiro	Moraceae	234	*Seora*	R/ L/ S	M
Synedrella nudiflora (Linnaeus) Gaertner	Asteraceae	4198		R/ L/ S	M
Syzygium cumini (Linnaeus) Skeels	Myrtaceae	364	*Jaam*	R/ L/ S	M
Tabernaemontana divaricata (Linnaeus) Roemer & Schultes	Apocynaceae	4147	*Tagar*	R/ L/ S	M
Telauma hodgsonii Hook.f. & Thomson	Magnoliaceae	3852	*Chiuri*	R/ L/ S	M
Tephrosia candida (Roxburgh) DC.	Fabaceae	3753	*Ban nim*	R/ L/ S	M
Terminalia bellirica (Gaertner) Roxburgh	Combretaceae	3789	*Bahera, Barra*	R/ L/ S	M
Terminalia chebula Retzius	Combretaceae	4511	*Haritaki, Harra*	R/ L/ S	M
Terminalia myriocarpa Heurck & Meuller	Combretaceae	4100	*Paani Saaj*	R/ L/ S	M
Tetracera sarmentosa (Linnaeus) Vahl	Dilleniaceae	3599	*Lata Chalta*	R/ L/ S	M
Thysanolaena maxima (Roxburgh) Kuntze	Poaceae	4148	*Phul jharu*	S	M
Tinospora sinensis (Loureiro) Merrill.	Menispermaceae	2477	*Padmagulancha*	R/ L/ S	M
Toddalia asiatica (Linnaeus) Lamarck	Rutaceae	3735	*Belkanta*	R/ L/ S	A
Toona ciliata Roemer	Meliaceae	145	*Toon*	R/ L/ S	M
Torenia cordata (Griffith) N.M. Datta	Scrophulariaceae	4529		R	M
Trema orientalis (Linnaeus) Blume	Ulmaceae	4161		R/ L/ S	M
Trewia nudiflora Linnaeus	Euphorbiaceae	4214	*Pithali*	R/ S	M
Trichosanthes cordata Roxburgh	Cucurbitaceae	4413		R/ L/ S	M
Trichosanthes tricuspidata Loureiro	Cucurbitaceae	3776	*Makal*	R/ L/ S	M
Triumfetta rhomboidea Jacquin	Tiliaceae	128	*Ban Okra*	R/ L/ S	M
Typhonium trilobatum (Linnaeus) Schott	Araceae	464	*Kharkon, Ghatkol*	R/ L/ S	M
Uraria picta Desv.	Fabaceae	3890	*Sankarjata*	R/ L/ S	M
Urena lobata Linnaeus	Malvaceae	3565	*Ban Okra*	R/ L/ S	M
Vallaris solanacea (Roth) O. Kuntze	Apocynaceae	19	*Haparmali*	R/ L/ S	M
Vernonia cinerea (Linnaeus) Less	Asteraceae	4127		R/ L/ S	M
Vitex negundo Linnaeus	Verbenaceae	4155	*Nisinda*	S	M
Vitex peduncularis Schauer	Verbenaceae	4359	*Charaigarua*	R/ L	M
Wattakaka volubilis (L.f.) Stapf	Asclepiadaceae	3751	*Chhint*	R/ L/ S	M
Wrightia arborea (Dennstaedt) Mabberley	Apocynaceae	3849	*Chhoto khira*	R/ L/ S	M
Xanthium strumarium Linnaeus	Asteraceae	4522	*Bon Onkra*	R/ L/ S	M
Zanonia indica Linnaeus	Cucurbitaceae	4312		R/ L/ S	M
Zanthoxylum armatum DC.	Rutaceae	4323	*Timbur*	R/ L	M
Zanthoxylum nitidum (Roxburgh) DC	Rutaceae	370	*Timbur*	R/ L/ S	A
Zanthoxylum rhetsa (Roxburgh) DC	Rutaceae	3738	*Timbur*	R/ L	M
Zingiber rubens Roxburgh	Zingiberaceae	4144		R/ L/ S	A
Zizyphus mauritiana Lamarck	Rhamnaceae	3763	*Kul, Ber*	R/ L/ S	M
Zizyphus rugosa Lamarck	Rhamnaceae	2049		R/ L/ S	M
Zornia gibbosa Spanoghe	Fabaceae	4102		R	M

Pleione 7(2): 441 - 448. 2013. **ISSN: 0973-9467**

Diversity of *Cinnamomum* Schaeffer (Lauraceae) in Terai and Duars region of West Bengal, India

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[Received 30.09.2013; accepted 14.12.2013]

Abstract

There are about 250 species in the genus *Cinnamomum* Schaeffer (Lauraceae), distributed mainly in the tropical and sub-tropical regions of the world. Among them, 26 species of *Cinnamomum* were reported from India. So far there is no comprehensive report published on this genus from Terai and Duars region, which is located at the foot of 'Himalaya Hotspot'. Present paper reported six species of *Cinnamomum* from Terai and Duars belt along with their local names, salient features, exsiccatus, status, flowering and fruiting periods and geographic distribution.

Key words**:** *Cinnamomum*, Terai, Duars, Diversity, Distribution, use.

INTRODUCTION

The genus *Cinnamomum* Schaeffer of Lauraceae contains about 250 species which occur naturally in Asia and some in South and Central America, and Australia (Mabberley 2008). Of these about 26 species occur in India (Anonymous 1992). The term *Cinnamomum* is derived from the Greek word 'Kinnamomon' which mean spice (Sharma & Nautiyal 2011). Several species of *Cinnamomum* are recognized as valuable spices as well as essential oil yielding plants having immense aromatic potential (Baruah 2012). These are mainly used for flavoring food and widely applied in pharmaceutical preparations because of their hypoglycemic, stimulant and carminative properties (Smerq & Sharma 2011). Leaf and bark of various species have astringent, warming stimulant, carminative, blood purifier, digestive, antiseptic, anti-fungal, anti-viral, anti-bacterial properties and can help to reduce cholesterol and blood sugar levels (Kamath *et al* 2003; Mir *et al* 2004; Jayaprakasha *et al* 2006; Cheng *et al* 2006)**. '**Camphor' is derived from *C. camphora,* employed in pharmaceuticals, especially liniments and insecticides (Seth 2004). So, the genus *Cinnamomum* is economically very important. Unfortunately, no complete floristic work is presently available which has been performed on these members in Terai-Duars of West Bengal. This region is situated at the foot hill region of 'Himalaya Conservation Hotspot' recognized by IUCN and very rich in biodiversity. Generally, the Northern part of West Bengal, west of the river Tista is referred as Terai (25º 57" to 26° 36" N latitude and 89° 54" to 88° 47" E longitude) and Duars (located between 26° 16" to 27° 0" N latitude and 88° 4" to 89° 53" E longitude) is referred to the foot-hill region located on the east of Tista (Ghosh 2006; Roy *et al* 2009; Das *et al* 2010).

Considering this knowledge gap, main aim of the present study was framed to investigate the distribution of *Cinnamomum* species in Terai and Duars region. Beside this, for proper identification of species an artificial key was made and for each species correct nomenclature, phenology, ecology and distribution were provided.

MATERIALS AND METHODS

During 2009 – 2012 extensive field survey were undertaken for documentation of the species of *Cinnamomum* available in different parts of Terai and Duars region. Plant specimens were collected at the vegetative, flowering and fruiting stages. The collected specimens were processed into mounted herbarium sheets following standard herbarium techniques (Jain & Rao 1977). The processed plant specimens were identified using the relevant taxonomic literature (Hooker 1886; Brandis 1906; Kanjilal *et al* 1940; Long 1984; Li *et al* 2008) and was later confirmed by matching with the authentic specimens housed in various herbaria *viz.* CAL and NBU. Identified specimens were deposited into NBU Herbarium. Recent literature like Long (1984), Ara *et al* (2007) and Li *et al* 2008 and websites including www.theplantlist.org were followed for nomenclatural treatment of the recorded taxa. Distributional status in the world of the identified species was also recorded from literature (Hooker 1886; Brandis 1906; Hara 1966; Long 1984; Alam 1988; Li *et al* 2008; Ara *et al* 2007) and herbarium studies at CAL. Local names and status of different species were documented during field work from the local people and some of the information was noted down from available literature (Cowan & Cowan 1929; Kanjilal *et al* 1940; Prain 1903; Matthew 1981; Banerjee 1993).

RESULT

From the present survey six species of *Cinnamomum* were collected from different parts of Terai & Duars region in West Bengal. One artificial dichotomous key has been prepared for their easy recognition and species were enumerated below alphabetically accompanied by local names, salient features, exsiccatae, availability status, flowering and fruiting time, occurrence in Terai & Duars region and geographic distribution.

Key to the recorded species of *Cinnamomum* Schaeffer

1a. Leaves opposite or sub opposite ……..…… **2**

1b. Leaves distinctly alternate ……...…… **5**

2a. Lamina elliptic, thickly–leathery, 15–30 cm long, obtuse or acute, base cuneate ..….. .... ***C. bejolghota***

2b. Lamina ovate to oblong ovate or ovate–lanceolate, sub-leathery, smaller, acute or acuminate but not obtuse, base acute or rounded ……..…..……. **3**

3a. Transvers veins reticulate, acuminate, base acute or rounded, fruits ovoid …..... .... ***C. verum***

3b. Transvers veins undulate, long acuminate, base acute or broadly cuneate, fruits obovoid or ellipsoid .....…….…… **4**

4a. Leaves lanceolate or ovate–lanceolate, part of perianth segments persist in fruit ...……..…… ***C. tamala***

4b. Leaves elliptic or ovate–elliptic, perianth segments completely deciduous …… …… ...…….. .... ***C. impressinervium***

5a. Leaves ovate–elliptic, perianth glabrous ……... ***C. camphora***

5b. Leaves elliptic or lanceolate, perienth densely tomentose ……. ***C. glaucescens***

Cinnamomum bejolghota (Buchanon–Hamilton) Sweet, Hort. Brit. 344. 1826; Long in Gierson & Long, Fl. Bhut. 1(2): 258. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 51. 1993. *Laurus bejolghota* Buchanon–Hamilton, Trans. Linn. Soc. London. 13(2): 559. 1822. *Cinnamomum obtusifolium* (Roxburgh) Nees, Pl. Asiat. Rar. 2: 73. 1831. *Laurus obtusifolia* Roxburgh, Fl. Ind., 2: 302. 1832; Hooker *f*., Fl. Brit. Ind. 5: 128. 1886; Prain, Beng. Pl. 2: 673. 1903; Matthew, Pl. Kurs. 89. 1981; Cowan & Cowan, Trs. N. Beng. 108. 1929.

