MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL STUDIES ON SELECTED SPECIES OF *CROTON* L. (EUPHORBIACEAE) IN SOUTHERN PENINSULAR INDIA

Thesis submitted to the
University of Calicut
For the award of the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY
Under the Faculty of Science

By

BHAVANA R.

Under the Guidance of

Dr. BINU THOMAS

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CHAPTER 1 INTRODUCTION Medicinal properties of plants and plant products have a great role in the development of human cultures around the whole world. Secondary metabolites which ar augmented with a wide range of medicinal applications (Krishnaraju et al., 2005). From a long time, herbal remedies continue to play a vital role in the preventive medicine. Several compounds f plant products that are helpful for human healthcare (Dutta et al., 2014). Isolation and characterization of pharmacologically active compounds from medicinal plants continue so far in a view to and cure for various health disorders in humans (Shantabi et al., 2017). Nowadays, the traditional medicine all over the world is evaluated by comprehensive activities of research on different tax populations of different parts of the world and their therapeutic principles. Based on indigenous theory, herbal medicines are belief and experiences that are passed down generations (Mohanasi properties of herbal medicines such as, effectiveness, accessibility, low cost and comparative freedom from serious side effects, makes herbal medicine not only popular but also an acceptable r modern times too (Dutta et al., 2014). Phytochemical screening leads to identification of various compounds and its procedure includes extraction, screening and identification of the compounc phytochemical screening, it is possible to locate the various secondary metabolites as well as its concentration in the plant. In addition to the morphological features, both anatomical and phyto authentication of various taxa, and are also extensively used to in taxonomical identification and classification which in turn helps in avoiding adulterations in the medicinal and aromatic plants. D materials, in the preparation of various herbal formulations, pharmaceutical industries are generally using adulterants. In this context, the anatomical and phytochemical studies on medicinal plants are highly useful to identify such adulterations in various herbal formulations (Wafaa, 2005). In India Euphorbiaceae is the 7 th largest family. Because includes a lot of plants having economic importance. The systematic studies of Euphorbiaceae have been less thoroughly carried out. The family Euphorbiaceae due to diverse selection pressur different habitats from arid regions to wet humid tropics have developed diverse growth forms from stunted succulents to tall canopy trees. Moreover, this family is also characterized by high rat 2007). On the basis of scientific classification, the species Croton included the family Euphorbiaceae, subfamily Crotonoideae. The tribe Crotoneae contains five genus such as Astraea, Brasilioc and Paracroton. Croton (Euphorbiaceae) is one of the largest genera of flowering plant of family Euphorbiaceae consists of herbs, shrubs, trees, and occasionally lianas that are ecologically pron secondary vegetation in the tropics and subtropics worldwide (Webster, 1993; Govaerts et al., 2000). Croton species has a wide range of medicinal uses, such as, for the treatment of cancer, col hypercholesterolemia, hypertension, inflammation, pain, malaria and ulcers (Garcia-Diaz et al., 2016). Many of the metabolites like terpenoids, essential oils, flavonoids and tannins, alkaloids, phe tannins), saponins, triterpenes and sterols were qualitatively detected (Garcia-Diaz et al., 2016). The initial identification of a plant species can be done with the help of easily observable morphol scientific methods like phytochemical analysis can assist our identification. The first step of the phytochemical screening is to determine qualitatively the main groups of chemical constituents p species. This analysis can lead to the subsequent extraction for the isolation of groups of interest. This would also give an indication to discover certain phytochemical compounds which are hav properties (Mirabean et al., 2013).

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CHAPTER 3 MATERIALS AND METHODS 3.1. AREA OF STUDY 3.1.1. SOUTHERN PENINSULAR INDIA South India includes, states of Kerala, Tamil Nadu, Karnataka, Andra Pradesh and Union territories of Pondicherry and Mahe (Fig.1). It covers an area of 4,67,186 sq K.M. and is bounded on the north by Maharashtra, Madhya Pradesh and Orissa, on the east by the Bay of Bengal, on the south by Indian ocean and on the west by Arabian sea. The study area can be subdivided in to two floristic regions, Malabar and the Deccan. Malabar is a long and narrow strip of land running parallel to the coast of Arabian Sea (Malabar Coast). West of the western ghat, this region is floristically rich and includes coastal plains and series of hill ranges of the western ghat traversing the states of Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. The more or less continuous hill ranges have a major discontinuity in the Palghat gap separately in the Nilgiri ranges from the Anamalais. The Ghat descends steeply in the west facing the Arabian Sea where they merge gradually through a series of hill with the Deccan Plateau in the east. Anamalai is the highest peak (2695m) south of the Himalayas. Of the 15000 species each of flowering plant estimated to occur in India, nearly 4000 species are found in the Western Ghat (Manickam & Indrajayaraj, 1992). It supports tropical moist evergreen forests and moist deciduous forests on the western slopes. The Deccan is the great table land of the south Indian peninsula, stretching from the Aravalis, Malwa, the Vindhyas, the Satpura and Chota Nagpur hills in the North, almost right down to Kanyakumari in the South. The western and Eastern Ghat flank it on either side. The northern part of the Plateau slopes west-wards while the south part slopes towards the south east. It is replaced by drought-resistant species and thorny shrubs. The eastern coastal pairs of the Deccan Plateau is road strip running parallel to the coast of Bay of Bengal (Coromandal Coast) and gradually rising from it. It consists of fertile coastal pair mainly formed by the deltas of the Kaveri in Tamil Nadu, Godaveri and Krishna in Andra Pradesh and a number of small rivulets and streams

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CHAPTER 4 RESULTS AND DISCUSSION 4.1. MORPHOLOGICAL CHARACTERIZATION OF SELECTED SPECIES OF CROTON 4.1.1. General characteristics of Family Euphorbiaceae Monoecious or dioecious, herbs (erect or prostrate), shrubs (erect of straggling), climbers or trees, armed or unarmed; branchlets terete or angular, often grained, compressed or simple, often zigzag, glabrous or pubescent or stellate tomentose; stems woody or succulent, often with acrid milky latex. Leaves alternate or distichous, entire or palmately lobed, opposite or alternate or opposite and alternate (rarely whorled), chartaceous or coriaceous or membranous or fleshy, persistent or caducous; entire or serrate, crenate, dentate at margin often glandular, penninerved or palmatinerved, glandular at base or eglandular; petioles present or absent; stipules present or absent, interpetiolar or intrapetiolar, persistent or deciduous. Inflorescence axillary, terminal or subterminal, simple or branched spikes, racemes or umbels, often pedunculate, unisexual or bisexual. Flowers actinomorphic, solitary or in glomerules, grouped into spiciform or capitate thyrses or in cyathia (pseudanthia), bracteate or ebracteate, often bracteolate, pedicellate. Perianth simple, usually calycine, rarely petaloid, sometimes petals also present, lobes free or connate, valvate or imbricate, or both, rarely reduced or even absent, caduceus or accrescent and sometimes enlarged in fruit. Disk-glands usually annular, entire or lobed or pulvinate or of free scales or absent. Stamens 1-many, free or connate, sometimes raised on a column, in 1 or 2 series, hypogynous; filaments equal or unequal; anthers mostly 2-celled, rarely 4-celled, parallel or divergent, basifixed or dorsifixed, simple or versatile. connective present, rarely absent, broad, narrow, or truncate, longitudinally or transversely dehiscent; pistillode central, columnar or absent. Ovary superior or half-inferior, usually 3 or 4 - 12-locular; ovules 1 or 2 per locule; placentation axile or pendulous or laterally pendulous; styles 3 or as many as locules, free or connate or halfunited, deeply or slightly bifid, rarely multifid, smooth or papillate, plumose or laciniate, erect or spreading. Fruits capsule, drupe or berry, globose or subglobose or

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL STUDIES ON SELECTED SPECIES OF *CROTON* L. (EUPHORBIACEAE) IN SOUTHERN PENINSULAR INDIA"** is an authentic record of the original research work accomplished by **Ms. Bhavana.R** under my supervision and guidance at the Centre for Post Graduate Studies and Research in Botany, St. Joseph's College (Autonomous) Devagiri, Calicut, Kerala and that no part of this thesis has been published earlier for the award of any other degree or diploma. Also certified that the contents in the thesis are subjected to **Plagiarism Check** using the software **Ouriginal** and that no text or data is reproduced from other's work.

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DECLARATION

I, **Bhavana R.,** do hereby declare that this Ph.D. thesis entitled **"MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL STUDIES ON SELECTED SPECIES OF *CROTON* L. (EUPHORBIACEAE) IN SOUTHERN PENINSULAR INDIA"** is the summary of the research work carried out by me under the supervision of **Dr. Binu Thomas,** Assistant Professor, Department of Botany, St. Joseph's College (Autonomous) Devagiri, Calicut, Kerala in partial fulfilment of the requirement for the award of the **Degree of Doctor of Philosophy in Botany** under the faculty of **Science** of the **University of Calicut**. I also declare that no part of this thesis has been submitted by me for the award of any other degree or diploma, and it represents original work done by me.

St. Joseph's College, Devagiri

Bhavana R.

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CHAPTER 1
INTRODUCTION

Medicinal properties of plants and plant products have a great role in the development of human cultures around the whole world. Secondary metabolites which are produced by the plants are augmented with a wide range of medicinal applications (Krishnaraju *et al*., 2005). From a long time, herbal remedies continue to play a vital role in the preventive medicine. Several compounds have been isolated from natural or plant products that are helpful for human healthcare (Dutta *et al*., 2014). Isolation and characterization of pharmacologically active compounds from medicinal plants continue so far in a view to search better conservative medicines and cure for various health disorders in humans. (Shantabi *et al*., 2017).

Nowadays, the traditional medicine all over the world is evaluated by comprehensive activities of research on different taxon consumed by the ethnic populations of different parts of the world and their therapeutic principles. Based on indigenous theory, herbal medicines are belief and experiences that are passed down generations (Mohanasundari *et al*., 2007). Many of the properties of herbal medicines such as, effectiveness, accessibility, low cost and comparative freedom from serious side effects, makes herbal medicine not only popular but also an acceptable mode of treating diseases even in modern times too (Dutta *et al*., 2014). Phytochemical screening leads to identification of various compounds and its procedure includes extraction, screening and identification of the compounds present in the plants. By means of phytochemical screening it is possible to locate the various secondary metabolites as well as its concentration in the plant.

In addition to the morphological features, both anatomical and phytochemical analysis are used for the authentication of various taxa and are also extensively used to in taxonomical identification and classification which in turn helps in avoiding adulterations in the medicinal and aromatic plants. Due to the lack of original source plant materials, in the preparation of various herbal formulations, pharmaceutical industries are generally using adulterants. In this context, the anatomical and phytochemical studies on medicinal plants are highly useful to identify such adulterations in various herbal formulations. (Wafaa, 2005).

In India Euphorbiaceae is the 7th largest family. Because of their diversity, the Euphorbiaceae includes a lot of plants having economic importance. The systematic studies of Euphorbiaceae have been less thoroughly carried out. The family Euphorbiaceae due to diverse selection pressures caused by their occurrence in different habitats from arid regions to wet humid tropics have developed diverse growth forms from stunted succulents to tall canopy trees. Moreover, this family is also characterized by high rate of endemism (Preetha & Binojkumar 2007).

On the basis of scientific classification, the species *Croton* included the family Euphorbiaceae, subfamily Crotonoideae. The tribe Crotoneae contains five genus such as *Astraea, Brasiliocroton, Croton, Mildbradia, Moacroton* and *Paracroton. Croton* (Euphorbiaceae) is one of the largest genera of flowering plant of family Euphorbiaceae consists of herbs, shrubs, trees, and occasionally lianas that are ecologically prominent and important elements of secondary vegetation in the tropics and subtropics worldwide (Webster, 1993; Govaerts *et al.,* 2000). *Croton* species has a wide range of medicinal uses, such as, for the treatment of cancer, colds, digestive issues, diabetes, hypercholesterolemia, hypertension, inflammation, pain, malaria and ulcers (Garcia-Diaz *et al*., 2016). Many of the metabolites like terpenoids, essential

oils, flavonoids and tannins, alkaloids, phenolic compounds (flavonoids and tannins), saponins, triterpenes and sterols were qualitatively detected (Garcia-Diaz *et al.,* 2016).

The initial identification of a plant species can be done with the help of easily observable morphological and anatomical studies. More scientific methods like phytochemical analysis can assist our identification. The first step of the phytochemical screening is to determine qualitatively the main groups of chemical constituents present in various parts of a plant species. This analysis can lead to the subsequent extraction for the isolation of groups of interest. This would also give an indication to discover certain phytochemical compounds which are having potential pharmacogonostic properties (Mirabean *et al*., 2013).

The genus *Croton* was established with a brief description of male and female flowers, fruits and seeds. In India, *Croton* consists of 16 taxa, of which 12 species from Southern Peninsular India. Five species namely *C. gibsonianus* Nimmo, *C. lawianus* Nimmo, *C. malabaricus* Bedd. *C. klotzschianus* (Weight) Thwaites and *C. scabiosus* Bedd. are endemic. *C. bonplandianum* Baill. And *C. hirtus* L. are introduced taxa, now naturalized, and frequently occur as weeds (Balakrishnan & Chakrabarty, 2007). *Croton* can be distinguished from its allied Paracroton by its non-appendage calyx lobes, female flowers devoid of petals, and buds with apically inflexed staminal filaments (Balakrishnan & Chakrabarty, 2007). According to the observation of many workers, the Genus *Croton* shows the existence of a wide range of morphological variations within the populations as well as the closely related species. The number of stamens are variable within a species and therefore not dependable. The features of female flowers are of great characteristic value (Chakrabarty& Balakrishnan, 1997).

Anatomical studies of taxa belonging to the family Euphorbiaceae were done to understand the foliar structural details in unwinding the taxonomical disputes if any. There is a range of characters which varies between genera and species. The presence of single, double and multiple layer of epidermis, palisade parenchyma, spongy parenchyma and other special tissues and storage organs are of taxonomic interest. The tribe Crotoneae shows generally, amphistomatic condition, In *Croton*, paracytic type stomata present. A mixture of these characters may be used to identify the specific species (Elumalai *et al.*, 2014).

Plant extracts and essential oils are the best sources of phytochemicals (Sun *et al*, 2015). They are provided with different phytochemical molecules such as terpenoids, phenolic acids, vitamins, lignin, stilbenes, tannins, amines, betalains, flavonoids, quinones, coumarins, alkaloids and other metabolites (Kumar & Deenadayalan, 2017).

Plants produce thousand types of chemicals. Some of the organic compounds like carbohydrates, fats, proteins, nucleic acids, chlorophylls, etc. required for their basic metabolic processes and present throughout the plant kingdom. These organic compounds are called primary metabolites or biomolecules. Many plants, fungi and microbes of certain genera and families synthesize a number of organic compounds which are not involved in primary metabolism and seem to have no direct function in growth and development of plants. Such compounds are called secondary metabolites, these compounds are produced small quantities and their extraction from the plants are costly. They accumulate in few amount only in specific parts of plants. These are derivatives of primary metabolites (Pretorius & Watt, 2001).

The Euphorbiaceae contain extraordinary diversity of organic compounds, possibly more than in any other plant family (Webster, 1968). Naturally, the genus *Croton* displays notable chemical importance. Species of

Croton contain a great diversity of chemical compounds, *viz*. several kinds of alkaloids, saponins, tannins and flavonoids. A number of non-alkaloid substances have also been reported in various species (Chakrabarty& Balakrishnan, 1997). .*Croton bonplandianum* Baill. (Euphorbiaceae), it is a rich bio resource in folk medicine and traditional Ayurvedic medicine as well as the pharmaceutical industry as an element of modern synthetic drugs. It is a native of South America and was first reported in India during the late 1890's (Kaul, 1967). It has many uses and actions. Its leaf juice is used to cure cough. Seed paste is applied locally on to cure eczema and ringworm. Latex is used to heal cuts and wounds. (Sivagnanam & Valliammai, 2016). *Croton caudatus* is a significant species in Dai folk medicine in the Indian traditional medicinal system, Ayurveda, recorded the medicinal properties of many a members of Euphorbiaceae.*C. persimilis.* Mull.Arg. and *Croton tiglium* have been used as the purgative for asthma, tumours, convulsions, rheumatism, and cancer. The stems and leaves of *C. caudatus,* which have been used for the treatment of malaria, ardent fever, convulsions, rheumatic arthritis and numbness (Zou *et al,*.2010).