Local name: *Tezpat, Ram tejpat*

Evergreen trees, up to 25m high. Leaves opposite or sub-opposite; lamina elliptic-oblong or elliptic, 15 – 30 × 4 – 9 cm; obtuse or acute, base cuneate, coriaceous, green and shiny adaxially; triplinerved; petioles 1 – 3 cm. Panicles 12 – 22 cm; perianth segments ovate, 2 – 3 mm, pubescent. Fruits ellipsoid, 6 – 12 mm long.

Exsiccatus: Garden of Medicinal Plants, NBU 134 m, *Dibakar Choudhury & AP Das 005*, dated 21.03.2009; Gorumara National Park, 98 m, *Dibakar Choudhury & AP Das 027*, dated 30.05.2009; North Sevoke 190 m, *Dibakar Choudhury & AP Das 064*, dated 26.06.2009; North Rajabhatkhawa, 88 m, *Dibakar Choudhury & AP Das 158*, dated 22.03.2010

Status: Common in forests

Flowers: February – March; **Fruits:** September – October

Local distribution: Throughout Terai & Duars.

General distribution: India, Bhutan, Nepal, Bangladesh, China, Myanmar, Thailand, Vietnam.

Cinnamomum camphora (Linnaeus) J. Presl, *Prir. Rostlin 2: 36. 1825*; Hooker *f*., Fl. Brit. Ind. 5: 134. 1886; Prain, Beng. Pl. 2: 899. 1903; Kanjilal *et al*, Fl. Ass. 4: 60. 1940. *Laurus camphora* Linnaeus, Sp. Pl. 369. 1753.

Local name: *Karpur*

Evergreen trees, up to 25 m high; whole plant strongly camphor–scented. Leaves alternate; lamina ovate-elliptic to elliptic, 5 – 9 × 2.5 – 5 cm, green or yellow-green and shiny adaxially, shortly acuminate, base cuneate, both surfaces glabrous; triplinerved sometimes inconspicuously 5-nerved; petioles 12 – 25 mm. Panicles 8 – 10 cm; perianth segments elliptic, 1.5 – 2 mm. Fruits ovoid or subglobose, 6 – 8 mm.

Exsiccatus: Garden of Medicinal plants, NBU 134 m, *Dibakar Choudhury & AP Das 175*, dated 10.05.2012

Status: Rarely planted

Flowers: March – April; **Fruits:** July – August

Local distribution: Planted in University of North Bengal campus, Sukna, etc.

General distribution: India, China, Japan, Korea, Vietnam and widely cultivated all over the world.

Cinnamomum glaucescens (Nees) Handel–Mazzetti, *Oesterr. Bot. Z. 85: 214. 1936; Long in* Gierson & Long, Fl. Bhu. 1(2): 259. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 51. 1993. *Laurus glaucescens* Buchanon–Hamilton *ex* Nees, *Pl. Asiat. Rar. 2: 70. 1831*. *Cinnamomum cecidodaphne* Meisner, *Prodr. 15(1): 25. 1864*; Hooker *f*., Fl. Brit. Ind. 5: 135. 1886; Cowan & Cowan, Trs. N. Beng. 108. 1929; Kanjilal *et al*, Fl. Ass. 4: 58. 1940.

PLATE - I: Species of *Cinnamomum* Schaeffer in Terai and Duars region of West Bengal: **A.** *C. tamala*; **B.** *C. verum*; **C.** *C. camphora*; **D.** *C. bejolghota*; **E.** *C. impressinervium*; **F.** *C. glaucescens*

Local name: *Malagiri*, *Gonserai*

Evergreen trees, up to 15 m high; young shoots tomentose, becoming glabrate. Leaves alternate; lamina ovate–elliptic, 7 – 15 × 3.5 – 8 cm, shortly acuminate, base broadly cuneate or rounded; lateral veins 4 – 6 pairs; petioles 6 – 12 mm. Panicles brownish–tomentose, densely clustered on young shoots. Fruits globose, 8 – 10 mm.

Exsiccatus: Sursuti Forest, Lataguri, 102 m, *Dibakar Choudhury & AP Das 115*, dated 20.02.2010

Status: Less common

Flowers: January – February; **Fruits:** April

Local distribution: Found only in Gorumara National Park – Lataguri area.

General distribution: India, Bhutan, Nepal, Bangladesh; endemic to Indian subcontinent.

Cinnamomum impressinervium Meisner, Prodr. 15(1): 21**.** 1864**.** Hooker *f.*, Fl. Brit. Ind. 5: 129. 1886; Cowan & Cowan, Trs. N. Beng. 108. 1929*; Long in* Gierson & Long, Fl. Bhut. 1(2): 258. 1984. *Cinnamomum albiflorum* Hooker *f.* & Thomson *ex* Meisner, Prodr. 15(1): 21. 1864. *Cinnamomum cacharense* R.N. Parker, Repert. Spec. Nov. Regni Veg. 31: 126. 1932.

Local name: *Sissi, Korsane*

Evergreen trees, up to 15 m high. Leaves opposite or sub-opposite; lamina elliptic or ovate–elliptic, 8 – 20 × 3 – 5 cm, finely acuminate, glossy adaxially with strongly impressed 3 vains; petioles 7 – 12 mm. Panicles 6 – 10 cm, pubescent, perianth segments 2 – 3 mm. Fruits ellipsoid, 10 – 12 mm long.

Exsiccatus: Sukna, Mahananda Wildlife Sanctuary, 215 m, *Dibakar Choudhury & AP Das 145*, dated 24.02.2010

Status: Less common

Flowers: July; **Fruits:** December

Local distribution: Found only in Mahananda Wild Life Sanctuary.

General distribution: India, Bhutan, Nepal; Endemic to Eastern Himalaya.

Cinnamomum tamala (Buchanon–Hamilton) T. Nees & Ebermaier, *Handb. Med.-Pharm. Bot. 2: 426. 1831*; Hooker *f.*, Fl. Brit. Ind. 5: 128. 1886; *Hara, Fl. E. Him.* 99. 1966; Prain, Beng. Pl. 2: 899. 1903; Cowan & Cowan, Trs. N. Beng. 107. 1929*; Long in* Gierson & Long, Fl. Bhut. 1(2): 258. 1984; Kanjilal *et al*, Fl. Ass. 4: 56. 1940. *Laurus tamala* Buchanon–Hamilton, *Trans. Linn. Soc. London 13(2): 555. 1822.*

Local name: *Tejpata, Tejpat*

Evergreen trees, up to 15 m high. Leaves opposite or sub-opposite; lamina lanceolate or ovate–lanceolate, 10 – 15 × 2.5 – 6 cm; shortly and bluntly acuminate, base cuneate, thinly leathery, both surfaces glabrous, triplinerved; petioles 7 – 13 mm. Panicles 5 – 10 mm; perianth segments ovate, 2 – 3 mm. Fruits obovoid or ellipsoid, 8 – 10 mm long.

Exsiccatus: Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 002*, dated 21.03.2009; Palashbari, 64m, *Dibakar Choudhury & AP Das 6*, dated 24.03.2009

Status: Widely planted for its aromatic leaves; also wild

Flowers: February – April; **Fruits:** July – August

Local distribution: Cultivated throughout Terai & Duars.

General distribution: India, Nepal, Bhutan, Tropical and sub-tropical Himalayan regions.

Cinnamomum verum J. Presl, *Prir. Rostlin 2: 36. 1823. Laurus cinnamomum* Linnaeus, Sp. Pl. 1: 369. 1753. *Cinnamomum zeylanicum* Blume, Bijdr. 11: 568. 1825; Hooker *f.*, Fl. Brit. Ind. 5: 131. 1886; Prain, Beng. Pl. 2: 899. 1903.

Local name: *Darchini, Daruchini, Dalchini*

Evergreen trees, up to 10 m high; bark blackish-brown with cinnamic aldehyde flavor. Leaves opposite or sub-opposite; lamina ovate to ovate-lanceolate, 8 – 13 × 4.5 – 6 cm, green and shiny adaxially; both surfaces glabrous, triplinerved; petioles 8 – 13 mm. Panicle axillary or terminal, 10 – 12 cm; perianth segments oblong, 2 – 3 mm. Fruits ovoid, 10 – 12 mm.

Exsiccatus: Garden of Medicinal plants, NBU, 134 m, *Dibakar Choudhury & AP Das 003*, dated 21.03.2009; Falakata, 62 m, *Dibakar Choudhury & AP Das 007*, dated 24.03.2009

Status: Less common

Flowers: March – April; **Fruits:** July – September

Local distribution: Cultivated several regions of Terai & Duars.

General distribution: India, Sri Lanka, China, Myanmar and also cultivated in many other countries in Asia.

DISSCUSSION AND CONCLUSION

This study indicates that several important spices as well as medicinal species of *Cinnamomum* Schaeffer are important assets of the vegetation of Terai and Duars region of West Bengal. One of these is *C. tamala*, which is widely cultivated in Terai & Duars for its commercially exploited *Tejpata* leaves or bay leaves. These leaves are used for cookery worldwide (Sobti & Bradu 1988). The essential oil of *C. tamala* leaves, known as 'Tejpat oil' is medicinally used as a carminative, anti-flatulent, diuretic and in cardiac diseases (Gulati 1982). Another important spice, cultivated in this region is *C. verum* which is known for world famous Cinnamon-bark and Cinnamon oil (Senanayake *et al* 1978;). This oil is rich in trans-cinnamaldehyde with antimicrobial property against several animal and plant pathogens and spoiled bacteria and fungi (Mastura *et al* 1999). In addition to these, another important species, *C. camphora*, is the key source of 'Camphor oil' (Lee *et al* 2006). This oil is used in perfume industry and treatment of nervous depression, acne, inflammation, arthritis, cold and fever. It is presently restricted only in *ex-situ* conservation areas of this region. *C. bejolghota* is frequently found in thick forests and forest edges of Terai & Duars region. Local people used its leaves as a substitute of tejpat and bark paste for preventing toothache. Besides, leaf and bark extracts are used in cough, cold and liver troubles (Barbhuiya *et al* 2009). Depending on growing region, *C. impressinervium* attains highest altitudinal (over 2000 m) areas among the recorded species. Leaves of this plant are used as hypoglycaemic, stimulant, carminative and antidiarrhoeal agent (Pullaiah 2006). Essential oils of *C. glaucescens* are used in perfumery and cosmetic preparations. Bark of this plant is used for the treatment of fever and kidney problem (Doley *et al* 2009). Local tribes used the juicy extract of flower for curing several skin diseases.

Hence, this study reveals that the species of *Cinnamomum* are very important for economic point of view. But over exploitation of some *Cinnamomum* species, especially collection of bark and leaves cause serious damage to the population of these plants. Besides this, due to loss of habitats caused by deforestation, monoculture and extensive tourism adversely affect the rich diversity of *Cinnamomum* in this region. So, an urgent attention is required to protect these valuable species from destruction in their original habitat.

Apart from the strictly cultivated ones (*C. camphora* and *C. verum*) the local species *C. tamala* is also widely cultivated. *C. impressinervium* is endemic to Eastern Himalaya, *C. glaucescens* is endemic to northeastern region of the Indian subcontinent and *C. tamala* is basically a tropical Himalayan plants.

It is also observed that, cultivated or not, all the species of *Cinnamomum* recorded from Terai and Duars region of West Bengal are economically important. Though none of these local species are under threat for their survival, even then it is important to look for the maintenance of their good population structure in the natural habitat.

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Asian Journal of Plant Science and Research, 2013, 3(1):99-107

ISSN : 2249-7412
CODEN (USA): AJPSKY

In vitro antioxidant activity of methanolic leaves and barks extracts of four *Litsea* plants

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ABSTRACT

As far as our literature survey could ascertain, a very few information were available on the in vitro antioxidant activities of the Litsea (Family: Lauraceae) plant. Therefore, the aim of this current investigation is to evaluate the in vitro antioxidant capacities of leaf and bark extracts of four Litsea spp. The antioxidant activity of Litsea extracts were evaluated by various antioxidant assays such as DPPH scavenging, nitric oxide scavenging, superoxide scavenging, metal chelating activity and reducing power potency. Phytochemical screening and the total phenol and flavonoids content were also estimated. A positive correlation between the antioxidant activities and physiochemical assays was observed and the highest scavenging activity was noticed in bark of Litsea monopetala. Results obtained in the present investigation indicate clearly that the extracts of Litsea spp possesses significant antioxidant properties and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Keywords: Free radicals, Antioxidant, Phytochemicals, DPPH, *Litsea*.

INTRODUCTION

The Laurels are economically very important as sources of medicine, timber, nutritious fruits, spices and perfumes. Different parts of these plants are famous for traditional medicines [1]. The genus *Litsea* belongs to the family Lauraceae and are a potential source of biologically-active compounds, such as flavonoids (leaves of *Litsea coreana* and *Litsea japonica*) [2], butanolides (leaves of *Litsea acutivena*) [3], sesquiterpene (leaves and twigs of *Litsea verticillata*) [4], 1,3-diarylpropan-2-ol (bark of *Litsea rotundifolia*) [5], butanolide, coumarin, syringaldehyde (bark of *Litsea akoensis*) [6], and essential oils (leaves of *Litsea cubeba*, fruits, flowers and bark of *Litsea monopetala*, fruits of *Litsea glutinosa*) [7, 8, 9]. These plant-derived products can scavenge free radical species, inhibit free radical formation, and prevent oxidative damage [10]. The reactive oxygen species, such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) radicals, are produced under oxidative stress. Reactive oxygen species play vital roles in degenerative or pathological processes like ageing [11], cancer, coronary heart disease, Alzheimer's disease [12], neurodegenerative disorders, atherosclerosis, diabetes and inflammation [13]. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease preventing capacities. It is well known that phytochemicals are efficient in combating or inhibiting disease due to their antioxidant effect [14, 15, 16, 17, 18, 19, 20]. Antioxidant protects molecules from oxidation and they are implicated in the etiology of many diseases and in food deterioration and spoilage [21]. In food industry synthetic antioxidants have been widely used but due to the possible toxicities of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), the improvement and use of more effective antioxidants of natural origin is highly desirable [22]. Several scientific reports suggest that the genus *Litsea* is the rich source of natural antioxidants [23].

The aim of the present study was to investigate the leaf and bark of four different *Litsea* species from Terai and Duars of West Bengal as a potential antioxidant source, as an alternative to synthetic compounds. In this study we have determined the radical scavenging efficacy of leaves and stem as well as the phytonutrients of these plants.

MATERIALS AND METHODS

5.1. Plant Samples

Leaf and bark of four species of Litsea genus viz. *Litsea glutinosa* (Loureiro) Robinson, *L. monopetala* (Roxburgh) Persoon, *L. assamica* Hooker f., *L. laeta* (Nees) Hooker f. were collected from Terai and Duars of West Bengal, India. Taxonomic position was authenticated in the Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The material has been deposited in the 'NBU Herbarium' and recorded against the accession number 9639, 9640, 9641, 9642 dated 11-06-11.

5.2. Preparation of extracts

The leaves and barks of four species of *Litsea* were cut into small pieces and were separately crushed with mortar and pestle. Under Soxhlet extractor, the crushed samples were separately extracted with methanol for eight hours. The supernatants of refluxed samples were isolated from the residues by filtering through Whatman No. 1 filter paper. The filtrates were dried *in vacuo* by rotary evaporator and their total extractive values were calculated on dry weight basis by the formula:

$$\% \text{ extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

The samples were then kept in freeze for further use.

5.3. Chemicals

Methanol (M), 2.2-diphenyl-1-picryl hydrazyl (DPPH), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), phenazine methosulphate (PMS), sulfanilamide, glacial acetic acid and napthylethylenediamine dihydrochloride, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeSO_4.7H_2O$, potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), ethylene-diamine tetra acetic acid (EDTA), 2-deoxyribose, potassium ferricyanide, ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), ferrozine, hydrogen peroxide (H_2O_2), sodium nitroprusside, gallic acid, Folin-Ciocaltue reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), petroleum ether, sodium hydroxide (NaOH), copper acetate, ninhydrin, chloroform, lead acetate, sulphuric acid, hydrochloric acid, Dragendroff's reagent and pyridine were either purchased from Sigma Chemicals (USA), or of Merck analytical grade.

5.4. Determination of DPPH radical scavenging assay:

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-1- picrylhydrazyl) was determined spectrophotometrically. The changes in color (from deep-violet to light-yellow) were measured at 517 nm wavelength. Radical scavenging activity of extracts was measured by standard method [24]. Two microliters of each sample, prepared at various concentrations (0.5, 1, 2.5, 5, 10, 25 mg/ml), were added to 1.8 ml of 0.2 mM DPPH solution. The mixture was shaken and incubated for 30 min at 20ºC, and then the absorbance was measured at 517 nm with UV-VIS spectrophotometer. The percentage inhibition activity was calculated by the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample}) / A_{control} \times 100]$$

Where, $A_{control}$ is the initial concentration of the stable DPPH radical without the test compound and A_{sample} is the absorbance of the remaining concentration of DPPH in the presence of methanol. IC_{50} values (mg/ml) were determined from a plotted graph of scavenging activity against the concentrations of the extracts, where IC_{50} is defined as the total amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

5.5. Determination of superoxide anions scavenging activity

Measurement of superoxide radical scavenging activity of *Litsea* spp were done by using standard method followed by Nishikimi *et al*., with minor modifications [25]. The reaction mixture contained 1 ml of NBT solution (312 µM

prepared in phosphate buffer, pH-7.4), 1ml of NADH solution (936 µM prepared in phosphate buffer, pH-7.4) and differentially diluted sample extracts. Finally, reaction were accelerated by adding 100 µL PMS solution (120 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixtures were allowed at 25^{o} C for 5 min and absorbance was measured at 560 nm against methanol as control. Percentage inhibition was calculated using the same formula mentioned above.

5.6. Reducing antioxidant power

The reducing antioxidant power of plant methanolic extracts was determined by the standard method [26]. Different concentrations of 1 ml of extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50^{o}C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis. Increased absorbance of the reaction mixture indicates increase in reducing power.

5.7. Metal chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al*., with slight modification [27]. To 0.4 ml of methanol extract, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_2$ (2 mM). After 30s, 0.8 ml ferrozine (5 mM) was added. Subsequently after 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

5.8. Determination of Nitric oxide activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction [28]. 320 µL methanol extract, 360 µL (5mM) sodium nitroprusside-PBS solution, 216 µL Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was mixed and incubated at 25^{o}C for one hour. Lastly 2 ml water was added and absorbance was taken at 546nm.

5.9. Total phenol estimation

Total phenolic compounds of leaves and bark extracts were determined by Folin-Ciocalteu method [29]. For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2 and 0.3 mg/ml methanolic gallic acid solution was mixed with 0.5 ml of Folin-Ciocaltue reagent and 4 ml sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 hr. at 20º C and the calibration curve was drawn. To the same reagent, 1 ml methanolic extracts was mixed as described above and after 1 hr. the absorbance was measured. Total phenolic content in methanolic plant extracts in Gallic Acid Equivalents (GAE) was measured by the formula:

C = c.V/m

Where, C - total content of phenolic compounds, mg/g of plant extract, in GAE; c - the concentration of gallic acid deduced from the calibration curve (mg/ml); V - the volume of extracts (ml); m - the dry weight of the plant material.

5.10. Total flavonoids estimation

Aluminum chloride spectrophotometric method was used for flavonoids determination [30]. Each methanol extracts were separately diluted with 4 ml double distilled water. Then the diluted extracts of plant were mixed with 5% (0.3 ml) $NaNO_2$. 10% aluminum chloride was then added with reaction mixture. After 6 minute 2ml (1.0 M) NaOH and 2.4 ml double distilled water was added and mixed well. Thereafter, absorbance was measured at 510 nm in spectrophotometer. Standard solution of quercetin (0-500 mg L^{-1}) was used as calibration curve.