Croton hirtus is native to tropical America. In Kerala *Croton hirtus* is distributed in Alappuzha, Thrissur, Palakkad, Kozhikode, Ernakulam, Kollam and Malappuram. In India, it was first reported from Tirunelveli Hills of Western Ghats (Ramachandran *et al*., 1992). Later it is reported to be common throughout the coastal regions of Kerala (Preetha & Binojkumar, 2007). *C. hirtus* have many bioactive secondary metabolites which includes alkaloids, tannins, flavonoids, steroids, phenols, glycosides, terpenoids, anthroquinone and saponins (Daouda *et al*, 2014).

Croton tiglium (Nirvalam) having a medicinal property, which used as the remedy for constipation, dyspepsia, dysenteries, gastrointestinal disorders, intestinal inflammation, rheumatism, peptic ulcer, visceral pain

and headache. During chromatographic analysis of *Croton tiglium* and *Jatropha curcas* seeds, the different peaks obtained by the quantitative analysis of protein in the seeds. Peak I and II from *Croton* and peak I from *Jatropha* were poisonous to mice.*C. tiglium* seed oil consists of phorbol esters and crotonic acid along with the fatty acids. *C. tiglium* and *Croton tiglium* are widely used in siddha formulation like soolaikudaram, Agasthiyarkuzhambu, Thazhamboo Mathirai, MeganathaKuligai, etc. *Croton* seeds undergo purification process, they are used for abdominal disorders constipation and dyspepsia. (Kanimozhi *et al*., 2017).

Croton persimilis Mull.Arg. is native from West Bengal, India. (Chakrabarty & Balakrishnan, 1997). *Croton persimilis* Mull.Arg. (Euphorbiaceae) has been used as a medicine for dyspepsia and dysentery. (Pudhom *et al.,* 2007). *C. persimilis* in combination with *C. sublyratus* have been used to treat gastric ulcers and gastric cancers (Wijesekera, 2017).

In Ayurvedic pharmaceutics, Jayapala (*Croton tiglium* Linn.), Therapeutic utility of Jayapala has been increased with advent of Ayurveda as a drastic purgative. Seed of Jayapala is a well-known virechaka (purgative) and is being administered for quick action in the form of Gulika (tablet) and Churṇa (powder) to treat various diseases. *C.tiglium* has a great role in toxicology, it is used to treat against poisonous bites (Vekariya *et al*., 2019).

In Ayurveda, siddha and Unani (ASU) system of medicinal plants, minerals, and animal products are used as main drugs to cure various ailments. There is a global renewal in the use of these medicines along with a progressing scientific interest in them as a source of new drugs. There has been a boom in the usage of ASU drugs and export is appreciably high in the last two decades. The usage of plants as medicines goes back to early treatment of wide variety of diseases.Due to the side effects of modern medicines, the herbal decoctions against human diseases are gaining

significance. The earliest mention of the medicinal use of plants is to be found in the Rig-Veda which dates back as early as 3500 BC. Currently about 80% of the world population depends on herbs, herbo-minerals and other plant-derived medicine for the first line of primary health care for human alleviation because it has less side effect. (Kanimozhi *et al*., 2017).

The present study is mainly focused on morphological, anatomical and phytochemical characterization of selected species of *Croton* to reveal more insights to its importance in the field of taxonomy and phytochemistry. In this context, In addition to the morphological features, the anatomical and phytochemical characterization can validate its authenticity and medico-potentiality of such potential plants, which are used for further pharmacological analysis. It may further leads to the discovery of active principle behind such plants and prospective to new drugs in future.

1.2. OBJECTIVES OF THE PRESENT STUDY

- To develop morphological parameters for the taxonomic identification of selected species of *Croton.*
- To develop anatomical markers for the correct identification of selected species of *Croton.*
- To compare preliminary phytochemical characteristics of different spp. of *Croton.*
- To develop chemical fingerprints of different spp. of *Croton* using chromatographic techniques.

CHAPTER 2
REVIEW OF LITERATURE

2.1 MORPHOLOGICAL STUDIES

A revision of *Croton* L. (Euphorbiaceae) of Indian subcontinent was given *by* Chakrabarty & Balakrishnan (1997). According to their observation, the genus *Croton* was well established. The generic name is derived from the word Greek *kroton* (which means, a tick, indicate to the affinity of the seed of *Ricinus*). Croziat (1942) states that, "While sincerely observing species of *Croton,* which are closely similar in their foliage turn out to have distinct female flowers and capsules". Airyshaw (1980) even warns that, "Specific characters in *Croton* are mostly connected and often – complicated. Therefore difficulty to name specimens without comparing scientifically named material"

In the study of taxonomic and phylogenetic assessment of the Euphorbiaceae by Thakur & Patil (2011), noticed that, the family Euphorbiaceae, exhibit taxonomic and phylogenetic variations and it lacks anatomical similarities, probably they are varying in habit and habitat.

Systematic distribution of foliar trichomes types in *Croton* (Euphorbiaceae) was evaluated by Webster *et al*. (1996). He reviewed that, diversity in foliar trichomes in *Croton,* and the terminology interpreted by descriptions and illustrations of terms. Evolution of trichomes types started from branched (stellate or fasciculate) hairs to simple and dendritic ones.

Pollen morphology of *Croton* sect. *Lamprocroton* (Müll. Arg.) Pax (Euphorbiaceae) and its taxonomic implications by Ribes de Lima *et al.* (2007), palynology of *Croton* tie up with taxonomic charecters, which leads to

identification of species, when the macro morphological features included in the key to the species, which makes easy to interpret a new taxon.

A revised infrageneric classification and molecular phylogeny of New World *Croton* (Euphorbiaceae) by van Ee *et al*. (2011). He reported that *Croton* (Euphorbiaceae) is a varying group of plants that is large number of species in the tropics. They revised the infrageneric typification of the species of *Croton* with new information from phylogenetic studies of DNA sequence data from the genomes. The connectivity of species that were once placed in a complicated positions by nuclear and chloroplast data, such as *C. cupreatus, C. poecilanthus* and *C. setiger*. They also allow the morphological descriptions and a key to the species.

Presence of Colleters on the Inflorescence axis of *Croton glandulosus* (Euphorbiaceae) structural and functional characters was studied by Machado *et al*, (2015). They reported that, most of the variations in the morphological structures with divergent distribution patterns has been ascribe to *Croton* species, including extra floral necteries in the petiole and leaf margins, floral necteries, idioblasts of lipophilic substances and laticifers. Glands structurally identical to the colleters, described in leaves of some genera of Euphorbiaceae including *Croton*.

Foliar secretory structures in *Crotoneae* (Euphorbiaceae), diversity, anatomy and evolutionary significance was studied by Vitarelli *et al.* (2015). Their studies revealed that, the secretory structures could be the area of synthesis and accumulation of metabolites with abundant of phytochemical and pharmacological value. These structures would be the major characters in many plants – animal ecological interactions.

Morpho-anatomical and histological characters in the systematics of the *Croton* species (Euphorbiaceae: *Crotonoideae*) in Southern Nigeria by

Nichodemus & Ekeke (2021), He studied on the taxonomic characters of plants with respect to morphology, anatomy and histology are well recorded. The importance of such data helps to understanding the phylogenetic connections and delimitation of species are highly important in plant taxonomy. This work was aimed to give more distinguishing features necessary for the delimitation of *Croton* species.

2.2 ANATOMICAL CHARACTERIZATION

Foliar anatomical studies of some taxa of Euphorbiaceae by Elumalai *et al.* (2014), reported that, foliar anatomical studies of fifteen taxa belonging to fifteen genera of Euphorbiaceae were done to analyse the foliar structural data transforming taxonomical dispute. The features which varies between genera and species. The presence of anatomical features would be significant in taxonomic studies. A combination of these characters are used for the key to the species.

Foliar secretory structures in *Crotoneae* (Euphorbiaceae) was studied by Vitarelli *et al.* (2015), they suggested that, indumentum is the most important character of *Croton*. The characteristic feature of trichomes depends up on its distribution, position and its topology. Molecular phylogenetics has an important role in the study of evolutionary pathway of *Croton* and other *Crotoneae*

Wood microstructure studies of indigenous species of spurge family Euphorbiaceae by Jangid, (2016), they reported that, the foliar epidermis and stomata of some genera of Euphorbiaceae have a great importance in the study of taxonomy. The anatomical features of wood of Euphorbiaceae, will be form an identification keys to analyses various genera under diverse climatic conditions.

2.3 PHYTOCHEMICAL ANALYSIS

Studies of Salatino *et al., (*2007) revealed that, *Croton* consists of some of the phytochemicals, clerodanes trans-dehydrocrotonin, including hypolipidemic, hypoglycaemic, antioestrogen and anti-cancer properties. Cytotoxic effects also have been observed in tests with alkaloids, secokaurene, labdane and cembranoid diterpenes etc. presented other properties of *Croton* elements have been recorded, comprising anti-hypertensive, anti-inflammatory, antimalarial, antimicrobial, and antispasmodic, antiulcer, antiviral and myorelaxant.

Anticancer and antioxidant activity of *Croton* species was studied by Nath *et al.* (2013). According to them phytochemicals present in the genus *Croton* are showing diversity. Terpenoids are the most important secondary metabolite, mainly diterpenoids, which belongs to the cembranoid, clerodanes, neoclerodane, halimane, isopimarane, kauranes, secokaurane, labdane, and phorbol and trachylobanes skeletal types. Triterpenoids, either pentacyclic or steroidal, have frequently been documented for *Croton* species. Volatile oils consists of mono and sesquiterpenoids. Some of the phytoconstituent present in different species of *Croton* includes volatile oil including eugenol, vanillin, crotsparinine, crotoflorine, oblongi-foliol, triterpenic acid, sparsiflorine, dotricontamol, b-amyrin and b-sitosterol.

Peralta *et al.* (2015). reported *Croton* is a broad genus of Euphorbiaceae and many species have medicinal value. They achieved experiments to interpret the flavonoid composition and the antioxidant potential of hydro alcoholic extracts of nine Argentinian species of *Croton*, to observe the alcoholic extracts of leaves by high-performance liquid chromatography and mass spectrometry. The antioxidant activity was studied by the 1, 1-diphenyl-2- picryl-hidrasil, Sigma (DPPH), ferric reducing antioxidant potential (FRAP) and Folin–Ciocalteu methods. Similarly flavonols (kaempferol, quercetin, and

isorhamnetin) and flavones (apigenin) were also studied in the samples. The phytochemical compounds like rutin was frequent and predominant in *Adenophylli* section. Acylglycosides of either quercetin or kaempferol (tiliroside) were detected in most samples.

In vitro antioxidant activity and phytochemical screening of *Croton zambesicus* was done by Abdalaziz *et al.* (2016). The antioxidant activities were conducted through DPPH radical scavenging assay on ethanolic extract. The given results of phytochemical screening exists the presence of Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpenes, Phytosterol, Anthraquinones and Carbohydrates.

Pharmacognostic and phytochemical studies on leaf extracts of *Croton linearis* Jacq. Conducted by Garcia-Diaz *et al*. (2016). They reported that ethanolic extracts of *C. linearis* give rise to large variation of metabolites such as alkaloids, triterpenes and carbohydrates, reducing sugars, amino acids, flavonoids and tannins.

Kumar & Deenadayalan, (2017) studied on *Croton macrostachyus* root extract to analyze for inorganic elements, proximate analysis, qualitative and quantitative characterization of phytochemicals, antioxidant activities and free radical scavenging activity. The phytochemical studies revealed that the presence of carbohydrates, reducing sugars, phenolics, flavonoids, glycosides, saponins and steroids. Quantitative characterization of phenols, flavonoids and Triterpenoids was further performed, and also inorganic elements such as iron, chloride and sulphate were found by total ash analysis.

The phytochemical extraction, isolation and identification of bioactive compounds from medicinal plant extracts were studied by Altemimi *et.al*, (2017). Their studies reported that, Plants are capable to produce a huge amount of phytochemicals. A huge concentrations of phytochemicals

accumulated in leaf, stem and root, which may secure against free radical damage. Plants consists of valuable phytoconstituent leads to progress the human body like natural antioxidants. Major studies have given that plants are the treasure of antioxidants, and also vitamins A, C, E, and phenolic compounds such as flavonoids, tannins and lignin found in plants, all can act as antioxidants.

Review on chemical constituents from *Croton* species and their biological activities were done by Xu *et al*. (2018). This review mainly give attention on the chemical constituents from *Croton* species and their biological activities, a total of 399 new compounds, were documented. Diterpenoids are characteristic element of the *Croton* species. These isolated compounds are introduced to a large spectrum of bioactivities, including cytotoxic, anti-inflammatory, antifungal, and acetylcholine esterase.

Phytochemical screening and evaluation of antibacterial activities of *Croton macrostachyus* stem bark extracts was done by Kiristos *et al.* (2018). Their results given that the ethanolic extracts of *C. macrostachyus* stem bark presented the importance of antibacterial activity. The phytochemical screening of the methanolic and ethanolic extracts of *C. macrostachyus* specified the presence of alkaloids, tannins, saponins, terpenoids and steroids while flavonoids were present only in the ethanolic extract.

Van Ee *et al*. (2015). Studied the molecular phylogeny of genus *Croton* L. (Euphorbiaceae) of Australia. The molecular phylogenetic results hold up the identification of six sections, to account for the 29 native Australian species. The monophyly of each of these sections was mainly helping in the Bayesian and maximum-likelihood studies of nuclear ITS and plastid *trn*L–F DNA sequences, likewise, their relationships to each other and to other groups were less well determined. *Croton* may constitute one, two or three

separate forms to Australia, with the help of hypotheses of equal dispersals from Australia to Pacific islands and to Asia.

De *et al.,* (2018) reported that the extract of plants and plant products derived some compounds give information to use as chemo preventive agents against various types of cancer. Recognition of phytochemicals are found to be an important chemo preventive agent in the area of cancer research. Pharmacological and clinical investigations of medicinal plants have given that a large diversity of natural compounds possessing an important cytotoxic as well as chemo preventive activity. The *In-vitro* cytotoxic activity of the methanolic extract of the leaves of *Croton caudatus* Geiseler on HeLa cells (human cervical cancer cell lines) and it further suggested for screening and isolation of natural active cytotoxic components.

2.3.1. *Croton aromaticus* L.

Assessment of phytochemicals and antifungal effects of *Croton aromaticus* against post-harvest fungal pathogens isolated from tropical fruits by Wijesundara *et al*. (2016). Plant extracts which are high in antimicrobial secondary metabolites such as terpenoids, alkaloids, saponins and flavonoids could be allowed to possible for synthetic fungicides. Present study was mainly studied on analysing the antifungal effect of ethanolic extract of *Croton aromaticus* (Kappettiya) leaves *in vitro* against mycelial growth and the spore germination of post-harvest fungal pathogens isolated from fruits of banana.

2.3.2. *Croton bonplandianum* Bail.

Evaluation of genotoxic and antimicrobial potential of *Croton bonplandianum* Baill. was conducted by Manjit *et al*. (2010). In which aqueous, acetone and methanolic leaf and fruit extracts of *Croton bonplandianum* Baill. were analyzed for genotoxicity and antimicrobial activity. Use of *Allium* roots analyzed that all the extracts were a bit mito depressive in nature. The

antimicrobial studies suggested that methanolic extract of leaf and fruit of *Croton bonplandianum* is more successful against tested microbes than aqueous and acetone extracts. The methanolic extract present maximum activity against gram positive bacteria and acetone extract of leaves showed maximum activity against gram negative bacteria.