5.11. Phytochemicals screening of the crude extracts

The methanolic crude extracts (500 mg/ml) of leaves and bark were subjected to various chemical tests in order to screening different phytochemicals like reducing sugars [31], resins [32], amino acid, anthraquinones, triterpenoids, alkaloids, glycosides [33], tannin, steroid [34], saponins and cardiac glycosides [35].

RESULTS AND DISCUSSION

Phenolic compounds are widely investigated and are naturally occurring antioxidant components of plants. These phenolic compounds are found in medicinal plants as well as fruits and vegetables and play important roles in preventing degenerative diseases, including inflammation, cancer, and arteriosclerosis [36, 37]. Figure 1 and Figure 2 presents the extractable total phenol and flavonoid contents of four different *Litsea* species of Terai and Duars region. The total phenolic contents of the leaf extracts were much higher than those of the bark extracts (except *L. assamica*). The contents of total extractable flavonoid compounds in the extracts were varied from 58.06 to 62.04 mg/100 g and showed almost similar trend to the total phenolics. In 2008, Muhammad *et al*. had worked on *Litsea monopetala* bark and they found four different phenolic compounds from the methanolic extract [38]. In several studies it was recommended that plant flavonoids, which showed antioxidant activity *in vitro*, also function as antioxidants *in vivo* [2, 39]. Naturally occurring polyphenols and flavonoids can prevent lipid peroxidation, low-density lipoprotein oxidation, and the development of atherosclerosis and heart disease [40]. According to Agrawal *et al*., [16] the genus *Litsea* contain several secondary metabolites. Our study (Table1) also proved these statements. In an earlier study, many medicinal plants contained high amounts of phenolic compounds and there was a positive linear correlation between the total phenolic content and antioxidant activity of the plants [37, 41]. This suggests that the genus *Litsea*, which contained higher levels of polyphenols might have high antioxidant properties. Figure 3, 4, 5, 6, 7 have confirmed this information. In 2009, Kshirsagar and Upadhyay found that the stem of *L. glutinosa* had high DPPH scavenging capacity than the twig of this plant [42]. In this present study the antioxidant activity of the methanolic extracts of the different parts (leaf and bark) of four *Litsea* plants were investigated by using DPPH scavenging, reducing power, metal chelating, superoxide scavenging and nitric oxide scavenging assay of the extracts. Methanolic extracts of every parts of *Litsea* plants have exhibited excellent antioxidant activity. As shown in the Figure 3, extracts from leaf had relatively strong DPPH scavenging activity (low IC_{50} value), thus exhibiting high antioxidant capacity compared to extracts from bark (except *L. monopetala*). Possible mechanism of DPPH scavenging was suggested to be through reduction of this radical by antioxidant molecule to a more stable DPPH form. Because of its unpaired or free electron, DPPH has absorption maxima at 517nm and as it gets reduced in the presence of free radical scavengers the absorbance decreases with respect to the number of electrons taken up. For the measurement of the reducing ability, Fe^{+3}-Fe^{+2} transformations in the presence of phenolic compounds of *Litsea* was found. The reducing ability of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 4 shows the reducing capability of the genus. Bark of the genus of *Litsea* is more potent in reducing capacity than leaf. The bark of *L. glutinosa* has high reducing power (0.02 mg Ascorbic acid Eq/gm FWT) than other extracts. Iron is known to generate free radicals through the Fenton and Haber–Weiss reaction. Metal ion chelating activity of an antioxidant compound prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity acts as significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in lipid peroxidation [43]. In 1990 Gordon reported that chelating agents form *s*-bonds with a metal, are effective as secondary antioxidants since they reduce the redox potential, stabilizing the oxidized form of the metal ion [44]. In the present study it was seen that all the extracts interfered with the ferrous-ferrozine complex formation, suggesting that it has chelating activity and captured ferrous ion before ferrozine. Figure 5 shows that IC_{50} of the bark extract of *L. glutinosa* and *L. laeta* for metal chelating activity are 15.25 and 16.14 mg/ml FWT respectively which is higher than the other extracts. An important messenger molecule involved in many physiological and pathological processes within the mammalian body is nitric oxide [45]. The plant products may have the property to counteract the effect of $NO^{\bullet}$ formation and in turn may be of considerable interest in preventing the ill effects of excessive $NO^{\bullet}$ generation *in vivo*. *In vitro* prevention of nitric oxide radical is a measure of antioxidant activity of plant drugs. The nitric oxide radical scavenging activity of leaf and bark extracts of four species of *Litsea* were studied and compared with each other. Figure 6 shows that *L. monopetala* plant has better nitric oxide radical scavenging activity than other plant extracts in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions. The toxicity of $NO^{\bullet}$ increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-) [46]. This superoxide radical is also very harmful to cellular components (Korycka-Dahl and Richardson, 1978). As shown in Figure 7, the superoxide radical scavenging activities of the plant extracts have significant amount of superoxide scavenging activity.

It is widely accepted that the antioxidant activity of a plant extract is correlated to its phenolic content with several authors showing this correlations by different statistic approaches [47, 48, 49]. To study the role of phenolic compounds in antioxidant or chelating properties, Pearson's correlation coefficient was performed and analyzed. High correlations were obtained between total phenol content (TPC) and IC_{50} of metal chelating (MC) activity (Table 2) [$p \leq 0.05$]; also TPC is significantly correlated with NO scavenging activity, suggesting that phenolic compounds are the major contributors of antioxidant activity. Rainha *et al*. proved the importance of phenolic compounds in the antioxidant behaviour of *Hypericum foliosum* extracts and also showed that phenolic compounds contribute significantly to the total antioxidant capacity [50].

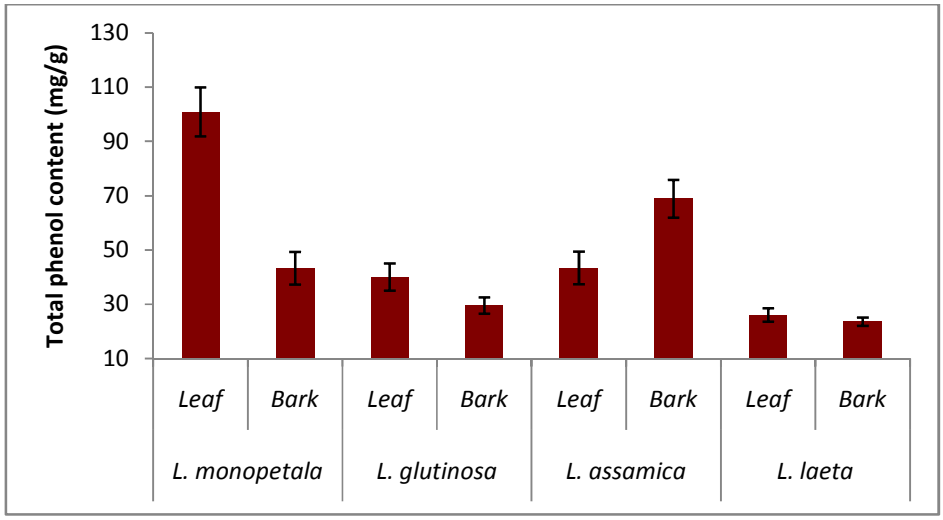

Figure 1: Total phenol content (mg/g FWT) of leaf and bark of *Litsea* spp.

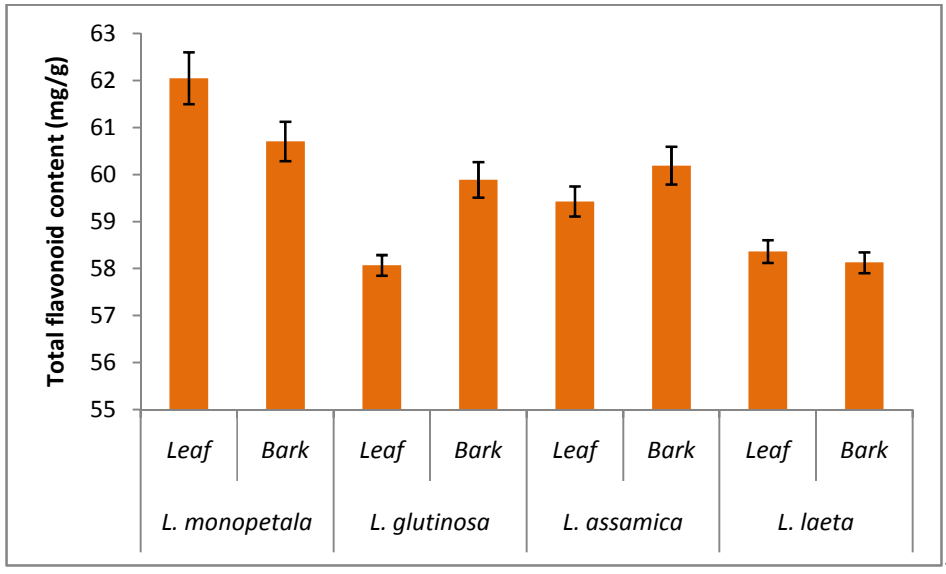

Figure 2: Total flavonoid content (mg/g FWT) of leaf and bark of *Litsea* spp.

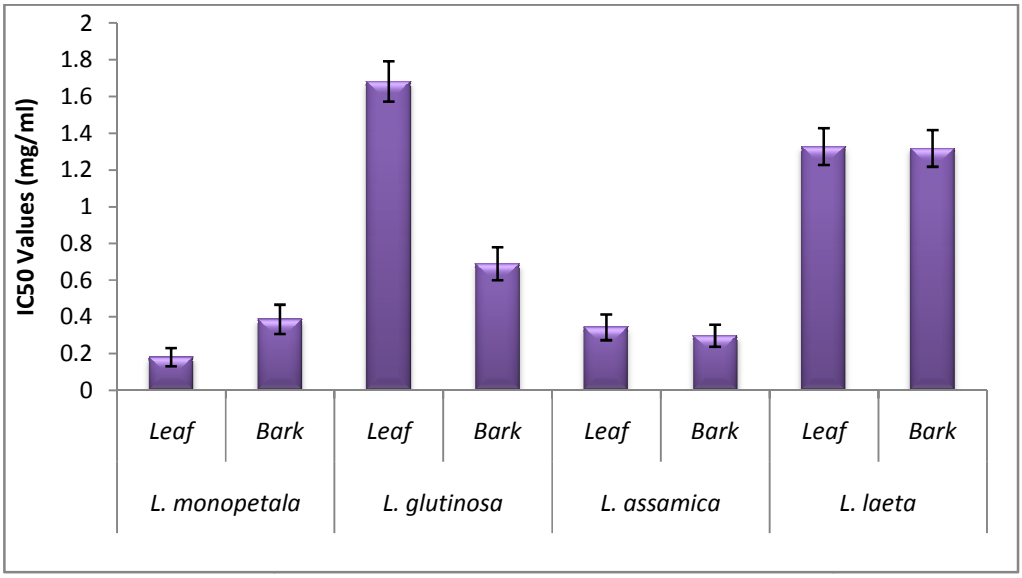

Figure 3: DPPH radical scavenging (IC_{50}) activity of leaf and bark of *Litsea* spp.

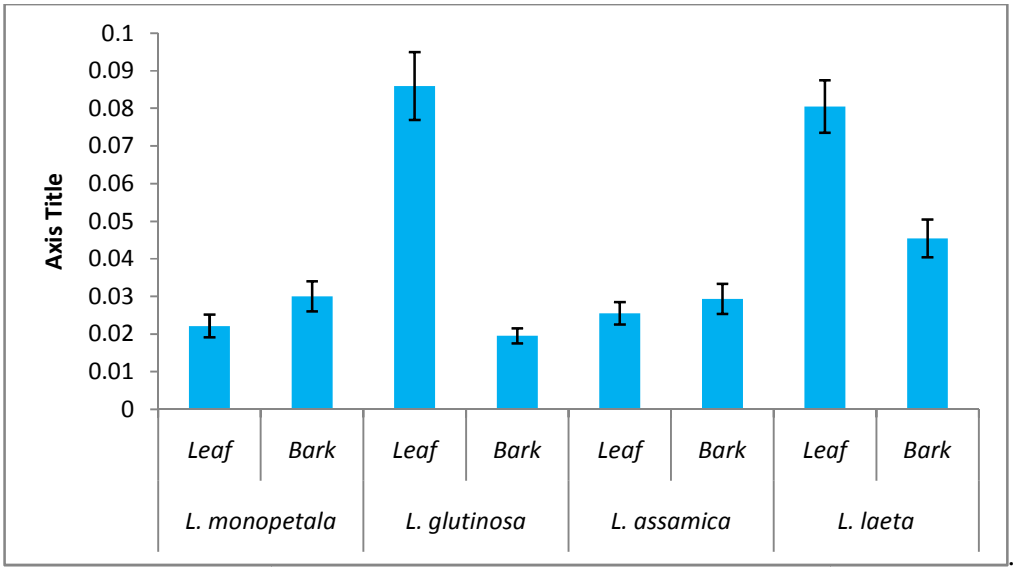

Figure 4: Reducing power of different parts leaf and bark of *Litsea* spp.

Table 1: Phytochemical profiling of *Litsea* spp (semi-quantitative)

Plants	Qualitative Phytochemical Test								
	Alkaloid	Steroids	Anthraquinones	Amino acid	Tanin	Tri terpenoids	Resin	Cardiac glycoside	Glycosides
L. glutinosa Leaf	++	++++	+	Nil	+	Nil	+++	Nil	Nil
L. glutinosa Bark	+	++	++	Nil	++++	+	+++	Nil	Nil
L. monopetala Leaf	+++	+++	+	Nil	++	Nil	++	Nil	Nil
L. monopetala Bark	+++	+++	+	+	+++	+	+	Nil	Nil
L. assamica Leaf	+++	++	++	Nil	++	+	++	Nil	Nil
L. assamica Bark	++++	+++	+++	Nil	++++	+	++	Nil	Nil
L. laeta Leaf	++++	+++	++	Nil	+++	+	+++	Nil	Nil
L. laeta Bark	+	++++	+	Nil	++	+	++	Nil	Nil

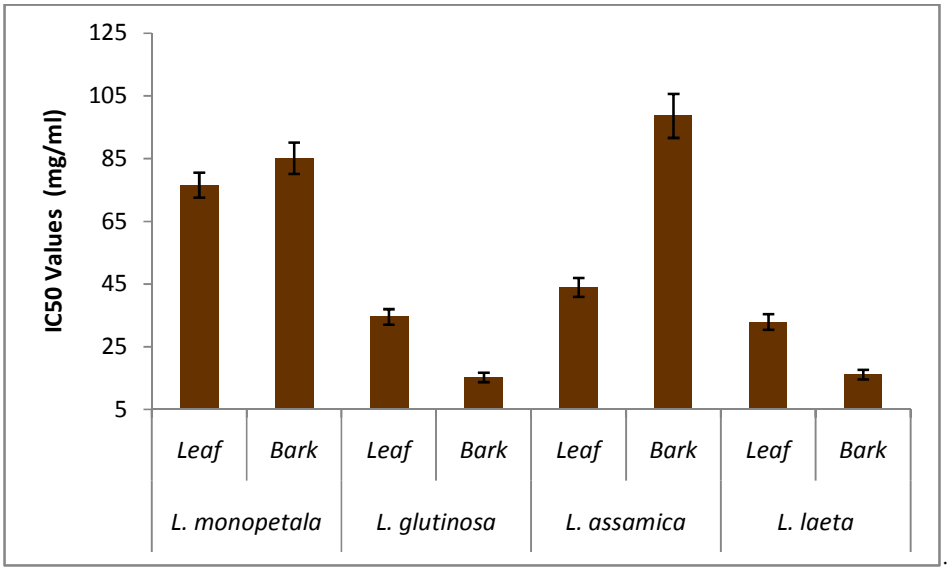

Figure 5: Metal chelating (IC_{50}) activity leaf and bark of *Litsea* spp.

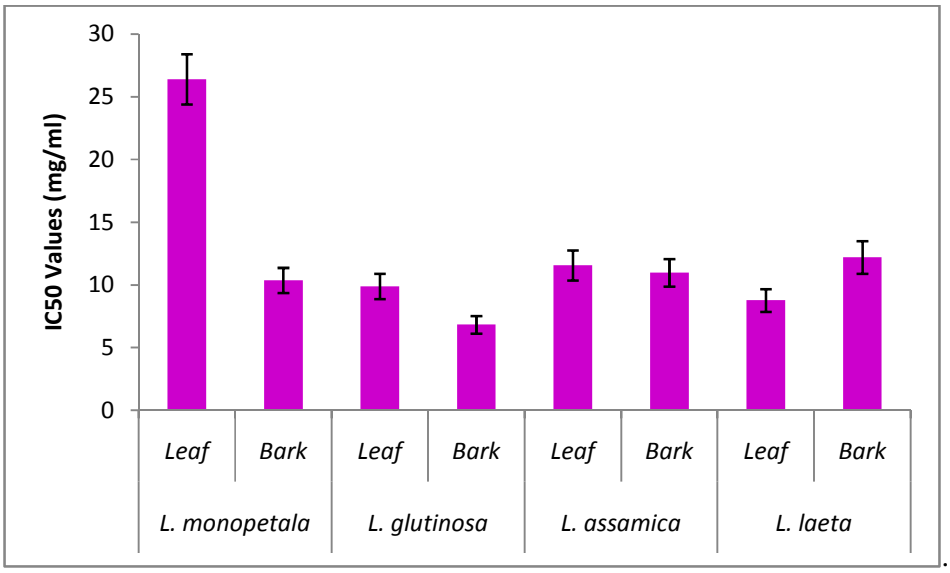

Figure 6: Nitric oxide scavenging (IC_{50}) activity leaf and bark of *Litsea* spp.

Table 2: Correlation Matrix of antioxidant activity and phytochemicals

	DPPH	SO	NO	RP	MC	TFC
SO	-0.120					
NO	-0.426	-0.322				
RP	**0.899(**)**	0.117	-0.341			
MC	-0.685	0.203	0.399	-0.395		
TFC	-0.649	0.181	0.271	-0.402	0.613	
TPC	-0.648	-0.039	**0.848(**)**	-0.462	**0.726(*)**	0.612

*** Correlation is significant at the 0.01 level (2-tailed).*
** Correlation is significant at the 0.05 level (2-tailed).*

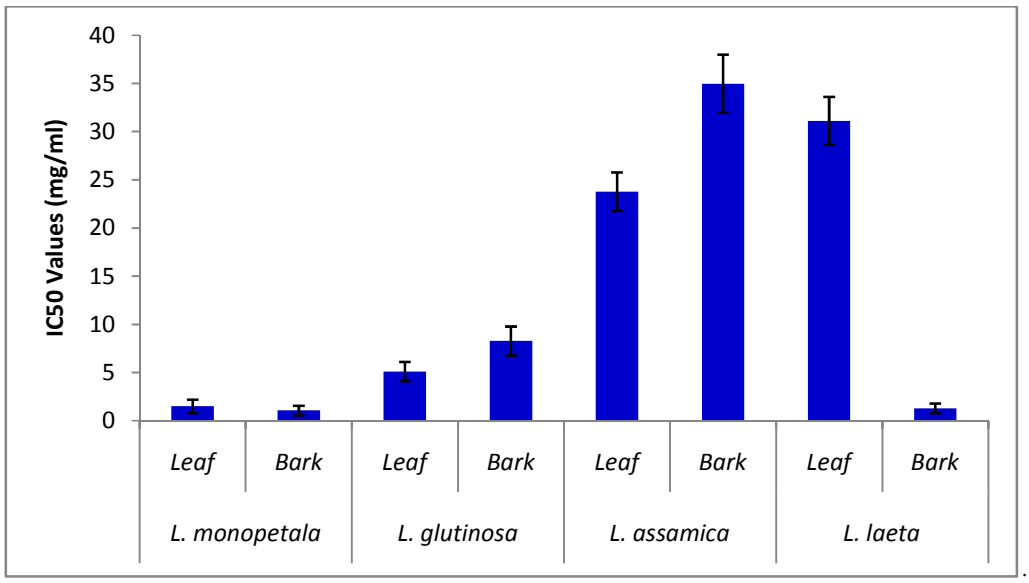

Figure 7: Superoxide scavenging (ic_{50}) activity leaf and bark of *Litsea* spp.