Phytochemical investigation and correlation study of *Croton bonplandianum* Baill. stem was done by Dutta *et al.* (2014). During their study various standard biochemical and spectrophotometric methods were employed. To analyse the phytochemical profiles of *C. bonplandianum* stem given that, the presence of alkaloids, flavanoids and phenolic compounds. In according to that, total riboflavin and ascorbic acid were present to be large quantity than thiamine content. These phytochemicals are majorly responsible for ethanomedicinal properties of a plant. Therefore, the presence of large quantity of phytocompounds showed that huge capacity of medicinal value of *C. bonplandianum* stem.

Studies on some phytochemical aspects like antioxidant, protease, catalase and peroxidase activities of *Croton bonplandianum* (Euphorbiaceae) was conducted by Rao& Raju, (2018). According to them, *Croton bonplandianum* is a one of the exotic weed and largely present in wastelands and it is often known as 'bantulasi'. Due to the large accessibility and pharmacological values, the study of the in-vitro antioxidant, protease, and catalase and peroxidase activities, resulted that the ethanomedicinal impact of *C. bonplandianum*.

Traditional uses, phytochemistry and pharmacological value of *Croton bonplandianum* Baill. were studied by Tripathy *et al*. (2017). They reported that, various parts of plants are largely used in ethanomedicinal properties like, hepatoprotective, swelling of the body, used against ring worms and skin disease, wound healing, antifungal, antimicrobial, antidiabetic, antitumor,

repellent property against insects, nematicide, anticoronary, anti-inflammatory, antihelmentic etc.

The preliminary phytochemical analysis of various extracts of *Croton bonplandianu*m was done by Selvakumar & Kumar, (2017). They reported that phytochemical screening of aqueous methanolic and ethanolic extracts were said to be the presence of Flavonoids, Tannins, Tri-terpenoids, Alkaloids, Triple sugars, cardiac glycosides and Steroids etc.

A review on traditional and pharmacologicaluses of *Croton bonplandianum* with special reference to phytochemical aspects by Ghosh *et al.* (2018). They observed that, the seed of *Croton bonplandianum* consists of diterpines, phorbolester, including 12- orthotrideconeoly-phorbol-13-acetate (TPA) and Myristoyl Phorbol Acetate (MPA). TPA having the property of carcinogen, affecting prostaglandin metabolism. The fresh plant extract used as the remedy of headache by ethnic groups. The latex having the healing effect on wounds and cuts. The study based up on the various types of phytochemicals, which are tannin, phlobatannin, terpenoid, glycoside, phenolic, flavonoid, steroid, anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein for sincere analysis regarding the phytochemical range of the stem.

2.3.3. *Croton caudatus* Geisel.

Studies on ethanolic extract of the stem-bark of *Croton caudatus* (Euphorbiaceae) leads to the isolation of a new furanoid norditerpene named Isocrotocaudin. Its spectral properties and chemical correlation with crotocaudin, helped in the elucidation of the stereochemistry and structure of it (Chatterjee *et al.* 1978).

A novel sesquiterpene, crocaudatol along with a known one, oplopanone (2) was isolated from the stems of *Croton caudatus* Geisel. *var. tomentosus* Hook. was studied by Yuan & Zhong-Mei (2008).

A new flavone, named crotoncaudatin, from the stems of *Croton caudatus* Geisel. var. *tomentosus* Hook., was identified by Guo-An Zou *et al.* (2010), along with their identification they elucidated their structures with the help of spectroscopic methods. They reported that all compounds were isolated and identified from this species for the first time and compounds 1-6 are new for the genus *Croton*.

According to the studies of Singha *et al.* (2011),the crude and solvent benzene and ethyl acetate extracts of two plants, *C. caudatus* (fruits) and *T. acuminata* (flowers) were analysed to study the synergistic effect against filarial vector *C. quinquefasciatus* larvae with gradually increasing concentration of crude extract.

Antioxidant and antimicrobial activity of *Croton caudatus* was studied by Lokendrajith *et al.* (2012), in their study they have taken different extracts of the leaves of *Croton caudatus* Geisel and evaluated for their antioxidant and antibacterial activity against human pathogenic gram positive and gram negative bacteria and also they have tested antifungal activity too. All the extracts exhibited different levels of antibacterial and antifungal activity against all the tested pathogens.

Review on anticancer and antioxidant activity of *Croton* was done by Rumki Nath *et al,* (2013), they said that *Croton caudatus* has curative medicinal properties for types of diseases like cancer, malaria, diabetes and indigestion. Leaves are asserted to have anti cancerous property. Phytochemical screening of the leaves ethanolic extract of the leaves of *Croton caudatus* disclosed the presence of, cyanogenetic glycosides, alkaloids, flavonoids and phenolic

compounds. Ethanolic extract of the *Croton caudatus* (leaves) has antioxidant activity, giving the idea that the leaves of *Croton caudatus* are very good source of natural antioxidant.

Zou *et al.* (2013), isolated a variety of compounds from *C. caudatus* var. *tomentosus* and the compounds were phenolic compounds flavonoids, sesquiterpenes, fatty acids, steroids etc. In their study they also isolated 5 ligans and described their structures as well. These compounds were first reported by them in the genus *Croton*.According to their observation *C. caudatus var. tomentosus* is commonly used for curing various diseases such as malaria, rheumatic arthritis, convulsions, and numbness.

Antioxidant activity of *Croton caudatus* was evaluated by Shantabi *et al*., (2014). Shade dried leaves are powdered and sequentially extracted in three different solvents viz. chloroform, ethanol and water. The antioxidant activity of these extracts was evaluated by their ability to inhibit the generation of DPPH, hydroxyl (OH), superoxide (O_2) and nitric oxide (NO) and FRAP free radicals in vitro. In association with this, they also estimated total flavonoid and the total phenol contents to understand their potential in free radical scavenging. Finally they concluded chloroform, ethanol, and aqueous extracts of *Croton caudatus* showed a concentration dependent inhibition in DPPH, OH, O_2, and NO and FRAP free radicals.

Dey *et al.* (2015) reported the active extract of *Croton caudatus* var. *tomentosus* Hook has effective medicinal power against the parasitic protozoans *in vitro* and *in vivo*. They also reported that these are used by Chakma and Hmar community, the local tribes of North-East India for medicinal and veterinary purposes.

Phytochemical basis for the medicinal uses *Croton caudatus* Geiseler was provided by Shantabi & Jagetia (2015). It is a commonly used plant

mainly used in the northeast region of India to treat several human diseases. The petroleum ether, chloroform, ethyl alcohol and water extracts showed the presence of alkaloids, phytosterols, saponins, phlobatannins, cardiac glycosides, flavonoids, phenolics and terpenoids in the leaves. The TLC profiling revealed presence of different chemical constituents indicated by different Rf values of various TLC spots in numerous solvent systems.

HPLC profiling and microscopic analysis on *Croton caudatus* Geiseler leaves by Shantabi *et al.* (2017). helped in standardizing correct sample identification. The detail microscopic analysis of powder revealed the presence of non-glandular trichome, covering trichome, starch grain, calcium oxalate crystals and stone cells. Powdered drug, treated with different chemicals and its extracts with different solvents showed colour changes when illuminated with UV light. HPLC profiling showed the presence of various phytochemicals.

Ayam *et al.* (2017), they studied the utility of certain medicinal plants on the missing tribe of Dhemaji district of Assam, India. By this study they collected 62 plant species belonging to 38 families that are being used to cure different diseases and ailments by the tribes. These plants were used in the treatment of approximately 56 ailments. Among these *C. caudatus* leaves are used to reduce inflammatory swellings.

Kumar & Kala (2018) they explored the reducing and capping potential of aqueous leaf extract from *Croton caudatus* Geisel for the synthesis of platinum nano particles. The reducing potential of *Croton caudatus* Geisel confirmed by FT - IR, XRD, SEM - EDX, and TEM

2.3.4 *Croton hirtus* L'Herit.

Phytochemical screening and antibacterial properties of *Croton hirtus* L'Her. plant against some important pathogenic bacteria was studied by

Subin & Reghu (2012). They demonstrated antimicrobial activity of different solvent extracts of *Croton hirtus* L'Her. against ten important bacterial strains. Phytochemical analysis and antimicrobial properties of shoot, root and whole plant extracts of *C. hirtus* were investigated separately in methanol, ethanol, chloroform, acetone and water extracts. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, steroids, phenols, glycosides, terpenoids, anthroquinone, saponins and cardiac glycosides. The methanolic extract exhibited higher and wider range of activity against majority of the test organisms than other kinds.

Daouda *et al.* (2014) studied the terpenes, antibacterial and modulatory antibiotic activity of essential oils from *Croton hirtus* L' Hérit. (Euphorbiaceae) from Ivory Coast. The chemical composition was determined by Gas Chromatography/Mass Spectrometry (GCMS). They reported that essential oil contained terpene derivatives like (15.55 % and 77.94%) monoterpenes and sesquiterpenes respectively. Hydrocarbons monoterpenes (14.79 %) were prevalent compared to oxygenated monoterpenes (0.76 %). Moreover among sesquiterpenes, the hydrocarbons species were also detected in a higher percentage (74.06 %) than oxygenated (3.88 %). Three major components found were (E)-caryophyllene (31.75 %), germacrene-D (22.57%) and α-humulène (7.42 %).

Studies on new diterpenoids from the roots of *Croton hirtus* (Euphorbiaceae) were conducted by Rosandy *et al*. (2017).The crude extracts obtained were analyzed using Thin Layer Chromatography (TLC) then fractionated via Vacuum Column Chromatography (VCC) and proceed the isolation using Radial Chromatography (RC) to get the pure compounds. The pure compounds obtained were elucidated by nuclear magnetic resonance (NMR), Fourier Transform Infrared (FT-IR) and mass spectroscopy to confirm their structures and from that analysis, the compounds were identified as

two new compounds naming (-)-5,8-dihydroxyjatrophan-3-one (1) and (+)-14,16,17-trihydroxykauran-1-on (2) with total weight 4.9 mg and 4.5 mg respectively.

2.3.5. *Croton persimilis* Mull.Arg.

Two new cembranoids, crotocembranoic acid (1) and neocrotocembranoic acid (2), were isolated by Roengsumran *et al*., (1998) from the stem bark of *C. persimilis*. The structures were established on the basis of spectroscopic analysis. Crotocembranoic acid was obtained from a hexane soluble crude extract from the stem bark of *C. persimilis* by Si gel column chromatography, eluting with hexane and ethyl acetate.

According to the studies of Roengsumran *et al.* (1999). Four new labdane diterpenoids, labda-7, 12(E), 14-triene, labda-7, 12(E), 14-triene-17-al, labda-7, 12(E), 14-triene-17-ol, labda7, 12(E), 14-triene-17-oic acid, were isolated from the stem bark of *C.persimilis*. The structure of these compounds was established by spectroscopic data and chemical transformation.

Roengsumran *et al.* (2001) isolated three labdane diterpenoids, 2-acetoxy-3-hydroxy-labda-8(17), 12(E)-14-triene, 3-acetoxy-2-hydroxy-labda-8(17), 12(E)-14-triene, and 2, 3-dihydroxy-labda-8(17), 12(E), 14-triene from stem bark of *C. persimilis* and the structures were established by spectroscopic data and each can also be tested for cytotoxicity against various human tumour cell lines. The latter compound showed non-specific, moderate, cytotoxicities against all the cell lines; whereas the first two compounds were less active.

A new furoclerodane, croblongifolin, together with one known clerodane, crovatin and one known labdane, nidorellol, were isolated from the stem bark of *C. persimilis* by Roengsumran *et al*. (2002). Structures were established based on spectroscopic and X-ray analysis. Croblongifolin

showed a significant cytotoxicity against various human tumour cell lines including HEP-G2, SW620, CHAGO, KATO3 and BT474.

Four novel furanocembranoids (1-4) were isolated from the stem bark of *C. persimilis* by Pudhom *et al*. (2007). The structures were elucidated on the basis of spectroscopic analysis, mainly NMR and MS. Compounds exhibited good cytotoxicity against several human tumour cell lines such as BT474 (human breast ductol carcinoma), CHAGO (human undifferentiated lung carcinoma), Hep-G2 (human liver hepatoblastoma), KATO-3 (human gastric carcinoma), and SW-620 (human colon adreno carcinoma).

Mandal and Bose, (2011) conducted the preliminary standardization of *C. persimilis* by pharmacognostic, morphological and microscopical investigations. They concluded that a good amount of tannin, flavonoid and a moderate amount of diterpenoids were present in this plant. Thus, *C. persimilis* may be an important herbal drug used to treat some challenging diseases to mankind in future life. Some important phytoconstituent and their amount which may act as marker compounds. Simultaneously, a preliminary standardization of this plant was performed by pharmacognostic, morphological and microscopical investigations. Here flavonoids, terpenoids and tannins were isolated and estimated to identify the marker compounds and different standardization parameters were also documented.

The properties of *C. persimilis* Müll.Arg. and *Antidesma puncticulatum* Miq. Interesting plants in the family Euphorbiaceae were investigated by Rattanapunya *et al*. (2021). The ethanolic plant extracts were established for phytochemicals screening, antioxidants activities, anticancer studies in five important human cancer cell lines (MCF-7, MDA-MB-231, HeLa, HepG2, and NCI-H187) and cytotoxic activity in normal cell line (hTERT-HME1). The leaf extracts of *C. persimilis* Mull. Arg. contained flavonoids, saponins, terpenoids

and tannins. They also noticed the total phenolic contents in selected plant extracts.

2.3.6. *Croton tiglium.* L.

The seperation, semi synthesis and structure derivation of a pure crystalline highly active tumour-enhancing principle from the seed of *Croton tiglium* L. Was reported by Arroyo & Holcomb, (1965) in their work 'Structural studies of an active principle from *Croton tiglium* L. It revealed that, the alkaline hydrolysis of the pure crystalline cocarcinogen called C-3 yielded myristic and acetic acids and polyhydroxy compound, $C_{20}H_{28}O_{6.}$ Crystalline derivatives of the active compound were prepared from extracts.

The poisonous activity of *Croton tiglium* seeds were studied by Hecker & Schmidt (1974), they reported that the seeds are toxic: 8 - 10 seeds may kill a dog and 15 seeds a horse; for human beings 4 seeds may be lethal. The seeds contain up to 20% of protein and 30 - 50% of lipids. Moreover Isoguanine-D-riboside (crotonoside) and saccharose were also isolated from the seeds.

Studies on the proteins *Croton tiglium* and *Jatropha curcas* by Stripe *et al*., (1975). Their ideas dedicated for the extraction of proteins from the seeds of *Croton tiglium* and *Jatropha curcas* and the three main peaks allocated (I, IIand III) by Sephadex chromatography. The basic protein from both seeds and and peak I from *Jatropha* peaks I and II from *Croton* were lethal to mice, to different extents.

El-Mekkawy *et al.* (2000) studied anti-HIV-1 phorbol esters from the seeds of *Croton tiglium*. Their studies revealed that, five phorbol diesters were derived from the methanolic extracts of the *Croton tiglium* seeds, and their structures were analysed by spectroscopic techniques and selective hydrolysis of acyl groups. The quantitative analysis of polyfunctional diterpene esters of the tigliane type in *Croton tiglium* studied by Glaser *et al*.,

(1988). They reported the resin and the seed oil of *C. tiglium* were usually used in traditional medicine as purgative, abortifacient and counter irritant. Nowadays, *C. tiglium* is still applied in homoeopathy and in combination with some kind of acupuncture, its main skin irritant component, 12-tetradecanoylphorbol 13-acetate (TPA), is used as a standard tumor promoter in mice in experimental cancer research.

Shahid *et al., (*2008) studied activity-guided isolation of a new protein from antifungal and antibacterial actions of *Croton tiglium.* Their studies motivated on the experiment directed based up on the purification of a new antimicrobial protein from the Croton *tiglium* seed was carried out by $(NH_4)_2SO_4$ precipitation, gel filtration and DEAE cellulose ion-exchange chromatography. SDS-polyacrylamide gel electrophoresis revealed that the purified protein was a monomer with molecular mass of 50 k Da. This is a first report on purification of a protein from *Croton tiglium*, which possesses a strong and broad spectrum of antimicrobial activity.

The properties of vital oils from *Croton tiglium* L. on intestinal transit in mice was studied by Wang *et al.* (2008). He defined that *Croton tiglium* (Euphorbiaceae) is widely used as a herb for curative of gastrointestinal disorders. According to his study, the minute dose of *C. tiglium oil* (CO) amplified the gastrointestinal transit of charcoal and barium meal as well as the production of fecal pellets in mice. Comparatively, maximum dose applied inhibitory effects. Colonic longitudinal bands in CO-treated mice were minor sensitive to electrical field motivation than those in control mice. The contraction of colonic longitudinal, colonic and jejunal circular strips in CO-treated mice was addedl sensitivity to atropine than that in control mice.

The poisonous properties of Crotocaudin extracted from the medicinal plant *Croton tiglium* was studied by Yadav & Singh (2010), their studies described that, The sub mortal doses of crotocaudin controlled over 24 hour

caused significant transitions in the carbohydrate and nitrogenous ingestion in nervous system, hepatopancreas and ovotestis tissues of *Lymnaea acuminata*. Likewise, *Channa punctatus* was also exposed to sublethal doses of crotocaudin for 96 h which represented an important modifications in the metabolism in muscle, liver and gonad tissues.

Studies of Wu *et al.* (2007) revealed novel analgesic pyrazine compound, named crotonine, was isolated from the leaves of *Croton tiglium* L. The structure of *Croton tiglium* (Nirvalam) is a considerable medicinal plant of the family Euphorbiaceae which is used for the therapy of constipation, dyspepsia, dysenteries, gastrointestinal disorders, intestinal inflammation, rheumatism, peptic ulcer, visceral pain and headache.

Bu *et al*. (2011), studied the sesquiterpenoids from the leaves of *Croton tiglium*. Two new compounds, badounoids A and B, combined with 13 known sesquiterpenes, were divided from the leaves of *Croton tiglium* L. The structural analysis of the new compounds were done by spectroscopic methods. The complete configuration of badounoid B was analysed by single-crystal X-ray diffraction method. All the identified compounds were isolated from *Croton* plants for the new chemical facet data, which added for this genus.

Tigliane diterpene esters from the leaves of *Croton tiglium* was studied by Ren *et al*. (2014). They successfully isolated three new tigliane-type diterpene esters, 1–3withunusual7-oxo-5-eneor7-hydroxy-5-enemoieties in their skeletons, from the leaves of *Croton tiglium*. Their structures were unambiguously elucidated on the basis of spectroscopic data.

Anticonvulsant properties of hydroalcoholic seed extract of *Croton tiglium* in rats and mice studied by Mudium & Kolasani, (2014). They reported that in electrically induced attacks produced by *Croton tiglium* that concerned

with the dosages prolonged with the time. There was a higher range of mortality in *Croton tiglium* group in chemically induced paroxysms when connected to sodium valproate.

Detoxification of *Croton tiglium* L. seeds by Ayurvedic process was studied by Pal *et al*. (2014). They observed that, *C. tiglium* seeds oil is reported to comprises of phorbol esters and crotonic acid besides of the fatty acids high-performance liquid chromatography (HPLC) of *C. tiglium* seed extracts be fond of the purification procedure were implemented for the documentation and quantification of the toxic values along with other physiochemical limitations.

Five new phorbol esters with cytotoxic and selective anti-inflammatory activities of *Croton tiglium* was studied by Wang *et al*. (2015). This also paper documented the existence of five new phorbol esters, (four phorbol diesters, 1–4, and one 4-deoxy-4a-phorbol diester, 5), as well as four known phorbol esters analogues (6–9) were isolated and identified from the branches and leaves of *Croton tiglium*. Compounds 2–5, and 7–9 showed potent cytotoxicity against the K562, A549, DU145, MCF-7,H1975,U937, HL60,SGC-7901,Hela, and MOLT-4 cell lines, with IC50 values ranging from 1.0 to 43lM, while none of the compounds exhibited cytotoxic effects on normal human cell lines 293T and LX-2, respectively. In addition, compound 3 exhibited moderate COX-1 and COX-2 inhibition, with IC50 values of 0.14 and 8.5lM, respectively.

A complete evaluation on curatively important plant, *Croton tiglium* L. was done by Dey *et al. (*2015). *Croton* seeds are used in curative phase in India before 450 BC. *C. tiglium* used to cure, gastrointestinal disorders, peptic ulcer, rheumatism, headache, intestinal inflammation and visceral pain. The seeds of *C. tiglium* have been utilized as crude drug for constipation, wound healing, a purgative and traditional dyspepsia and dysentery. The essential oil of *C. tiglium* also documented to have purgative, antimicrobial, analgesic,

insecticidal and inflammatory properties. The leaves of *C. tiglium* have been used to treat diarrhea, linea, pain and hurts. In Ayurvedic treatments croton seed oil used as a therapeutic agent for dropsy, constipation, cold, cough, and asthma and fevers.

The various part of *Croton tiglium* possess different biological and chemical perspectives such as anti- tumor, anti- HIV, antidermatophytic, anti-inflammatory, antioxidant activities in case of biological perspective and toxicity, phytochemistry, cytotoxic, detoxification activities in chemical perspectives. This plant has great prospects for development of Ayurvedic and modern medicines. This has been reported by Sinsinwar *et al.* (2016).

Research were carried out in the study to estimate the antidermatophytic activity of the leaves, stems and seeds of *Croton tiglium*. The stems, leaves, and seeds of *C. tiglium* extracted in ethanolic solution were arranged by cold soak or heat reflux procedures. The antidermatophytic actions of the extracts were considered by disc diffusion and micro dilution susceptibility assays against *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum*. The active constituents in the extracts were determined and recognised by GC-MS. Entire *C. tiglium ethanolic* extracts presented antifungal activities compared to three dermatophytes to a range. The ethanolic stem extract had the maximum inhibitory actions compared to *T. mentagrophytes* and *E. floccosum.* The ethanolic extracts of stem or seed of *C. tiglium* revealed severe antidermatophytic activities, thus,it could be reflected for presentation on curing skin fungal infections after suitable processing. This was reported by Lin *et al*. (2016).

Regulation and authentication of traditional siddha purification method for detoxifying *Croton tiglium* seeds by modern investigative techniques was standardized by Shanmugapriya *et al*. (2019). Their works was established on the methods like GCMS and HPLC seeds of *C. tiglium*

possess certain toxic values which have to be detoxified and purified before presence of the same for therapeutic disorders. Siddha system of medicine has exclusive method of purification without co-operating the biologically active constituents present in the drugs. The major goal of the present examination is to carry out the complete phytochemical and refined instrumental analysis of the *Croton* seeds with HPLC and GCMS including entire stages of purification techniques as listed in the Vedic literature. The results of the study has observed that there was an important reduction of Phorbol, a lethal organic compound from 5.18 to 3.86% and also the saturated fatty acids like, Behenic acid, Stearic acid Arachidic acid and Palmitic acid.

Assessment of antioxidant capability of *Croton tiglium* L. seed extracts after including silver nanoparticles was done by Aboulthana *et al. (*2019). Their studies targeted to rise the efficiency of the various *Croton tiglium* seed extracts when including silver nanoparticles (Ag-NPs) through more cytotoxicity compared to growth of human colon cancer cells. It was established that the average toxic dose (LD50) of the ethanolic, petroleum ether and aqueous seeds extract-Ag nano mixtures was about 7.95, 5.2 and 65 ml/Kg, respectively.

2.3.7. *Croton zeylanicus* Mull.Arg.

Inhibitory activities of several plant extracts compared to germination of uredospore of *Uromyces Ciceris arietini* (Grognot) Jacz. &Boyer was studied by Patil & Kamble, (2014). Phytofungicides are tested for their inhibitory activities compared to urediospore germination. *Croton zeylanicus* leaf extracted in aqueous medium. *Croton zeylanicus* leaves were collected from cultivated lands. The leaf extracts of *C. zeylanicus* has utilized to the curative concentration 1%, 2% and 3% correspondingly. The current interpretations shown that, *C. zeylanicus* indicated the 65.32% and 59.36% inhibitory effect on spore germination of particular fungi.

CHAPTER 3
MATERIALS AND METHODS

3.1. AREA OF STUDY

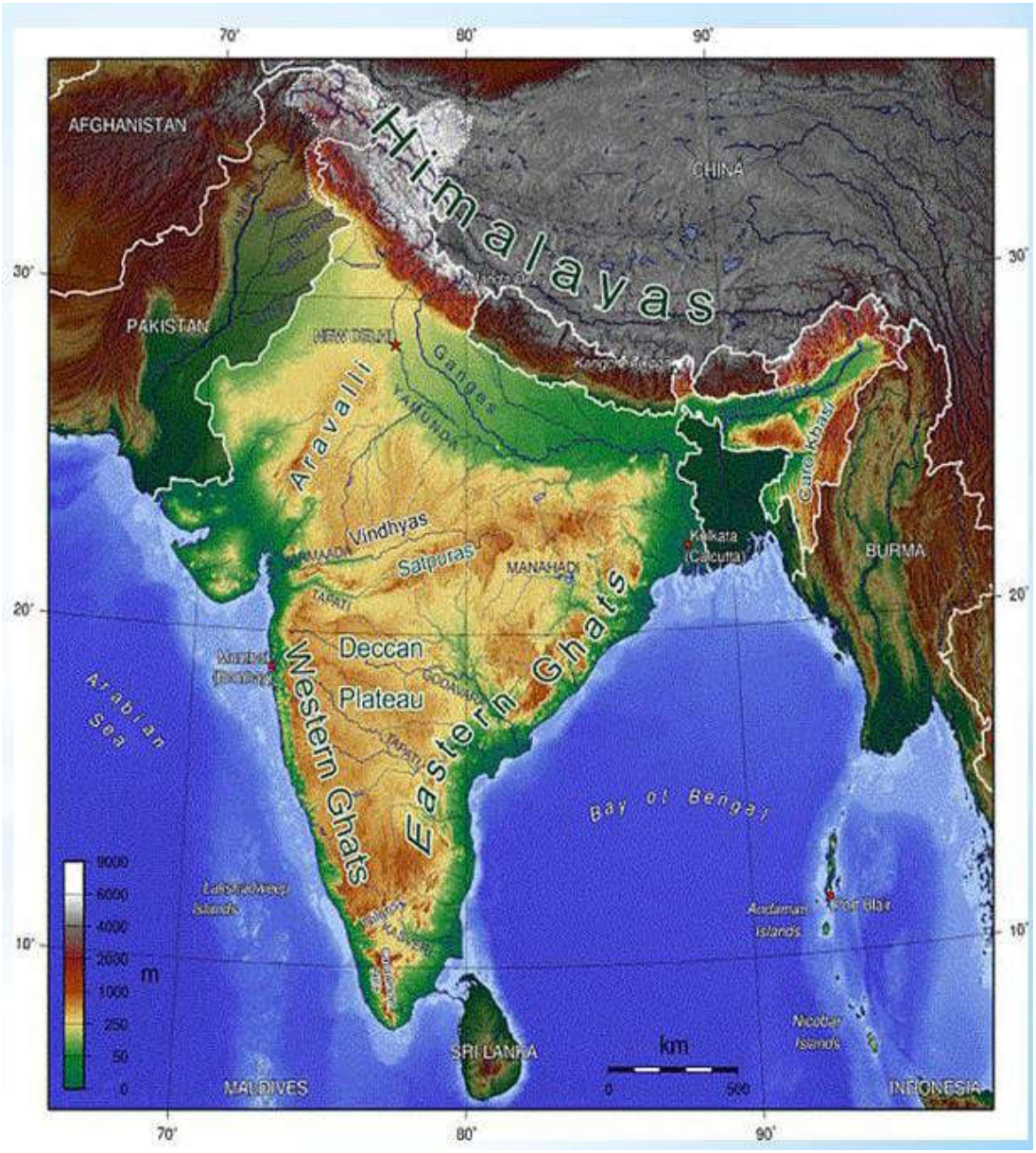
3.1.1. SOUTHERN PENINSULAR INDIA

South India includes, states of Kerala, Tamil Nadu, Karnataka, Andra Pradesh and Union territories of Pondicherry and Mahe (**Fig.1**). It covers an area of 4,67,186 sq.K.M. and is bounded on the north by Maharashtra, Madhya Pradesh and Orissa, on the east by the Bay of Bengal, on the south by Indian ocean and on the west by Arabian sea. The study area can be subdivided in to two floristic regions, Malabar and the Deccan. Malabar is a long and narrow strip of land running parallel to the coast of Arabian Sea (Malabar Coast). West of the Western Ghat, this region is floristically rich and includes coastal plains and series of hill ranges of the western ghat traversing the states of Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. The more or less continuous hill ranges have a major discontinuity in the Palghat gap separately in the Nilgiri ranges from the Anamalais. The Ghat descends steeply in the west facing the Arabian Sea where they merge gradually through a series of hill with the Deccan Plateau in the east. Anamalai is the highest peak (2695m) south of the Himalayas. Of the 15000 species each of flowering plant estimated to occur in India, nearly 4000 species are found in the Western Ghat (Manickam & Indrajayaraj, 1992). It supports tropical moist evergreen forests and moist deciduous forests on the western slopes. The Deccan is the great table land of the south Indian peninsula, stretching from the Aravalis, Malwa, the Vindhyas, the Satpura and Chota Nagpur hills in the North, almost right down to Kanyakumari in the South. The western and Eastern Ghat flank it on either side. The northern part of the Plateau slopes

west-wards while the south part slopes towards the south east. It is replaced by drought-resistant species and thorny shrubs. The eastern coastal pairs of the Deccan Plateau is road strip running parallel to the coast of Bay of Bengal (Coromandal Coast) and gradually rising from it. It consists of fertile coastal pair mainly formed by the deltas of the Kaveri in Tamil Nadu, Godaveri and Krishna in Andra Pradesh and a number of small rivulets and streams

The Malabar and the Deccan regions together provide a wide variety of climate and edaphic zones with mountain ranges, hill rocks, valleys, swamps, marshy low lands, sandy sea- coasts, fresh water streams, rivers, ponds and back waters on the sea- front and harbour diverse type of vegetation.

Peninsular India receives both North- East monsoons (October-December) and South – West monsoon (January- September). The former is more achieve in Tamil Nadu, Pondychery and Andra Pradesh, while later is more vigorous in Kerala and coastal Karnataka, the climate in peninsular India is in general, megathermal (Subrahmanyam *et al*., 1965; Rao *et al*., 1972). Chaudhury & Sarwade (1982) classified the homoclimatic regions of India into 5 categories namely arid regions, semi-arid –regions, sub-humid- region, humid regime and super –humid regime. Among them, South Indian falls under four homoclimatic types. The coastal districts of Andra Pradesh, interior Karnataka and some districts in Tamil Nadu come under semi-arid climate. Northern coastal Andra Pradesh, southern districts of Karnataka and Northern Tamil Nadu experience dry, sub-humid climate – while coastal Karnataka and Northern Kerala have moist sub – humid type of climate. The humid region predominates in southern districts of Kerala and at higher elevations around Coonoor and Ootty (Tamil Nadu), whereas super-humid climate region occupies only at Kodaikanal in Tamil Nadu (Sivarajan & Pradeep, 1996).



Study area

3.2. MORPHOLOGICAL STUDY

3.2.1. Literature Survey

Literature survey has been done by analyzing various Flora's, Protologues, Herbarium sheets and other available literature.

3.2.2. Details of various Flora's referred

Morphological characters are identified by the help of Flora's, such as Flora of India vol: 23 (Balakrishnan *et al*., 2012), Flora of The Presidency of Madras (Gamble, 1936), A hand book of Coimbatore (Somasundaram, 1963), An Excursion Flora of Central Tamilnadu (Matthew, 1991), Flora of Palani Hills (South India) (Matthew, 1999), A Pocket Flora of Sirumalai Hills, South India (Pallithanam, 2001), Flora of Coimbatore (Chandrabose & Nair, 1987), Flora of Andhra Pradesh (India) (Pullaiah & Moulali, 1997), Flora of Karnataka Analysis (Sharma *et al*., 1984), Flora of Udupi (Bhat, 2003), Flora of Coorg (Murthy & Yoganarasimhan, 1990), Flora of Agasthyamala (Mohanan & Sivadasan, 2002), Flora of Alappuzha (Sunil & Sivadasan, 2009), Flowering plants of Trissur forest (Sasidharan & Sivarajan, 1996), Flora of Palghat (Vajravelu, 1990), Flora of Calicut (Manilal & Sivarajan, 1982), Flora of Cannonore (Ramachandran & Nair, 1988), Flora of Thiruvananthapuram (Mohanan & Henry, 1994), Flora of Thenmala (Subramanian, 1995).