CONCLUSION

In conclusion, the data obtained from the present study showed that the leaf and bark extracts of *Litsea* spp are the potential sources of natural antioxidant which might help in preventing the progress of various oxidative stresses. It can be assumed that these plants possesses the significant antioxidant activity compared to other well characterized, standard antioxidant systems *in vitro* and could serve as free radical inhibitors which might be due to the presence of phenol, flavonoids, alkaloids, steroids, anthraquinones, tannins, resin. These finding suggests that these plants are the potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases.

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Pleione 8(1): 68 - 78. 2014. **ISSN: 0973-9467**

Diversity of *Litsea* Lamarck [Lauraceae] in Terai and Duars regions of West Bengal, India

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[Received 23.03.2014; Revised 12.05.2014; Accepted 29.05.2014; Published 30.06.2014]

Abstract

There are about 622 species in the genus *Litsea* Lamarck (Lauraceae), distributed mainly in Australia, New Zealand, North & South America and Asia. Among those, 45 species of *Litsea* were reported from India. No comprehensive report is available on this genus from Terai and Duars regions, which are located at the foot of the Eastern Himalaya and located within the Himalaya Hotspot. Present paper reported the occurrence of nine species of *Litsea* from Terai and Duars belt along with their local names, salient features, exsiccatus, status, flowering and fruiting periods and geographic distribution. Collection of *L. assamica* Hooker *f.* from the Jaldapara National Park in Duars forms the new record for the state flora of West Bengal.

Key words: Genus *Litsea*, Terai, Duars, West Bengal, *Litsea assamica*, new record

INTRODUCTION

The Terai and Duars region politically constitute the plains of Darjeeling and whole of Jaipaiguri districts of West Bengal. Northern part of West Bengal touching the feat of Eastern Himalaya is generally referred as Terai (25º 57' to 26° 36' N, Latitude and 89° 54' to 88° 47' longitude) and Duars (located between 26° 16' to 27° 0' N latitude and 88° 4' to 89° 53' E longitude) (Ghosh 2006; Das *et al* 2010; Roy *et al* 2009). Terai and Duars are famous for the tea gardens, which were first developed by the British planters. The beauty of the region lies not only in its tea gardens but also in the dense jungles those make up the countryside. Famous wildlife sanctuaries and national parks like Mahananda Wildlife Sanctuary, Gorumara National Park, Chapramari Wildlife Sanctuary, Buxa Tiger Reserve and Jaldapara National Park are located in this region. The vegetation of Terai and Duars are floristically very rich and covers all major groups of plants including several members of endemic and RET species (Chatterjee 1940; Das & Chanda 1987; Ghosh & Das 2009). Also, this area is falling under the IUCN recognized 'Himalaya Biodiversity Hotspot' (Conservation International 2005). The wide diversity in habitat structure helped numerous plant families to settle in this area (Kadir 2001; Rai & Das 2008). Lauraceae is one of the dominant families in this region (Banerjee 1993; Das *et al* 2010; Cowan & Cowan 1929).

The Lauraceae or Laurel family comprises a group of flowering plants included in the order Laurales of kingdom Plantae. Laurels contain about 55 genera and 2850 species world-

wide (Werff & Richter 1996; Mabberley 2008) of which *Litsea* Lamarck is the largest (Bhuinya *et al* 2008). *Litsea* has about 622 species distributed mainly in Australia, New Zealand, North America, South America and Asia (Agrawal *et al* 2011; Si *et al* 2012), of which about 45 species are growing in India (Bhuinya *et al* 2009).

Species of *Litsea* are economically important as sources of medicine, timber and nutritious fruits. Numerous species of *Litsea* contain several biologically-active compounds like alkaloids (Zhang *et al* 2012), flavonoids (Lee *et al* 2005), steroids (Choudhury *et al* 2013), terpenes, triterpenoids and essential oils (Wang *et al* 1999; Choudhury *et al* 1997). Several studies using modern techniques have authenticated it's apply to cure diarrhea, dysentery, rheumatism and as an aid to longevity. Also, it has antibacterial (Hosamath 2011), antifungal (Yang *et al* 2010), antiseptic, anti-inflammatory, wound healing (Devi & Meera 2010) and antioxidant (Choudhury *et al* 2013) effects. Most importantly, numerous studies have shown its efficiency as anticancer (Ho *et al* 2010), cytotoxic and anti-HIV agents (Agrawal *et al* 2011).

So, the genus *Litsea* is economically very important. Unfortunately, there is no detailed floristic work available on these members in Terai-Duars belt. So, in the present study an attempt has been undertaken to investigate the distribution of *Litsea* species in Terai and Duars region.

MATERIALS AND METHODS

Extensive random collections of *Litsea* Lamarck species were done during 2009 – 2012 from different parts of Terai and Duars region of West Bengal covering different seasons (mainly pre-monsoon, post-monsoon and winter) of the year. Collected specimens were processed into mounted herbarium sheets following conventional techniques (Jain & Rao 1977). The processed specimens were identified taking help of relevant taxonomic literature including Hooker (1886), Brandis (1906), Kanjilal *et al* (1940), Long (1984), Li *et al* (2008) and by matching with the previously identified specimens at NBU and CAL. Digital images of type sheets were acquired from K and E to confirm the identity of some specimens. Identified specimens were deposited in NBU Herbarium. www.theplantlist.org was mostly pursued for correct nomenclature of the recorded taxa. Distributional status in the world of the identified species was recorded also from different literature (Hooker 1886; Brandis 1906; Momiyama 1966; Long 1984; Alam 1988; Li *et al* 2008; Ara *et al* 2007). Uses, local names and status of different species were documented during field work from the local people and some of the information was noted down from available literature (Cowan & Cowan 1929; Kanjilal *et al* 1940; Prain 1903; Matthew 1981; Banerjee 1993).

RESULT

The present study is the first taxonomic revision of genus *Litsea* Lamarck from the floristically very rich Terai & Duars belt of West Bengal. It is separated from the other genera by having umbellate inflorescences, solitary or clustered in leaf axis; unisexual and trimerous flowers, 9 – 12 stamens in 3 or 4 whorls of 3 each; 4-locular anthers; reduced tepals; superior ovary and seated fruit on enlarged perianth tube.

From the present survey, nine species of *Litsea* were recorded. An artificial Key for the recorded species ware constructed based on significant vegetative, flower and fruit characters. All these species were enumerated below alphabetically accompanied by local names, salient features, exsiccatus, availability status, flowering and fruiting time, occurrence in Terai & Duars region and geographic distribution.

Key to the studied species of *Litsea* Lamarck

1a.	Umbels peduncled, arranged usually in clusters, sometimes solitary or peduncles borne on a short stout axis upto 4 mm ……... ……	**2**
1b.	Umbels peduncled, arranged in racemes or corymbs with a conspicuous slender axis 5 – 70 mm ……... ……... ……... ……	**7**
2a.	Shoots with scaly terminal vegetative bud and ring of bud scale scars …	***L. elongata***
2b.	Scaly terminal vegetative buds and ring of bud scale scars absent …	**3**
3a.	Lamina lanceolate or narrowly elliptic-oblong, 2 – 5 cm broad (up to 8.5 cm broad in *L. salicifolia*) ……... ……... ……... ……	**4**
3b.	Lamina ovate, obovate or broadly elliptic, 6 – 12 cm broad ……...	**6**
4a.	Leaves coriaceous, pale or yellowish green above when dry, lateral veins 5 – 7 pairs ……... ……... ……... ……... …….	***L. laeta***
4b.	Leaves rather membranous, dark green or brown above when dry, lateral veins 8 – 15 pairs ……... ……... ……... ……...	**5**
5a.	Lamina lanceolate, glabrous beneath except on veins, lateral veins 8 – 12 pairs ……... ……... ……... ……... ……...	***L. cubeba***
5b.	Lamina elliptic–oblong, minutely silky–pubescent beneath, lateral veins 10 – 15 pairs ……..….. …..…….. ….…….. …….…..	***L. salicifolia***
6a.	Lamina broadly ovate/obovate to ovate oblong, apex obtuse or apiculate, base rounded, tormentose beneath, lateral veins 6 – 13 pairs …….	***L. monopetala***
6b.	Lamina elliptic obovata, apex shortly acuminate, base cuneate, pubescent on veins beneath, lateral veins 9 – 15 pairs …….....	***L. hookeri***
7a.	Lamina 4 –12 cm long; petioles 8–14 mm long; fruits narrow-ellipsoid …	***L. assamica***
7b.	Lamina 8 – 32 cm long; petioles 10 – 28 mm long; fruits globose or depressed globose ……... ……... ……... ……..	**8**
8a.	Lamina oblong or lanceolate, apex acuminate or shortly acute ……..	***L. panamanja***
8b.	Lamina ovate–lanceolate, apex obtuse or rounded ……... ……..	***L. glutinosa***

ENUMERATION

Litsea assamica Hooker *f.*, Fl. Brit. Ind. 5: 161. 1886; Kanjilal *et al*, Fl. Ass. 4: 85. 1940.

Local name: *Timur*

Evergreen trees, up to 15 m high; branchlets glabrous, blackish brown. Leaves alternate; lamina elliptic, 4 – 12 × 2.5 – 6 cm, acute to bluntly acuminate, base cunate, thinly coriaceous, rather glabrous, lateral veins 5 – 9 pairs; petioles 8 – 14 mm. Umbels axillary solitary; peduncle 9 – 15 mm. Fruits narrowly ellipsoid, 6 – 9 mm long.

Exsiccatae: Chilapata 88 m, *Dibakar Choudhury & AP Das 095*, dated 10.11.2009

Status: Less common

Flowers: May – June; **Fruits:** August – September

Local distribution: Found only in Jaldapara National Park.

General distribution: India **[**North–East India, West Bengal]; Endemic; a new record for West Bengal.

*Note***:** Wood is used for making match boxes.

Litsea cubeba (Loureiro) Persoon, Syn. Pl. 2: 4. 1807; Momiyama in Hara, Fl. E. Him. 1: 101.1966; Long in Gierson & Long, Fl. Bhut. 1(2): 274. 1984. *Laurus cubeba* Loureiro, Fl. Cochinch. 1: 252. 1790.