3.2.3. Herbarium Analysis

Specimen deposited various herbaria analyzed. BSI Coimbatore, Calicut University Herbarium (CALI) Malappuram, Kerala Forest Research Institute (KFRI), Thrissur and Madras Herbarium (MH) were consulted and studied.

3.2.4. Taxa selected for the present study:

There are about 8 taxa selected for the present analysis, they are

1. *C. aromaticus* L.

2. *C. bonplandianum* Bail.

3. *C. caudatus* Geisel.

4. *C. hirtus* L' Herit.

5. *C. malabaricus* Bedd.

6. *C. persimilis* Mull.Arg.

7. *C. tiglium* L.

8. *C. zeylanicus* Mull.Arg.

From several localities of the study area, specimens were collected for morphological analysis. The floral parts, fruit, seed are preserved in formalin and photographs were also taken. Labels are prepared for the collection and filled up the field notes. Morphological characterizations of various parts were carried out. Details of habit, stem, stipule, leaves, pubescent, venation pattern, petiole, inflorescence, bract, flower, calyx, sepal, androecium, gynoecium, ovary, placentation, seed, etc. were recorded for each species. Morphological characters were analyzed by using LABOMED CSM2 and photographs were taken by using Leica EZ4Hd.

3.3. ANATOMICAL STUDIES

During collection, root, stem and leaf of each species were collected and preserved in formalin. Anatomical studies were done for identifying and comparing the species. The cross sections of leaf, petiole, stem, and root of selected species were taken by hand sectioning. The thin sections were stained with Saffranin and Toluidine blue (Prasanna & Karpagam, 2015). The stained materials were mounted using glycerine and observed under compound microscope-LABOMED CXLPLUS and photographs are also taken.

3.4. PREPARATION OF EXTRACT

The methanolic extract of leaf, stem and root of selected species of *Croton* were prepared for the phytochemical analysis. The collected material

carried out to cleaning, drying and powdering. The extraction was done using Reflex condenser. 10g of each powdered material of leaves, stem and root were taken in the RB flask containing methanol (200ml). Boiling temperature was kept for three hours. The extracts in which obtained were filtered and concentrated to 30ml in a water bath. The extract that obtained is diluted and subjected to preliminary phytochemical tests. Concentrated extract were subjected to HPTLC analysis.

A liquid reaction mixture is placed in a vessel open at the top. This vessel is connected to a condenser, like that vapors given off to cool back to the liquid and fall back into the reaction vessel. The vessel is then heated vigorously for the course of the reaction. The purpose is to thermally accelerate the reaction by conducting it at an elevated temperature. The goodness of this technique is that it can be left for a long time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapor is immediately condensed in the condenser.

3.5. PHYTOCHEMICAL ANALYSIS

The stored extract was diluted and used for the different phytochemical studies. using preliminary phytochemical tests preliminary phytochemical analysis done to determine the presence of phytochemical components which are alkaloids, carbohydrates, reducing sugars, flavonoids, saponins, tannins, steroids, proteins, glycosides, phenols, amino acids and terpenoids.

3.5.1. Tests for Alkaloids

1) Mayer's test: To a 2-3 ml of extract, add two drops of Mayer's reagent along the sides of test tube. Occurrence of white creamy precipitate indicates the appearance of alkaloids (Tiwari *et al*., 2011).

2) Hager's test: To 2-4 ml of extract, two drops of Hager's reagent are added along the sides of test tube. Presence of yellow precipitate indicates the presence of alkaloids (Tiwari *et al*., 2011).

3) Wagner's test: 2-5 drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the presence of alkaloids. (Tiwari *et al*., 2011).

3.5.2. Tests for Carbohydrates

1) Molish's test: To 2 ml of extract, to add two drops of alcoholic solution of α- naphthol. The mixture is shaken well and 2-3 drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the test of carbohydrates. (Banu & Cathrine, 2015).

3.5.3. Tests for reducing sugars

1) Fehling's test: Fehling A and Fehling B reagents are mixed and 2-3 drops of extract was added and boiled. A brick red coloured precipitate of cuprous oxide forms, if reducing sugars present (Joseph et *al*., 2013).

2) Benedict's test: 0.5ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool fastly. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar (Rishikesh *et al*., 2013).

3.5.4. Tests for Flavonoids

1) Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with extract, intensive yellow color was formed, which turned into colorless then added 2 drops of diluted acid to solution.This result indicated the presence of flavonoids (Jaradat *et al*., 2015).

2) Lead acetate test: Extracts are treated with 2-3 drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids (Tiwari *et al*., 2011).

3.5.5. Tests for Saponins

1) Foam test: The stock solution (1 ml) was taken in a test tube. It was shaken by hand for 15 minutes. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins (Hossain *et al*., 2013).

3.5.6. Tests for Tannins

1) Braymer's test: 5ml solution of the extract was taken in a test tube. Then 1ml of 5% Ferric Chloride solution was added. Greenish black precipitate was formed and represents the presence of tannins (Rishikesh *et al*., 2013).

3.5.7. Tests for Steroids

1) Salkowski tests: To 2 ml of extract, add 2ml chloroform and 2 ml concentrated H_2SO_4 and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence represent the presence of steroids (Joseph *et al*., 2013).

3.5.8. Tests for Proteins

1) Millon's test: 5ml of Millon's reagent is added to 3ml of aqueous solution of extract sample. The appearance of white precipitate which slowly turns to pink or red when heated gently indicates the presence of proteins (Morsy, 2014).

3.5.9. Tests for Glycosides

1) Keller Killiani's test: To the test solution, 2ml of glacial acetic acid consisting 2-3 drops of $FeCl_3$ solution was added. 1ml of conc. H_2SO_4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxy sugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer (Singh & Bag, 2013).

3.5.10. Tests for Phenols

1) Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol (Santhi & Sengottuvel, 2016).

2) Lead acetate test: 10mg extracts was used with 2-3 drops of lead acetate solution. Formation of yellow colour precipitate represents the presence of phenol (Santhi & Sengottuvel, 2016).

3.5.11. Tests For Amino acids

1) Ninhydrin test: 2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour as the test for amino acids (Prasad *et al.,* 2015).

3.5.12. Tests for Terpenoids

1) Copper Acetate test: Extracts is dissolved in water and treated with a few drops of copper acetate solution. Formation emerald green color represents the presence of diterpenes (Morsy, 2014).

3.5.13. Quantitative phytochemical analysis High Performance Thin Layer Chromatography (HPTLC) studies

HPTLC is a sophisticated and automated form of TLC. It is the fastest of all chromatographic methods. In HPTLC Aluminium plate precoated with silica gel 60 F 254 was used as stationary phase. Chromatograms were developed in a saturated twin trough chamber. Mobile phases employed in this study were prepared by mixing toluene, ethyl acetate and methanol in the ratio 7:3:1 respectively. 10 µl of the samples were applied on precoated plate using Camag automatic TLC sampler 4. The plates were dried and the spots were visualized sequentially under UV light at 254 and 366 nm. Densitometric scanning of the plates was done by using Camag TLC scanner at 254 nm and 366 nm (Banu & Cathrine, 2015).

CHAPTER 4
RESULTS AND DISCUSSION

4.1. MORPHOLOGICAL CHARACTERIZATION OF SELECTED SPECIES OF *CROTON*

4.1.1. General characteristics of Family Euphorbiaceae

Monoecious or dioecious, herbs (erect or prostrate), shrubs (erect of straggling), climbers or trees, armed or unarmed; branchlets terete or angular, often grained, compressed or simple, often zigzag, glabrous or pubescent or stellate tomentose; stems woody or succulent, often with acrid milky latex. Leaves alternate or distichous, entire or palmately lobed, opposite or alternate or opposite and alternate (rarely whorled), chartaceous or coriaceous or membranous or fleshy, persistent or caducous; entire or serrate, crenate, dentate at margin often glandular, penninerved or palmatinerved, glandular at base or eglandular; petioles present or absent; stipules present or absent, interpetiolar or intrapetiolar, persistent or deciduous. Inflorescence axillary, terminal or subterminal, simple or branched spikes, racemes or umbels, often pedunculate, unisexual or bisexual. Flowers actinomorphic, solitary or in glomerules, grouped into spiciform or capitate thyrses or in cyathia (pseudanthia), bracteate or ebracteate, often bracteolate, pedicellate. Perianth simple, usually calycine, rarely petaloid, sometimes petals also present, lobes free or connate, valvate or imbricate, or both, rarely reduced or even absent, caduceus or accrescent and sometimes enlarged in fruit. Disk-glands usually annular, entire or lobed or pulvinate or of free scales or absent. Stamens 1-many, free or connate, sometimes raised on a column, in 1 or 2 series, hypogynous; filaments equal or unequal; anthers mostly 2-celled, rarely 4-celled, parallel or divergent, basifixed or dorsifixed, simple or versatile,

connective present, rarely absent, broad, narrow, or truncate, longitudinally or transversely dehiscent; pistillode central, columnar or absent. Ovary superior or half-inferior, usually 3 or 4 - 12-locular; ovules 1 or 2 per locule; placentation axile or pendulous or laterally pendulous; styles 3 or as many as locules, free or connate or halfunited, deeply or slightly bifid, rarely multifid, smooth or papillate, plumose or laciniate, erect or spreading. Fruits capsule, drupe or berry, globose or subglobose or ellipsoid-ovoid, smooth or verrucose or tuberculate, 1 - 3- lobed. Seeds 1 or 2 per locule, with or without a caruncle, globose or trigonous, or ellipsoid-ovoid, glabrous or appressed hairy; testa smooth, crustaceous, pitted, striate or marbled (Gamble, 1936).

4.1.2. General Charecters of Genus: *Croton*

Trees or shrubs, rarely scandent, some herbs, monoecious or rarely dioecious, densely or sparsely clothed with stellate hairs or lepidote scales. Leaves simple, alternate, entire or lobed, petiolate, often with a pair of glands at the base of the blade, stipulate. Inflorescences terminal or axillary; racemose or spicate; androgynous, with female flowers usually below the male flowers or unisexual, bracteate. Male flowers: sepals 3-6, valvate or imbricate; petals 3-6; disk glands 3 - 6 or absent; stamens 5-6, free; pistillode absent. Female flowers: sepals 4-6, persistent; petals 4 - 6; ovary glandular, 2 - 3-celled; ovule one per cell; styles bifid or bipartite. Fruits schizocarpic warty capsule; seeds oblong or ellipsoid, smooth, carunculate (Gamble, 1936).

4.1.3. SYSTEMATIC TREATMENTS

4.1.4. Key to the Species

1a. Introduced weedy herbs, stem herbaceous.....**2**

1b. Native trees, shrubs or under shrubs, stem woody.....**3**

2a. Leaves penninerved; glands at the base of the lamina sessile ....**2** ***C. bonplandianum***

2b. Leaves trinerved at base; glands at the base of the lamina shortly Stipitate ..... 4 ***C. hirtus***

3a. Plants large, scandent shrubs.....***3 C. caudatus***

3b. Plant erect, shrubs, trees **4**

4a. Foliar glands sessile; leaves never separated by longitudinal ....**5** ***C. malabaricus***

4b. Foliar glands stipitate; leaves separated by long internodes ....**8** ***C. zeylanicus***

5a. Petioles, rachis and flowers lepidote.....**6** ***C. persimilis***

5b. Petioles, rachis and flowers stellate pubescent.....**1**

6a. Leaves hispid, style branches usually quadrified, Fruit muricate..... .....**1** ***C. aromaticus***

6b. Leaves glaberous above; style branches usually bifid, Fruit smooth..... .....**7** ***C. tiglium***

4.1.4. (1) ***CROTON AROMATICUS*** L.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: ***Croton aromaticus*** L.

Croton aromaticus L., Sp. Pl. 1005. 1753, "*lacciferum*"; Gamble, Fl. Pres. Madras 1936. 1925; Manilal & Sivar., Fl. Calicut 267. 1982; Manilal, Fl. Silent Valley 247. 1988; Antony, Syst. Stud. Fl. Kottayam Dist. 358. 1989; Babu, Fl. Malappuram Dist. 710. 1990; Sasidh. & Sivar., Fl. Pl. Thrissur For. 399. 1996; Sasidh., Fl. Periyar Tiger Reserve 368. 1998; Sasidh., Fl. Parambikulam WLS 286. 2002; Kumar *et al*., Fl. Pathanamthitta 441. 2005. *Croton aromaticus* sensu

Hook. f., Fl. Brit. India 5: 338. 1887,p.p. non L. 1753; 1984; Vajr., Fl. Palghat Dist. 425. 1990; N.P. Balakr. & Chakrab., Fam. Euphorbiaceae India 212. 2007., Fl. South Indian Hillstation. 534, 1977; P.F.Fyson., Fl. Chickmagalur. Dist. 293. 1981; S.N.Yoganarasimhan, *et al*.; Fl. India 23: 228. 2012;Balakrishnan *et al.* **(Plate 2).**

Trees, branchlets stellate, brown tomentose. Leaves to 12 x 6 cm, simple, ovate, acuminate, cordate at base, 3-ribbed from base, glands 2 pairs; upper leaves with acute base; petiole 2-3 cm long. Inflorescence 10 cm long, densely stellate hairy, white; Male flower 3-5 mm long, above, unisexual, actinomorphic, bracteate, Perianth biseriate, tepel 10, outer layer 1-2mm,5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.6 -1mm, 5, fused, white, stellate hairs , Stamens 0.8-1mm, 10, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flower 7 mm across; sepals ovate, acute; petals obovate, ciliate; styles 3, bifid, glabrous. Capsule4-8mm long, ovoid, trilocular, green, hirsute; seeds 3-4mm, 3, black and cream patches, glaberous.

Fl. & Fr.: April-Auguest

Vernacular Name: *Keppetiya, Theppadi*

Habit: Tree

Habitat: Terrestrial.

Distribution:IndiaandSri Lanka. Kerala: Palakkad, Kottayam, Idukki, Kollam, Pathanamthitta.

4.1.4. (2). *CROTON BONPLANDIANUM* Bail.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: ***Croton bonplandianum*** Baill.