Local name: *Siltimur*

Deciduous shrubs to small aromatic trees, up to 10 m high; branchlets glabrous. Leaves alternate; lamina lanceolate, 4 – 14 × 2 – 4 cm, long acuminate, base cuneate, dark green above when dry, pale beneath, both surfaces glabrous or sericeous-pubescent on veins; lateral veins 8 – 12 pairs; petioles 6 – 20 mm. Umbels solitary or clustered; peduncles 3 – 8 mm. Fruits subglobose, 6 – 7 mm.

Exsiccatae: Dhupjhora 127 m, *Dibakar Choudhury & AP Das 113*, dated 20.02.2010; North Sevoke 190 m, *Dibakar Choudhury & AP Das 142*, dated 24.02.2010; Sal Bagan, NBU 143 m, *Dibakar Choudhury & AP Das 155*, dated 15.03.2010.

Status: Less common

Flowers: February – March; **Fruits:** July – August

Local distribution: Found in Gorumara National Park, Mahananda Wild Life Sanctuary & NBU Campus.

General distribution: India **[**Arunachal Pradesh, Assam, Meghalaya, West Bengal, Sikkim**]**, Nepal, Bhutan, Myanmar, Java, China.

*Note***:** Fruit oil is added to food for flavouring and also as bio-pesticide.

Litsea elongata (Nees) Hooker *f*., Fl. Brit. Ind. 5: 165. 1886; Momiyama in Hara, Fl. E. Him. 1: 101. 1966; Matthew, Pl. Kurs. 90. 1981; Cowan & Cowan, Trs. N. Beng. 110. 1929*;* Long in Gierson & Long, Fl. Bhut. 1(2): 275. 1984; Kanjilal *et al*, Fl. Ass. 4: 86. 1940. *Daphnidium elongatum* Nees, Pl. Asiat. Rar. 2: 63. 1831.

Local name: *Thulo pahenlay*

Evergreen trees, robust upto 18 m tall; branchlets often tomentose, brownish. Leaves alternate; lamina elliptic to oblanceolate or obovate, 8 – 18 × 2 – 6 cm, acute or obtuse, sometimes acuminate, base cuneate, lateral veins 6 – 13 pairs, much prominent beneath; petioles 6 – 16 mm. Umbels solitary, on slender peduncles, 10 – 15 mm. Fruits ellipsoid 10 – 14 mm, with minute apical point.

Exsiccatus: Sukna 220 m, *Dibakar Choudhury & AP Das 092*, dated 07.10.2009

Status: Less common

Flowers: July – September; **Fruits:** October – November

Local distribution: Found only in Mahananda Wild Life Sanctuary.

General distribution: India **[**Arunachal Pradesh, Assam, West Bengal, Sikkim, Himachal Pradesh] Nepal, Bhutan, Myanmar, Tibet, China.

*Note***:** The species is a good fodder for cattle and wood is used for construction works, making furniture, etc.

Litsea glutinosa (Loureiro) C.B. Robinson, Philipp. J. Sci. 6(5): 321. 1911; Matthew, Pl. Kurs. 90. 1981; Long in Gierson & Long, Fl. Bhut. 1 (2): 277. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993. *Sebifera glutinosa* Loureiro, Fl. Cochinch. 2: 638. 1790. *Litsea sebifera* Persoon, Syn. Pl. 2: 4. 1807; Hooker *f*., Fl. Brit. Ind. 5: 124. 1886; Prain, Beng. Pl. 2: 902. 1903; Cowan & Cowan, Trs. N. Beng. 109. 1929; Kanjilal *et al*, Fl. Ass. 4: 82. 1940.

Local name: *Kawala*

Evergreen, aromatic trees, up to 18 m high; young branchlets gray-yellow tomentose. Leaves alternate, both surfaces tomentose when young; lamina elliptic-oblong or ovate-lanceolate,

7.5 – 22.5 × 3.5 – 10 cm, obtuse or rounded, base cuneate, obtuse or rotund, lateral veins 5 – 12 pairs, petioles 10 – 28 mm. Umbels solitary or several on short branchlets; peduncle 9 – 15 mm. Fruits globose, 7 – 9 mm.

Exsiccatae: Lataguri 102 m, *Dibakar Choudhury & AP Das 026*, dated 30.05.2009; Rajabhatkhawa 80 m, *Dibakar Choudhury & AP Das 051*, dated 09.06.2009; Sevoke 188 m, *Dibakar Choudhury & AP Das 063*, dated 26.06.2009; NBU campus 134 m, *Dibakar Choudhury & AP Das 174*, dated 10.05.2012; Salkumar 78 m, *Dibakar Choudhury & AP Das 060*, dated 15.09.2009

Status: Common

Flowers: March – June; **Fruits:** September – October

Local distribution: Found throughout the Terai and Duars region.

General distribution: Pakistan, India [almost throughout- Arunachal Pradesh, Assam, Nagaland, Tripura, Meghalaya, West Bengal, Sikkim, Bihar, Jharkhand, Orissa, Andhra Pradesh, Andaman & Nicobar Islands, Karnataka, Maharashtra, Madhya Pradesh, Uttarakhand, Punjab, Himachal Pradesh], Nepal, Bhutan, Sri Lanka, China, Myanmar, Philippines, Thailand, Vietnam.

Note: Bark is used for treatment of diarrhea, dysentery, rheumatic joint pain etc. and bark powder is used as an adhesive paste in incense stick production.

Litsea hookeri (Meisner) Long, Notes Roy. Bot. Gard. Edinburgh. 41: 510. 1984; Long in Gierson & Long, Fl. Bhut. 1(2): 276. 1984. *Cylicodaphne hookeri* Meisner, Prodr. 15(1): 209. 1864.

Local name: *Dude Lampate*

Evergreen trees, up to 12 m high; branchlets brownish tomentose. Leaves alternate; lamina elliptic-obovate, 12 – 26 × 6 – 10 cm, shortly acuminate, base cuneate, pubescent on veins beneath; lateral veins 9 – 15 pairs; petioles 8 – 15 mm. Umbels densely pubescent, clustered on shortest branchlets; peduncle 4 – 8 mm. Fruits ellipsoid, 11 – 17 mm long.

Exsiccatae: North Sevoke 214 m, *Dibakar Choudhury & AP Das 009*, dated 10.04.2009; Lataguri, Mahakaldham 127 m, *Dibakar Choudhury & AP Das 024*, dated 30.05.2009

Status: Less common

Flowers: May – June; **Fruits:** August – September

Local distribution: Found in Mahananda Wildlife Sanctuary & Gorumara National Park.

General distribution: India **[**Arunachal Pradesh, Assam, West Bengal] Bhutan, Thailand.

Note: Timber is used for constructing houses and for making furniture.

Litsea laeta (Nees) Hooker *f.*, Fl. Brit. Ind. 5: 169. 1886; Matthew, Pl. Kurs. 90. 1981; Cowan & Cowan, Trs. N. Beng. 111. 1929*;* Long in Gierson & Long, Fl. Bhut. 1 (2): 275. 1984; Kanjilal *et al*, Fl. Ass. 4: 88. 1940. *Tetranthera laeta* Nees, Pl. Asiat. Rar. 2: 67. 1831.

Shrub or small trees up to 8 m high; young shoots generally finely ferruginous-pubescent. Leaves alternate, coriaceous; lamina oblong-elliptic, 10 – 20 × 3 – 5 cm, acute, base cuneate, glabrous; lateral veins 5 – 7 pairs; petioles 10 – 15 mm. Umbels axillary clusters, rarely solitary; peduncles 4 – 10 mm. Fruits obovoid or subglobose, 5 – 10 mm long.

Exsiccatae: Sukna 220 m, *Dibakar Choudhury & AP Das 090*, dated 07.10.2009; Garden of Medicinal plants, NBU 134 m, *Dibakar Choudhury & AP Das 154*, dated 15.03.2010

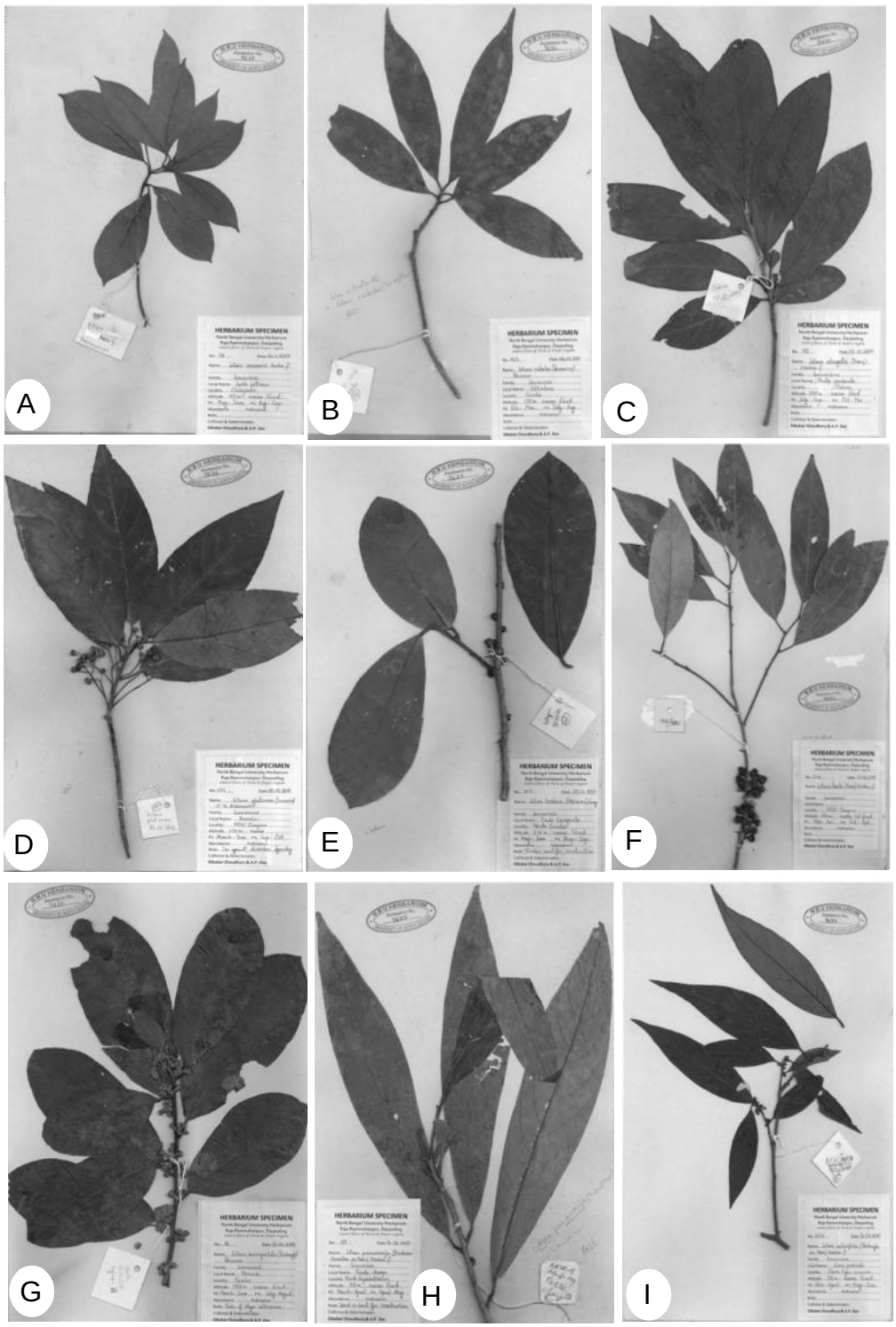

PLATE – I: Species of *Litsea* Lamarck in Terai and Duars region of West Bengal: **A.** *L. assamica*; **B.** *L. cubeba*; **C.** *L. elongata*; **D.** *L. glutinosa*; **E.** *L. hookeri*; **F.** *L. laeta*; **G.** *L. monopetala*; **H.** *L. panamanja*; **I.** *L. salicifolia*

Status: Less common

Flowers: November – January; **Fruits:** February – April

Local distribution: Found in Mahananda Wildlife Sanctuary and University of North Bengal campus.

General distribution: India **[**Arunachal Pradesh, Assam, West Bengal, Sikkim, Andhra Pradesh], Bhutan, Bangladesh.

Note: Seed oil is with high antioxidant activity (Choudhury *et al* 2013).

Litsea monopetala (Roxburgh) Persoon, Syn. Pl. 2: 4. 1807; Momiyama in Hara, Fl. E. Him. 1: 102. 1966; Matthew, Pl. Kurs. 89. 1981; Long in Gierson & Long, Fl. Bhut. 1(2): 276. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993. *Tetranthera monopetala* Roxburgh, Pl. Coromandel. 2: 26. 1798. *Litsea polyantha* Jussieu, Ann. Mus. Natl. Hist. Nat. 6: 211. 1805; Hooker *f*., Fl. Brit. Ind. 5: 162. 1886; Prain, Beng. Pl. 2: 903. 1903; Cowan & Cowan, Trs. N. Beng.110. 1929; Kanjilal *et al*, Fl. Ass. 4: 83. 1940.

Local Name: *Bonsum, Kutmero, Patmero*

Evergreen trees, up to 15 m high, with spreading crown. Leaves alternate; lamina ovate-oblong, oblanceolate or elliptic-oblong, 7 – 25 × 6 – 12 cm, obtuse or apiculate, base rounded, lateral veins 6 – 13 pairs; petioles 8 – 20 mm. Umbels densely pubescent on tormentose peduncles, 3 – 10 mm. Fruits globose to ellipsoid, 7 – 12 mm long; blackish when ripe.

Exsiccatae: Lataguri 98 m, *Dibakar Choudhury & AP Das 109*, dated 20.02.2010; North Rajabhatkhawa 88 m, *Dibakar Choudhury & AP Das 050*, dated 09.06.2009; Sevoke 188 m, *Dibakar Choudhury & AP Das 011*, dated 10.04.2009; NBU campus 134 m, *Dibakar Choudhury & AP Das 172*, dated 10.05.2012; Jaldapara 80 m, *Dibakar Choudhury & AP Das 075*, dated 02.10.2009

Status: Abundant

Flower: March – June; **Fruit:** July – August

Local Distribution: Found throughout the Terai and Duars region.

General Distribution: Pakistan, India [Arunachal Pradesh, Assam, Tripura, Meghalaya, West Bengal, Sikkim, Bihar, Jharkhand, Orissa, Andhra Pradesh, Andaman & Nicobar Islands, Maharashtra, Madhya Pradesh, Uttarakhand], Nepal, Bhutan, China, Myanmar, Thailand, Malaysia, Vietnam, Cambodia.

Note: The leaves are used topically to treat arthritis and is a good food for rearing larvae of muga-silk moth.

Litsea panamanja (Buchanon–Hamilton *ex* Nees) Hooker *f*., Fl. Brit. Ind. 5: 175. 1886; Prain, Beng. Pl. 2: 903. 1903; Long in Gierson & Long, Fl. Bhut. 1(2): 277. 1984; Kanjilal *et al*, Fl. Ass. 4: 90. 1940. *Tetranthera panamanja* Buchanon–Hamilton *ex* Nees, Pl. Asiat. Rar. 2: 67. 1831.

Local name: *Painle champ, Dudhi lampati*

Evergreen trees, up to 25 m high; branchlets pubescent and becoming glabrous. Leaves alternate; lamina oblong or lanceolate, 15 – 32 × 3 – 7 cm, acuminate or shortly acute, base cuneate, both surfaces glabrous, coriaceous; lateral veins 7 – 11 pairs; petioles 13 – 22 mm. Umbels 13 – 18 cm, racemosely arranged on short branchlets, pubescent. Fruits depressed globose, 6 – 8 mm in diameter.

Exsiccatae: Sursuti 102 m, *Dibakar Choudhury & AP Das 039*, dated 31.05.2009; North Rajabhatkhawa 88 m, *Dibakar Choudhury & AP Das 049*, dated 09.06.2009; Sevoke 210 m, *Dibakar Choudhury & AP Das 140*, dated 24.02.2010

Status: Less common

Flowers: March – April; **Fruits:** April – May

Local distribution: Found in forest areas throughout Terai and Duars.

General distribution: India **[**Arunachal Pradesh, Assam, Nagaland, Tripura, West Bengal, Sikkim, Andaman & Nicobar Islands**]**, Nepal, Bhutan, Bangladesh, China, Myanmar, Vietnam, Malay Peninsula.

*Note***:** Wood is used for house construction, making furniture and as fire wood.

Litsea salicifolia (Roxburgh *ex* Nees) Hooker *f*., Fl. Brit. Ind. 5: 167. 1886; Prain, Beng. Pl. 2: 903. 1903; Cowan & Cowan, Trs. N. Beng. 110. 1929; Momiyama in Hara, Fl. E. Him. 2: 39. 1971; Long in Gierson & Long, Fl. Bhut. 1 (2): 275. 1984; Banerjee, Pl. Res. Jal. Rhi. Sanc. 52. 1993; Kanjilal *et al*, Fl. Ass. 4: 87. 1940. *Tetranthera salicifolia* Roxburgh *ex* Nees, Pl. Asiat. Rar. 2: 66. 1831.

Local name: *Sanu pahenle*

Evergreen trees, up to 10 m high; branchlets glabrous. Leaves alternate; lamina elliptic–oblong, 12 – 30 × 2.5 – 8.5 cm, acuminate or acute, base acute, dark brown above when dry, lateral veins 10 – 15 pairs, prominent beneath; petioles 8 – 12 mm. Umbels 7 – 16 in dense axillary clusters on 2 – 6 mm long peduncles. Fruits ellipsoid, 10 – 11 mm in diameter.

Exsiccatae: Dhupjhora 127 m, *Dibakar Choudhury & AP Das 112*, dated 20.02.2010; Buxa 96 m, *Dibakar Choudhury & AP Das 104*, dated 08.02.2010; Sevoke 190 m, *Dibakar Choudhury & AP Das 013*, dated 10.04.2009; NBU campus 134 m, *Dibakar Choudhury & AP Das 156*, dated 15.03.2010; Hollong 87 m, *Dibakar Choudhury & AP Das 077*, dated 02.10.2009

Status: Frequent in forests

Flowers: February – April; **Fruits:** May – June

Local distribution: Found in forest areas throughout Terai and Duars.

General distribution: Pakistan, India **[**Arunachal Pradesh, Assam, West Bengal, Sikkim, Bihar**]**, Bangladesh, Nepal, Bhutan, China, Vietnam, Myanmar.

Note: Seed oil is used as bio-pesticide and leaves are good food for rearing larvae of muga-silk moth.

DISCUSSION AND CONCLUSION

After intensive scrutiny of literature (Prain 1903; Hooker 1886; Brandis 1906; Cowan & Cowan 1929; Banerjee 1993; Das *et al* 2010) it is revealed that out of the recorded nine species of *Litsea* Lamarck only four species viz., *L. cubeba*, *L. glutinosa*, *L. monopetala* and *L. salicifolia* were reported earlier (without specifying locality in the earlier works) and other five species viz., *L. assamica*, *L. elongate*, *L. hookeri*, *L. laeta* and *L. panamanja* were not reported earlier from Terai and Duars belt of West Bengal. However, apart from *L. assamica*, all other species were known to grow from different other localities of the state. The distribution of *L. assamica* was earlier known only from North–East India

(Kanjilal *et al.* 1940; Bhuinya *et al.* 2009); or, in other words, the species was known as endemic to that region. The present collection of the species from Terai and Duars is a new record of its occurrence in West Bengal. Present study also indicates that several medicinal as well as economically useful species of *Litsea* are important assets in the vegetation of Terai and Duars belt.

However, with the rapid extension of human settlement areas, establishment of tea gardens, metalled roads, illegal timber extraction, monoculture plantations (mostly with fast growing exotic species), extensive tourism related activities and other socio-economic developmental activities adversely affecting the rich diversity of pristine vegetation of the entire area in which most of the presently recorded species of *Litsea* are surviving. So, the pressure for removal or death or extinction of many of these species, along with numerous other important and interesting species, is increasing at every moment. The activities in the name of 'eco-tourism' are creating havoc in many places especially in the Lataguri – Gorumara region. Active steps for the conservation under proper surveillance are deemed essential since a thorough scientific research is certain to reveal their benevolent aspects as well as ecological functions. Every one needs to remember that conservation is best when a species is permitted to grow undisturbed in its own home!

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