Croton bonplandianum Baill., Adansonia 4: 339. 1864. *Croton sparsiflorus* Morong, Ann. New York Acad. Sci. 7:221. 1893; Mani. & Sivar., Fl. Calicut 266.1982; Matthew, Fl. Tam. Carnatic 3: 1420. 1983; Ramachandran and Nair, Fl. Cannanore. 272. 1988; Gamble, Fl. Pres. Madras1936; Croizat, J. Bombay Nat. Hist. Soc. 41: 573. 1940; Vajravellu, E. Fl. Palghat Dist. 426. 1990; Mohanan & Henry, Fl. Thiruvananthapuram 411. 1994; Subramanian, Fl. Thenmala 326. 1995; Sivar. & Mathew, Fl. Nilambur 613. 1996; Sasi. & Sivar., Fl. Pl. Trissur For. 399. 1996; Dassanayake, A Revised handbook to the Fl. Ceylon 11: 90. 1997; Pattithanam, A Pocket Fl. Siramalai Hills, South India 228-229. 2001; Suryanarayana & Rao, Fl. Nellore Dist. Andhra Pradesh 477. 2002; Mohanan & Sivad., Fl. Agasthyamala 604. 2002; N. Mohanan & M. Sivadasan, Fl. Udupi 560. 2003; K.Gopalakrisna Bhat, Fl. Pathanamthitta 441. 2005**;** Sunil & Sivadasan, Fl. Alappuzha 624-625. 2009; Balakrishnan *et al.,* Fl. India 23: 228. 2012; N.P. Balakrishnan *et al.,* Fl.Courtallum.167, 1986; K.K.N. Nair & M.P. Nair. Fl. Devanagari.Dist.346.2004, B.K. Manjunatha, V. Krishna**(Plate 3).**

Aromatic shrub, perennial, erect up to 80 cm, dichotomously branched, monoecious; Stem floccose, green , watery latex, tender parts warty; Leaves 2.5-5 × 1-2.5 cm, simple, alternate, exstipulate, petiolate; Petiole 1.3- 1 cm, cauline, floccose , green; lamina lance - ovate, base obtuse, venation cladodromous, serrulate, acute adaxial side dark green and glabrous, abaxial side light green and pubescent; Inflorescence 8-15 cm, terminal raceme, white, pubescent; Male flower 3-2 cm, above, unisexual, actinomorphic, bracteate and pedicillate; Pedicel 1-2mm, cauline, white, floccose; Bract 1 -2mm, green,

hairy , triangular, green; Perianth biseriate, tepels 10, Outer layer 1-2 mm, 5, fused , toothed, green, pubescent; Inner layer 2mm, 5, fused, pubescent, white; Stamens 0.5-1.5 mm, 15, basifixed, white; Anther globose, longitudinal dehiscence, light yellow; Female flower 3-2 cm long, below, few in number, unisexual, actinomorphic, hypogynous , bracteate and pedicillate, each flower with a gland at the base of pedicel; Pedicel - 1mm long, cauline, green, floccose; Bract 1mm, triangular, hairy, green; Perianth 1-2 mm, one seriate, tepals 5, green, pubescent, lanceolate; Ovary superior, green, pubescent, sub globose, 3 loculed, placentation axile; Style short, 3, white; Stigma 3, each forked into 6 lobes, brown; Capsules 6 × 4mm, epicarp warty, trigonous, green, floccose; Seeds 3, 4 × 3 mm, greyish black, shiny**.**

Fl. & Fr.: Throughout the year.

Vernacular Name: *Bantulsi*

Habit: Herb

Habitat: Terrestrial

Distribution: Found in waste lands and road side areas in India, Bangladesh and all other countries of South Asia, Kerala: All districts,

4.1.4. (3). ***CROTON CAUDATUS*** Geisel.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: ***Croton caudatus*** Giesel.

Croton caudatus Geisel. Crot. Monogr. 73. 1807; Gamble, Fl. Pres. Madras 1936.; Subram., Fl. Thenmala Div. 327. 1995; Sivar. & Mathew, Fl. Nilambur 614. 1997; Sasidh., Fl. Periyar Tiger Reserve 368. 1998; Anil Kumar *et al*., Fl. Pathanamthitta 441. 2005; N.P.Balakr. & Chakrab., Fam. Euphorbiaceae India 213. 2007; *Croton caudatus* Geisel. var. obovoideus Chakrab.& Balakr. Bull. Bot. Surv. India 25: 190. 1983; Sasidh. &Sivar., Fl. Pl. Thrissur For. 399. 1996. Fl.Chickmagalur.Dist.293.1981; S.N.Yoganarasimhan, K.Subramanyan., Fl. India 23: 228. 2012; N.P.Balakrishnan *et al* **(Plate 4).**

Scandent woody climber; bark greyish or blackish brown; all parts coarsely ochraceous or often greyish tomentellous or scattered scabrid-pubescent, hispid or hirsute from erect central rays .Leaves narrowly to broadly ovate, cordate, elliptic to oblong or orbicular, 3-25 x 2-18 cm, cordate or sometimes truncate or rounded at base, coarsely dentate-serrate to subserrulate along margins, acuminate or apiculate or cuspidate or caudate at apex (acumen or cauda 5-25 mm long), membranous to thinly coriaceous, remaining green or turning yellow, brown, reddish-brown or blackish on drying and becoming brittle, strongly trinerved at base; lateral primary veins ascending 60-85% way up the lamina; lateral nerves (above the basal)2-6 pairs, faint to prominent above, conspicuous beneath; tertiary nerves obscure to prominent above, conspicuous beneath, scalariform; basal glands 2-4, stipitate, marginal glands present; petioles 0.5-7 cm long, stipules lacerate with glandular tips, 4-15 mm long. Inflorescences 8-35 cm long, sometimes unisexual; bracts subulate or lanceolate or linear, 1-10 mm long, often fringed with stipitate glands. Male flowers', pedicels 3-9(-16) mm long; sepals 5<-6), ovate, oblong to elliptic or triangular, 2-4.5 x 1-3.5 mm; petals 5(-6), narrowly oblong elliptic to spathulate, 2-4 x 1-3 mm; stamens 18-40, 3.5-6 mm long; anthers oblong or obovoid, 0.8-1.3 mm ; long. Female flowers: pedicels 1-5 x 1-1.8 mm; sepals 5, oblong, elliptic to ovate, 2-6 x 1-4 mm, often fringed with subsessile glands; petals 0-5, filiform or subulate, 0.5-2.5 mm long; ovary

globose or oblong or obovoid, 2-4 x 2-4 mm, densely ochraceous hirsute or hispid (from central rays); styles 5-13 mm long, free, bifid (rarely quadrifid) almost to the base. Capsules globose or oblong or obovoid, 1.5-3 x 1.5-2.5 cm, bluntly 3-or 6-angled, often muricate or verruculose, scattered pubescent; seeds oblong or ellipsoid or ovoid or suborbicular, 8-20 x 5-15 mm, brown, scattered pubescent.

Fl. & Fr.: March- June.

Vernacular Name: *Umithinnikodi*

Habit: Woody Climber

Habitat: Terrestrial

Distribution: Common in evergreen forests or mixed forests or deciduous forests or 'scrub and thickets and sacred grooves often along streams, up to 1500maltitude,Kerala:Kannur, Kozhikode, Malappuram, Wayanad, Palakkad, Thrissur, Idukki, Kollam and Pathanamthitta.

4.1.4. (4). ***CROTON HIRTUS*** L ' Herit.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: ***Croton*** **L.**

Species: ***Croton hirtus*** L.

Croton hirtus L. Herit., Strip. Nov. 17, t. 9.1785; Webster in Ann. Missouri Bot. Gard. 54: 262. 1968; Ramachandran *et al.*, in Indian For. 15 (2): 183. 1992;

Sunil& Sivadasan, Fl. Alappuzha Dist. 625. 2009; Balakr. & Chakrab *et al*., in Bull. Bot. Surv. India 23: 228. 2012. Fl. India 23: 228. 2012; N.P.Balakrishnan *et al* **(Plate 5).**

Aromatic herbs, annual, erect up to 30cm, dichotomously branched monoecious; stem terete, pubescent with watery latex; The leaves 5.5 – 6.2× 3.5-4.7cm simple, alternate, stipulate, petiolate; Petiole 2.5-3.1cm cauline, green, stellate hairs; stipules5-7×1-2mm, 2,filiform, free, opposite, tomentose; lamina ovate-lanceolate, base round with a pair of glands, 3-5 palmately nerved at base and pinnately nerved at lamina, serrate, acute, chartaceous, stellate hairs,green on both sides;Inflorescence 5-10cm, terminal raceme, white, densely hirsute; Male Flowers2-4 mm, above, unisexual, actinomorphic, bracteate and pedicellate; Pedicel 0.6-1 mm long ,cauline,white, stellate hairs with glands; bract 1-2mm, linear, green, hairy, fringed with 2-5 capitate glands, Perianth biseriate, tepel 10, outer layer 1-2mm,5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.5 -1mm, 5, fused, white, stellate hairs , Stamens 0.9-1mm, 10, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flowers 2.5-5mm, below, unisexual, actinomorphic, hypogynous, bracteate and pedicellate; pedicel 0.5 mm long, cauline, stellate hairs, green, bract 1-2mm, linear, green, hairy, Perianth uniseriate, tepel 0.5-1 mm, 5, fused , obovate, green, persistent, stellate hairy, ovary 1mm, ovoid, syncarpous with free stigma, superior,axile, green, densely hirsute , style 3, 2mm long, bifid; capsule3-6mm long , ovoid, trilocular , green, hirsute; seeds 3-4mm, 3, black and cream patches, glaberous**.**

Fl. & Fr.: Throughout the year.

Common Name: *Hairy Croton*

Habit: Herb

Habitat: Wastelands

Distribution: Weed of waste places, Plantations and roadsides of Kerala, Tamil Nadu. Native to West Indies and Central and South America, which has become aggressive weed in Tropical Asia and Africa, Kerala: All districts.

4.1.4. (5). ***CROTON MALABARICUS*** Bedd.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: ***Croton malabaricus*** Bedd

Croton malabaricus Bedd., Fl. Sylv. 204. 1873; Gamble, Fl. Pres. Madras 1936. 1925; Mohanan, Fl. Quilon Dist. 362. 1984; Vajr., Fl. Palghat Dist. 426. 1990; M. Mohanan & Henry, Fl. Thiruvanthapuram 412. 1994; Subram., Fl. Thenmala Div. 326. 1995; Sasidh. & Sivar., Fl. Pl. Thrissur For. 399. 1996; Sasidh., Fl. Shenduruny WLS 284. 1997; Sivar. & Mathew, Fl. Nilambur 614. 1997; Sasidh., Fl. Periyar Tiger Reserve 368. 1998; Sasidh., Fl. Parambikulam WLS 286. 2002; Mohanan & Sivad., Fl. Agasthyamala 605. 2002; Anil Kumar et al., Fl. Pathanamthitta 442. 2005; N.P. Balakr. & Chakrab., Fam. Euphorbiaceae India 214. 2007; Narayanan, Fl. Stud. Wayanad Dist. 732. 2009. Fl. India 23: 228. 2012; N.P.Balakrishnan *et al* **(Plate 6).**

Trees, to 20 m high, bark greyish-white, smooth; branchlets stellate-hairy. Leaves simple, alternate, 7-24 x 3-12 cm, rhombic-ovate, broadly ovate or elliptic, apex acuminate, base cuneate, obtuse or round, margin entire, glabrous or with silvery stellate hairs and reddish glands beneath,

chartaceous; stipules 12-18 mm long, lateral, filiform, scarious; petiole 10-125 mm long, slender, stellate scales present; prominently, 3-4-ribbed from base; lateral nerves 4-6 pairs, pinnate, prominent, intercostae scalariform, obscure. Flowers 5-10 mm , white, in erect terminal racemes ; bracts small; Male flower1-3 mm, above, unisexual, actinomorphic, bracteate and pedicellate; Pedicel 0.3-1 mm long, cauline, white, stellate hairs, 2-seriate; 5-partite, glandular at base; tepel 10, outer layer 1-3mm,5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.4 -1mm, 5, fused, white, stellate hairs , Stamens 0.7-1mm, 10 -15, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flowers 3-5mm, below, unisexual, actinomorphic, hypogynous, bracteate and pedicellate; pedicel 0.7 mm long, cauline, stellate hairs, green, bract 1-3mm, green, hairy, Perianth uniseriate, tepel 0.6-1 mm, 5 ovary stellate hairy, 3-celled, ovules one in each cell; styles long, slender, pistillode absent. Fruit capsule 2.5 x 2 cm, obovoid, depressed above, brown tomentose; seeds 13 x 8 mm, oblong, mottled with brown.

Fl. & Fr.: April-November

Vernacular Name: *Chunnambumaram*

Habit: Tree

Habitat: Terrestrial.

Distribution: Palakkad, Idukki, Kollam, Pathanamthitta, Malappuram, Thiruvananthapuram, Thrissur, Wayanad, Kannur

4.1.4. (6). ***CROTON PERSIMILIS*** Mull.Arg.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: ***Croton persimilis*** Mull.Arg.

Croton persimilis Muell.-Arg., Linnaea 34: 116. 1865 & DC., Prodr. 15: 618. 1866 emend. Philcox in Dassan. & Clayton, Rev. Handb. Fl. Ceylon 11: 92. 1997; Chakrab. & Balakr., J. Econ. Tax. Bot. 30: 298. 2006; N.P.Balakr. & Chakrab., Fam. Euphorbiaceae India 215. 2007. *Croton oblongifolius* Roxb.,; Gamble, Fl. Pres. Madras 1936. 1925. Croton roxburghii Balakr., Bull. Bot. Surv. India 3: 39. 1961; Ansari, Fl. Kasaragod Div. 337. 1985; Sivar. & Mathew, Fl. Nilambur 616. 1997., Fl. India 23: 228. 2012; N.P.Balakrishnan *et al* **(Plate 7)**.

Small trees; bark grey or brownish; young shoots covered with minute orbicular silvery scales. Leaves simple, alternate, spiral, turning red before falling; 11-23 x 3.5-7.5 cm; elliptic, obovate, elliptic-obovate, elliptic-oblanceolate or oblong-lanceolate, base acute or cuneate, apex acute, margin more or less crenate or young, coriaceous; lateral nerves 8-13 pairs, pinnate, slender, prominent, intercostae reticulate, prominent; stipules lateral; petiole 15-40 mm long, stout, slightly grooved above, swollen at tip and base, silver lepidote scales present. Flowers unisexual, pale yellowish-green, solitary or fascicled in the axils of minute bracts on long erect often fascicled racemes, the males in the upper part of the raceme, the females in the lower part; male flowers: pedicels of variable length, slender; sepals 5, ovate, obtuse; petals elliptic-lanceolate, obtuse, woolly; stamens 12, inflexed in bud; lower half of the filaments hairy; female flowers: pedicels short, stout; sepals 5, ovate, acute, ciliate; petals 5, obovate, margin densely woolly; ovary 3-celleed, ovule 1 in each cell; styles 3, each again divided into 2 longer slender curled branches.

Fruit a capsule, subglobose, depressed, slightly 3-lobed, lepidote scaly; seeds 3.

Fl. & Fr.: March-June

Common Name: Somaraaji, Thomarayam, Vellapine.

Habit: Small Tree

Habitat: Terrestrial.

Distribution: India to China, Kerala: Kannur, Alappuzha, Kasaragod, Malappuram and Thrissur.

4.1.4. (7). *CROTON TIGLIUM* L.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: *Croton tiglium* L.

Croton tiglium L., Sp. Pl. 1004. 1753; 1887; Gamble, Fl. Pres. Madras. 1936. 1925; Manilal, Fl. Silent Valley 247. 1988; Nicols., S. Ramach. & V.J. Nair, Fl. Cannanore 413. 1988; Vajr., Fl. Palghat Dist. 426. 1990; Sivar. & P. Mathew, Fl. Nilambur 616. 1997; Anil Kumar, Sivad. & N. Ravi., Fl. Pathanamthitta 442. 2005. *Tiglium officinale* Klotzsch, Nov. Acta Phys. Med. Acad. Caes. Leop. Carol. Nat. Cur. 19, Suppl. 1: 418. 1843. *Croton officinalis* (Klotzsch) Alston in Trimen, Handb. Fl. Ceylon 6: 264. 1931. *Cadel-avanacu* Rheede, Hort. Malab.2:

61-62, t.33-1679., Fl. Udupi 560. 2003; K.Gopalakrisna Bhat., Fl. India 23: 228. 2012; N.P.Balakrishnan *et al* **(Plate 8).**

Small trees, annual, up to 15m height; Stem terete, glaberous, green; Leaves 10.6-13.8 × 3.6-4.3cm simple, alternate, ovate, stipulate, petiolate –3.5-4 cm, hairy with stellate hairs, trinerved at base; lateral nerves 1 - 6 pairs, membranous, yellowish green, acuminate, serrulate, round, pinnately veined glaberous, green; Inflorescence 5 - 18 cm long, pedicels 2 - 5 mm long, terminal raceme. Flowers pedicellate. Male flowers: 8-8.5mm, biseriate, outer layer 1.5 - 4 x 1, 2.5-3mm, 5 fused hairy with stellate hair green; inner layer 2 - 4 x 0.7 - 2 mm; Stamen 3-4 mm long , 14-20 basifixed ,yellow; Female flowers: pedicels 2 - 8 mm long; tepals oblong, triangular or lanceolate, 2 - 4.5 x 0.7 - 3 mm ovary obovoid , 2.5 - 4 x 2 - 3.5 mm, tomentose; styles 3.5 - 7 mm long, free, bifid. Capsules obovoid to oblong, 3 lobed, 1.6 - 2.5 x 1.3 - 2 cm, subglabrous; seeds 3-4mm, black.

Fl. & Fr.: January – November.

Vernacular Name: Nirvalam

Habit: Small Tree

Habitat: Terrestrial.

Distribution: Indo-Malesia. Occasional; in evergreen forests, Kerala:Kannur,Wayanad, Malappuram,Palakkad.

4.1.4. (8). ***CROTON ZEYLANICUS*** Mull.Arg.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species*: Croton zeylanicus* Mull.Arg.

Croton zeylanicus Muell.-Arg., Linnaea 34: 107. 1865 & in DC., Prodr. 15: 581. 1866;. 1984; Manilal, Fl. Silent Valley 247. 1988; Vajr., Fl. Palghat Dist. 426. 1990; M. Mohanan & Henry, Fl. Thiruvanthapuram 412. 1994; Sasidh. & Sivar., Fl. Pl. Thrissur For. 400. 1996; Sasidh., Fl. Shenduruny WLS 285. 1997; Sasidh., Fl. Periyar Tiger Reserve 369. 1998; Sasidh., Fl. Parambikulam WLS 286. 2002; Mohanan & Sivad., Fl. Agasthyamala 605. 2002; Anil Kumar et al., Fl. Pathanamthitta 442. 2005; N.P.Balakr. & Chakrab., Fam. Euphorbiaceae India 216. 2007; Ratheesh Narayanan, Fl. Stud. Wayanad Dist. 732. 2009. *Croton reticulatus* Heyne *ex* Muell.-Arg. in DC. Prodr. 15: 580. 1866; Hook. f., Fl. Brit. India 5: 380. 1887; Gamble, Fl. Pres. Madras 1314(919). 1925., Fl. India 23: 228. 2012; N.P. Balakrishnan *et al.* **(Plate 9).**

Small tree, up to 4m tall, branchlets terete with stellate scally.densely covered with brown, fimbriate scales. Leaves to 2- 10 cm, ovate-lanceolate, acuminate, rounded at base, bracteate; lateral nerves 6-10 pairs, glands 1 pairs, sessile; petiole 1-3 cm long. Racemes 6 cm long. Male flowers 5 tepals; filaments glaberous ; 3-5mm, biseriate, hairy with stellate hair green; Stamen 1-2 mm long , 12-14 anther, bifid, pubescent, cream; Female flowers pedicelled, 4 mm across; sepals ,5,ovate, obtuse; petals 5,obovate, smaller, tomentose, green; ovary green stellate hairy; styles 3, each 3 fid and then 2 fid, glaberous. Capsule 1.3 x 1 cm, ovoid; seeds 8 x7 mm, oblong, mottled with brown.

Fl. & Fr.: March-October

Vernacular Name: Porivatta

Habit: Small Tree

Habitat: Terrestrial.

Distribution: Peninsular India and Sri Lanka,Kerala:Palakkad,Thrissur,Wayanad,Kollam,Idukki.

4.2. ANATOMICAL CHARACTERIZATION OF SELECTED SPECIES OF *CROTON*

Anatomical studies were done for identifying and comparing the *Croton* species. In the present analysis, anatomy of leaf, petiole, stem and root of selected taxa had been observed. They are as follows.

4.2.1. LEAF ANATOMY

Epidermis is Single layered, continuous except at the region of sunken stomata, composed of elongated barrel shaped uniform cells, thick walled, compactly arranged without intercellular space, covered by thick cuticle. Trichomes present at lower and upper epidermis which is stalked, glandular, unicellular and branched at base. In the mid rib, lower epidermis convex and upper epidermis is linear. Trichomes are absent in the leaves of *C. persimilis* and *C. tiglium*. The upper epidermis is slightly concave and lower epidermis is convex in *C. bonplandianum*. Both epidermis convex in *C. hirtus*.

Mesophylls are differentiated into palisade and spongy parenchyma. Palisade parenchyma oriented towards the upper epidermis, single layered, made up of vertically elongated cells, compactly packed, abundant chloroplast, covered with tannin cells; spongy parenchyma oriented towards lower epidermis, 2-3 layered, made up of cells with circular outline and arranged with intercellular space, few of chloroplast, tannin cells present. Tannin cells are absent and calcium crystals present in *C. bonplandianum*. Spicular cells are present in *C. caudatus, C. tiglium*. Chloroplasts are covered with tannin cells in *C. hirtus*. and also calcium oxalate, spicular cells present.

Chloroplasts abundantly arranged in *C. caudatus*, *C. persimilis* and *C. zeylanicus.*

In midrib, outer layers of cells are made up of 3-4 small sclerenchymatous cells and inner layers of cells are made up of 7-8 layers of parenchyma cells.In *C. caudatus*, midrib is below the upper epidermis sclerenchymatous patch of 6-7 layer of cells at the centre of midrib, followed by 5-6 layer of parenchymatous cells, 9-11 layer of parenchyma cells above the lower epidermis, discontinuous patches of sclerenchyma surrounding the vascular bundle, vascular bundles arranged in a wavy outline, Tannin cells are abundant.

In *C. persimilis*, a small patch of sclerenchyma below the upper epidermis at the centre. It is followed by a continuous layer of chlorenchyma at the upper epidermis, which consist of 2-3 layers of cells. It is then followed by a massive zone of parenchyma all around the midrib. Scattered patches of sclerenchyma cells are present in this parenchymatous zone.

In *C. tiglium*, midrib consist of a sclerenchymatous cap, palisade parenchyma seen just beneath the sclerenchymatous cap, above the lower epidermis 3-4 layered sclerenchymatous cells and inner region made up of parenchymatous cells.

Vascular bundle arranged as two sets of bundles; it is 'U' shaped towards the lower epidermis and seen as another small patch towards the upper epidermis, conjoint, collateral, endarch and closed. Calcium oxalate crystals and sclerids present at vascular region of *C. bonplandianum*. In *C. hirtus*, Vascular bundle 2, arranged oppositly, conjoint, collateral, endarch and closed. Abaxial bundle larger than adaxial bundle. In *C. malabaricus*, vascular bundles are conjoint, collateral, endarch, closed, towards the lower epidermis it is 'U' shaped and towards the upper epidermis two bundles are seen.

Xylem is made up of xylem tracheids, xylem vessels, xylem parenchyma and xylem fibers. In abaxial bundle xylem arranged in U shape, Protoxylem oriented towards centre and metaxylem oriented towards periphery. Protoxylem oriented towards centre and shows spiral thickening, metaxylem oriented towards periphery and shows boardered pitted thickening in *C. bonplandianum* and *C. hirtus*.

Phloem seen outside the xylem and made up of phloem parenchyma which are irregular, unequal in size and compactly packed.

Stomata are paracytic with two unequal subsidiary cells, stomatal pore elliptical in outline and surrounded by two subsidiary cells (**Table 1**).(**Plates 10-17**).

4.2.2. PETIOLE ANATOMY

Epidermis is single layered, composed of elongated barrel shaped uniform cells, thick walled, compactly arranged without intercellular spaces, covered by thick cuticle, epidermal cells gives rise to numerous trichomes which is stalked, glandular, long, unicellular and branched at base. Trichomes are absent in *C. tiglium*.

Cortex consists of 7 – 8 layered parenchyma cells, spherical in shape, unequal in size and arranged without inter cellular spaces. At adaxial side 1 – 2 layer chlorenchyma cells present, unequal in size, irregularly shaped, chloroplast present and arranged with inter cellular space. Calcium oxalate crystals are distributed in *C. hirtus*. 10-11 layers of cells, tannin cells and calcium oxalate crystals are present in *C. aromaticus*. Cortex made up of parenchymatous cells, reduced, 2-3 layered, two bundles present at the cortex, few tannin cells, calcium oxalate crystals and spicular cells are present in *C. malabaricus*. Cortex of *C. tiglium*, reduced, made up of parenchymatous cells, two bundles present at one side, few calcium oxalate crystals and tannin cells

present, small thick walled patches of cells are present as discontinuous ring. In *C. caudatus*, made up of parenchymatous, thin walled, 9-11 layered, large spherical cells with intercellular space, small patches of spicular cells and tannin cells lactiferous are present.

Vascular bundles of *C. bonplandianum*, 4 – 5, triangular in outline, abaxial bundles are much larger than adaxial bundles, bundles are conjoint, collateral, endarch and closed. Calcium oxalate crystals and sclerieds are distributed below the vascular bundle. 6 – 7 vascular bundles, arranged as a circle, conjoint, collateral, endarch and closed in *C.hirtus*. Bundles are 7 in number in which 5 in a circle with wavy outline and 2 are at the two upper sides, conjoint, collateral, endarch and closed in *C. aromaticus.* Vascular bundles of *C. malabaricus*, xylem and phloem seen as continuous ring, two bundles present outside this ring, conjoint, collateral, endarch and closed. Xylem and phloem present as ring, conjoint, collateral, endarch and closed in *C. malabaricus*. Numerous bundles, xylem and phloem present as wavy ring, conjoint, collateral, endarch and closed in *C.caudatus*. arranged as a broken ring, have heart shaped outline in *C. persimilis*, *C. tiglium* and *C. zeylanicus*.

Xylem made up of xylem parenchyma and xylem vessels, vessels are arranged in rows, protoxylem oriented towards center and shows spiral thickening, metaxylem oriented towards the periphery and shows boarderd pitted thickening.

Phloem arranged beneath the xylem as discrete patches, made up of phloem parenchyma, which are small in size and irregular in shape. Arranged just below the xylem as patches. *C. hirtus*, phloem made up of irregular shaped, unequal sized small cells which are compactly packed without inter cellular space. Sclereids and calcium oxalate crystals seen just below the phloem. *C. aromaticus*, phloem seen outside the xylem as wavy ring in the central bundle and circular around outer bundle, tannin cells present.

Pith is parenchymatous with large and spherical cells, tannin cells and calcium oxalate crystals are present in *C. aromaticus*. Massive, parenchymatous, spicular cells and few calcium oxalate crystals are present in *C. malabaricus*. Pith of *C. tiglium* massive, parenchymatous, few tannin cells present. Massive, parenchymatous, thin walled large cells in *C. caudatus.* Similarly parenchymatous, massive tannin cells are present in *C. persimilis* and *C. zeylanicus* (**Table 2**),(**Plates 18-25**).

4.2.3. STEM ANATOMY

Epidermis single layered, composed of elongated barrel shaped uniform cells, thick walled, compactly arranged without inter cellular spaces, covered by thick cuticle and epidermal cells gives rise to numerous trichomes which is stalked, glandular, unicellular and branched at base. Outline wavy or circular. In *C. bonplandianum*, *C. caudatus*, C. *persimilis* and *C. tiglium* trichomes are absent.

Cortex differentiated**.** Outer cortex; 4 – 5 layered, made up of alternate patches of parenchyma and chlorenchyma cells. Parenchyma cells are spherical, unequal in size, thin walled loosely packed and seen just below the ridges. Chlorenchyma cells are 1 -2 layered, uneven, irregularly shaped, chloroplast present, loosely packed and seen just below the furrows. Inner cortex; 5- 6 layered, made up of polygonal parenchyma cells. Which is thin walled, unequal sized, spherical in shape and arranged with inter cellular space. Several patches of sclereids present in between parenchyma cells. Calcium oxalate crystals are unevently distributed. In *C. hirtus*, Outer cortex – 4 – 5 layered, collenchymatous, spherical in shape, uniform, compactly packed without inter cellular space. Inner cortex composed of 5 – 6 layered, parenchymatous, polygonal, unequal in size, arranged with inter cellular space. Calcium oxalate crystals and sclereids distributed in inner cortex.

In *C. aromaticus* and *C. malabaricus*, cortex reduced, differentiated as outer, middle and inner cortex. *C. tiglium* consists of outer cortex is made up of sclerenchymatous cells, 2-3 layered. Inner cortex is chlorenchymatous, 8-9 layered, circular with tannin cells. Patches of spicular cells are seen as discontinuous ring.

Vascular bundle are conjoint, bicollateral, endarch and open. In *C. hirtus*, conjoint, bicollateral, endarch and open.

Xylem is massive, made up xylem tracheids, xylem vessels and xylem parenchyma, arranged in a wavy ring. Protoxylem arranged towards the pith and shows spiral thickening. Metaxylem arranged towards the periphery and shows boarderd pitted thickening. Tannin cells present in *C. aromaticus* and *C. malabaricus*.

Phloem arranged on either side of xylem. Made up of small sized, irregularly shaped cells which are compactly packed.

Pith consists of massive, made up of spherical shaped parenchyma cells which are thin walled, unequal sized and arranged with inter cellular spaces. In *C. aromaticus*, massive, wavy in outline, sometimes seen as intruded into the xylem, made up of parenchymatous cells, round outline, peripheral region has small sized cells and large sized cells are present towards the inner side, tannin cells and calcium oxalate crystals are also present. Few tannin cells present in *C. malabaricus* and *C. persimilis*. Chlorenchymatous cells present in *C.persimilis* and *C.zeylanicus* (**Table 3**),(**Plates 26-33).**

4.2.4. ROOT ANATOMY

Periderm ruptured and forming wide fissures, circular in outline. In *C. hirtus* and *C. caudatus*, periderm is ruptured and discontinuous layer of cells.

Cortex reduced, made up of laterally compressed parenchyma cells which are thin walled and arranged with inter cellular spaces. Sclerenchyma cells are unevenly distributed on cortex. In *C. hirtus*, cortex is 5 – 6 layered, made up of laterally compressed elongated parenchyma cells, thin walled and arranged with inter cellular spaces. Sclerieds present. In *C. caudatus*, 8-10 layers of chlorenchymatous cells followed by irregular shaped collenchymatous cells with starch grains, small and large patches of sclerieds are seen above the phloem. Tannin cells present in *C. persimilis* and *C. zeylanicus*.

Vascular bundles are conjoint, collateral, exarch and open. In *C. caudatus*, numerous vascular bundles present, arranged in a circular fashion, open.

Xylem consists of massive, made up of xylem vessels, xylem tracheids and xylem parenchyma. Metaxylem oriented towards center and shows boarderd pitted thickening. Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present. Medullary rays are uniseriate in *C. hirtus*.

Phloem arranged above the xylem as layer. Which is small sized, irregularly shaped and compactly packed.

Pith reduced, made up of irregularly shaped parenchyma cells which are loosely packed in *C. hirtus*, *C. caudatus* and *C. zeylanicus.* Pith is highly reduced and attached to the vascular bundle in C. *aromaticus*, *C. bonplandianum*, *C. malabaricus*, *C. persimilis* and *C. tiglium* (**Table 4**)**, (Plates 34-41).**

Table 1: Anatomical comparison of different species of *Croton* (Leaf)

Anatomical Features	CA	CB	CC	CH	CM	CP	CT	CZ
Outline	Concave on the upper side and convexly on lower side	Convexly arranged on both side.	Slightly Concave on upper side and convexly on lower side.	Convexly on both side.	Linear on upper side and round at lower side	Concave on upper side and round at lower surface	Concave on the upper side and slightly centrally depressed on lower side	Concave on the upper side and slightly convexly on lower siiude.
Epidermis	Glandular and stalked, unicellular trichomes present on both surface. Paracytic Stomata with two subsidiary cells.	Small sized simple trichomes rarely on the lower epidermis. Paracytic Stomata with two subsidiary cells.	Without any trichomes on both lower and upper epidermis. Paracytic Stomata with two subsidiary cells.	Numerous unicellular trichomes on both lower and upper epidermis. Paracytic Stomata with two subsidiary cells.	Glandular and stalked, unicellular trichomes present on both surface. Paracytic Stomata with two subsidiary cells.	Trichomes absent. Paracytic Stomata with two subsidiary cells.	Trichomes absent. Paracytic Stomata with two subsidiary cells.	Glandular and stalked, unicellular trichomes present on lower surface. Paracytic Stomata with two subsidiary cells.
Vascular bundle	'U' shaped towards the lower	Single, conjoint, collaretal,	conjoint, collateral, endarch,	Oppositly, conjoint, collateral,	Conjoint, collateral, endarch,	Conjoint, collateral	Conjoint, collateral, endarch,	conjoint, collateral,

	epidermis and seen as another small patch towards the upper epidermis, conjoint, collateral, endarch and closed.	endarch, closed and arranged in 'U' shape. Calcium oxalate crystals and sclerieds present at vascular region.	closed, wavy in outline	endarch and closed. Abaxial bundle larger than adaxial bundle.	closed, towards the lower epidermis it is U shaped and towards the upper epidermis two bundles are seen.	and closed, have a wavy outline.	closed, towards the lower epidermis 'U' shaped and towards the upper epidermis bundles are in linear line.	endarch, closed,
Meso phyll	tannin cells; spongy parenchyma oriented towards lower epidermis, 2-3 layered, made up of cells with circular outline and arranged with intercellular space, few	Tannin cells are absent and made up of small irregular cells, arranged with intercellular spaces, chloroplast is very few, Calcium oxalate crystals are present in	Chloroplast present abunduntly. Tannin cells and spicular cells are present.	Chloroplast abund Calcium oxalate crystals present in between palliside and spongy parenchymatous,	Abundant chloroplast, covered with tannin cells; tannin cells and spicular cells present.	Few of chloroplast present.	Abundant chloroplast, covered with tannin cells, spicular cells.	Abundant chloroplast, tannin cells present.

	of chloroplast, tannin cells present.	between palliside and spongy parenchyma.						
Observations	Presence of Tannin cells	Presence of calcium oxalate crystals and sclerieds	Presence of Chloroplast, Tannin cells and spicular cells.	Calcium oxalate present.	Tannin cells and spicular cells present.	Trichomes abs Few of chloroplast present.	Trichomes absent. Tannin cells, spicular cells present.	Tannin cells present.

CA: *Croton aromaticus*, **CB:** *Croton bonplandianum*, **CC:** *Croton caudatus*, **CH:** *Croton hirtus*, **CM:** *Croton malabaricus*, **CP:** *Croton persimilis*, **CT:** *Croton tiglium*, **CZ:** *Croton zeylanicus*.

Table 2: Anatomical comparison of different species of *Croton* (Petiole)

Anatomical Features	CA	CB	CC	CH	CM	CP	CT	CZ
Outline	Almost kidney shaped	Slightly heart shaped	Oval shaped	Slightly Oval shaped	Oval shaped	Oval shaped	Oval shaped	Slightly heart shaped
Epidermis	Numerous trichomes which is stalked, glandular, unicellular and branched at base.	Numerous trichomes which is stalked, glandular, unicellular and branched at base.	Trichomes orientating from epidermal cells are branched at base, glandular.	Numerous trichomes which is stalked, glandular, long, unicellular and branched at base.	Trichomes which is stalked, glandular, unicellular and branched at base.	Numerous trichomes which is stalked, glandular, long, unicellular	Trichomes absent.	Trichomes present.
Cortex	10-11 layers of cells, tannin cells and calcium oxalate crystals are present in *C. aromaticus*.	7 – 8 layered, made up of parenchyma cells, chloroplast present	Made up of parenchymatous, thin walled, 9-11 layered, calcium crystals with intercellular space, small patches of spicular cells and tannin cells lactiferous are present.	7 – 8 layered, made up of parenchyma cells, Calcium oxalate crystals are distributed	made up of parenchymatous cells, reduced, 2-3 layered, two bundles present at the cortex, few tannin cells, calcium oxalate crystals and spicular cells are present	Massive paranchymatous zone, numerous calcium crystals present	reduced, made up of parenchymatous cells, two bundles present at one side, few calcium oxalate crystals and tannin cells present	Made up of parenchymatous cells, two bundles present at both sides and tannin cells present.

Vascular Bundle	Bundles are 7 in number, conjoint, collateral, endarch and closed	4 – 5 vascular bundle, triangular in outline, conjoint, collateral, endarch and closed	Numerous bundles, conjoint, collateral, endarch and closed.	6–7 vascular bundle, arranged as a circle, conjoint, collateral, endarch and closed.	Continuous ring, two bundles present outside this ring, conjoint, collateral, endarch and closed.	7-8 bundles, Conjoint, collateral and closed. Have a wavy outline.	Continuous ring, conjoint, collateral, endarch and closed.	Continuous ring, conjoint, collateral, endarch and closed.
Observations	Numerous trichomes, tannin cells and calcium oxalate crystals are present.	chloroplast present	Tannin cells lactiferous are present.	Calcium oxalate crystals present.	Tannin cells, calcium oxalate crystals	Numerous calcium crystals present	calcium oxalate crystals and tannin cells present	Tannin cells present.

CA: *Croton aromaticus*, **CB:** *Croton bonplandianum*, **CC:** *Croton caudatus*, **CH:** *Croton hirtus*, **CM:** *Croton malabaricus*, **CP:** *Croton persimilis*, **CT:** *Croton tiglium*, **CZ:** *Croton zeylanicus*.

Table 3: Anatomical comparison of different species of *Croton* (Stem)

Anatomical Features	CA	CB	CC	CH	CM	CP	CT	CZ
Shape	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**	**Circular**
Epidermis	Numerous trichomes, stalked, glandular, unicellular and branched at base.	Trichomes absent.	Trichomes absent.	many unicellular trichomes	Numerous trichomes, stalked, glandular, unicellular and branched at base.	Trichomes absent.	Tannin cells are seen as a continuous layer just beneath the epidermis. Trichomes absent.	Trichomes absent.
Cortex	Reduces, 4-5 layered, tannin cells present, spicular cells present. Middle cortex is chlorenchymatous, 4-5 layered, completely filled with tannin cells, calcium oxalate crystals are present.	Uneven, irregularly shaped, chloroplast present, loosely packed and seen just below the furrows. Several patches of sclereids present in between parenchyma cells. Calcium oxalate crystals are	Spicular cells seen in discontinuous patches, tannin cells present abundantly.	Calcium oxalate crystals and sclereids distributed in inner cortex.	Reduced, 5-6 layered, few tannin cells, spicular cells are seen as discontinuous ring.	Tannin cells scattered, Sclerenchymatous patches present.	Inner cortex is chlorenchymatous, 8-9 layered, circular with tannin cells. Patches of spicular cells are seen as discontinuous ring.	8-9 layersed paranchymatous cells. Tanni cells absent

		unevently distributed.						
Vascular Bundle	conjoint, collateral and open	Conjoint, bicollateral, endarch and open.	conjoint, collateral and open	Conjoint, bicollateral, endarch and open.	conjoint, collateral, open and reduced	conjoint, collateral and open	conjoint, collateral and open	conjoint, collateral and open
Phloem	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.	Made up of small sized, irregularly shaped cells which are compactly packed.
Xylem	Bordered pitted thickening, tannin cells present.	Bordered pitted	Tannin cells absent	Bordered pitted	Tannin cells present.	Tannin cells absent	Tannin cells absent	Tannin cells absent
Pith	Tannin cells and calcium oxalate crystals are present.	Tannin cells absent	Tannin cells absent	Tannin cells absent	Tannin cells are abundantly present.	Tannin cells absent	Few tannin cells present	Tannin cells absent
Observations	Presence of tannin cells and calcium oxalate.	Calcium oxalate crystals present.	-	Calcium oxalate crystals and sclerieds present.	Numerous trichomes Tannin cells present.	Trichomes absent	-	Trichomes absent.

CA: *Croton aromaticus*, **CB:** *Croton bonplandianum*, **CC:** *Croton caudatus*, **CH:** *Croton hirtus*, **CM:** *Croton malabaricus*, **CP:** *Croton persimilis*, **CT:** *Croton tiglium*, **CZ:** *Croton zeylanicus*.

Table 4: Anatomical comparison of different species of *Croton* (Root)

Anatomical Features	CA	CB	CC	CH	CM	CP	CT	CZ
Outline	Circular	Circular	Circular	Circular	Circular	Circular	circular	circular
Periderm	Ruptured and forming wide fissures.	Ruptured and forming wide fissures.	Ruptured and discontinuous layer of cells.	Ruptured and forming wide fissures.	Ruptured and forming wide fissures.	Ruptured and forming wide fissures.	Ruptured and forming wide fissures.	Ruptured and forming wide fissures.
Cortex	Parenchymatous, few sclerenchymatous patches present.	Reduced, made up of laterally compressed parenchyma cells, sclerenchyma cells are unevenly distributed.	8-10 layers of chlorenchymatous cells followed by irregular shaped collenchymatous cells with starch grains, small and large patches of sclerieds are seen above the phloem.	Made up of laterally compressed elongated parenchyma cells, Sclereids present.	Parenchymatous.	Parenchymatous, Sclerenchymatous patches present. Tannin cells are scattered.	Parenchymatous, few sclerenchymatous patches present.	Parenchymatous. Tannin cells present

Vascular Bundle	conjoint, collateral, exarch and open	conjoint, collateral, exarch and open	Numerous vascular bundles present, arranged in a circular fashion, open	conjoint, collateral, exarch and open	conjoint, collateral, exarch and open	conjoint, collateral, exarch and open	conjoint, collateral, exarch and open	conjoint, collateral, exarch and open
Phloem	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	parenchyma cells with deposition of starch grains are present outer to xylem,	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.	Made up of phloem parenchyma cells, seen just above the xylem which is made up of small sized, irregularly shaped cells and arranged compactly.

Xylem	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are uniseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.	Protoxylem oriented towards periphery and shows spiral thickening. Medullary rays are biseriate. Growth rings are also present.
Pith	Highly reduced, attached to vasclarbundle.	Highly reduced, attached to vasclarbundle.	reduced, made up of irregularly shaped parenchyma cells which are loosely packed	reduced, made up of irregularly shaped parenchyma cells which	Highly reduced, attached to vasclarbundle.	Highly reduced, attached to vasclarbundle.	Highly reduced, attached to vasclarbundle.	reduced, made up of irregularly shaped parenchyma cells which are loosely packed

				are loosely packed				
Observations	-	-	Numerous vascular bundles	-	-	-	-	-

CA: *Croton aromaticus*, **CB:** *Croton bonplandianum*, **CC:** *Croton caudatus*, **CH:** *Croton hirtus*, **CM:** *Croton malabaricus*, **CP:** *Croton persimilis*, **CT:** *Croton tiglium*, **CZ:** *Croton zeylanicus*.

4.3. PHYTOCHEMICAL CHARACTERIZATION OF SELECTED SPECIES OF CROTON

4.3.1. QUALITATIVE PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis results detailed aspects of phyto chemical constituents present in the selected species of genus *Croton*. Alkaloids were present in most of the species while it is absent in stem of *C. malabaricus*, *C. tiglium* and *C. persimilis* and leaf of *C. zeylanicus*. Similarly Coumarins are present in root extracts of *C. aromaticus, C. bonplandianum*, *C. persimilis* while it was absent in remaining species. In the case of stem extracts coumarins are absent in *C. hirtus* and *C. malabaricus.* But it is present in remaining taxon. In the case of leaf extracts, coumarins were absent in *C. malabaricus*, *C. tiglium* and *C. zeylanicus*, and it is present in other species. Flavonoids are major phyto constituents, it is present in root extracts of *C. caudatus*, *C. persimilis* and *C. zeylanicus*. While Flavonoids are present in all stem extracts of selected species of *Croton* except in *C. hirtus* and *C. zeylanicus*. Similarly flavonoids are present in leaf extracts of *C. aromaticus*, *C. caudatus* and *C. persimilis*. Likewise glycosides are other kind of phyto-constituents which are present in all of them except *C. zeylanicus*. Besides that, Phenols were present in all of them except root extracts of *C. bonplandianum, C. aromaticus*, *C. caudatus*, stem extracts of *C. aromaticus, C. bonplandianum, C. caudatus*, *C. hirtus*, and *C. zeylanicus*. and also leaf extracts of *C.aromaticus*, *C.bonplandianum*, *C.caudatus* and *C.zeylanicus*. The Phyto constituent like quinones were absent in all root extracts of *Croton* species except in *C. bonplandianum*, *C. aromaticus*, *C. malabaricus* and *C. tiglium*. In the case of phytochemical analysis of stem, quinones are present in *C. bonplandianum*, *C. caudatus* and *C. tiglium* whereas it was absent in remaining species. During the analisis of phytochemical constituents in the leaf revealed that, quinones are present in *C. aromaticus*, *C. hirtus* and *C. persimilis* whereas it becomes absent

in *C. bonplandianum*, *C. caudatus*, *C. malabaricus*, *C. tiglium* and *C. zeylanicus*. Another important phytochemicals like saponine are absent in most of the taxa, and they were present in *C. caudatus,* leaf of *C. zeylanicus*, *C. malabaricus* and *C. tiglium*. Similarly steroids are present in most of them, in which they are absent in root of *C. caudatus*, stem of *C. persimilis*, *C. bonplandianum and* leaf of *C. zeylanicus*. Also tannins were present in most of them like the roots of *C. caudatus* and *C. malabaricus*, stem of *C. aromaticus*, *C. caudatus*, *C. persimilis*, *C. malabaricus* and *C. tiglium*. Similarly tannins were absent in the leaves of *C. hirtus*, *C. malabaricus* and *C. zeylanicus etc*. Terpenoids were present in all of them whereas it was absent in roots of *C. aromaticus*, *C. malabaricus, C. tiglium, C. zeylanicus*, stem of *C. zeylanicus* and leaf of *C. bonplandianum* respectively (**Table-5**).

Table 5- Preliminary phytochemical analysis of selected species of *Croton*

Sl. No	Phytochemical constituents	Name of test	Root								Stem								Leaf							
			CA	CB	CC	CH	CM	CT	CP	CZ	CA	CB	CC	CH	CM	CT	CP	CZ	CA	CB	CC	CH	CM	CT	CP	CZ
1	Alkaloids	Mayer's test	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-
		Hager's test	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-
		Wagner's test	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-
2	Coumarins		+	+	-	-	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-
3	Flavonoids	Alkaline reagent test	-	-	+	-	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	-	-	-	+	-
		Lead acetate test	-	-	+	-	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	-	-	-	+	-
4	Glycosides	Keller killani's test	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
5	Phenol	Ferric chloride test	-	-	+	-	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	+	-
		Lead acetate test	-	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	+	+	+	-
6	Quinones		+	+	-	-	+	+	-	-	-	+	+	-	-	+	-	-	+	-	-	+	-	-	+	-
7	Saponins	Foam test	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	+	-	+	+	-	+
8	Steroids	Salkowski's test	-	+	-	+	-	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-
9	Tannins	Braymer's test	-	-	+	-	+	-	-	-	+	-	+	-	+	+	-	-	+	+	+	-	-	+	+	-
10	Terpenoids	Copper acetate test	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+

CA: *Croton aromaticus*, **CB:** *Croton bonplandianum*, **CC:** *Croton caudatus*, **CH:** *Croton hirtus*, **CM:** *Croton malabaricus*, **CP:** *Croton persimilis*, **CT:** *Croton tiglium*, **CZ:** *Croton zeylanicus*.

4.3.2. Quantitative Phytochemical Analysis

Comparative High Performance Thin Layer Chromatography (HPTLC) Analysis

The comparative HPTLC analysis results the quantity of phytoconstituents, which are present in the selected plant extracts. The details of the obtained results are given below (**Table 6, 7 &8**).

Table. 6 Comparative analysis of R_f values (HPTLC) observed under UV 254 nm

Sl. No	Rf	254nm																							
		CAL	CAS	CAR	CBL	CBS	CBR	CCL	CCS	CCR	CHL	CHS	CHR	CML	CMS	CMR	CPL	CPS	CPR	CTL	CTS	CTR	CZL	CZS	CZR
1	0.01	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
2	0.02	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.03	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
4	0.04	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
5	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
6	0.06	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0.07	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0.09	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
9	0.10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
10	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
11	0.12	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
12	0.13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
13	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
14	0.15	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
16	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0.19	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
18	0.20	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	0

Sl. No	Rf	254nm																						
		CAL	**CAS**	**CAR**	**CBL**	**CBS**	**CBR**	**CCL**	**CCS**	**CCR**	**CHL**	**CHS**	**CHR**	**CML**	**CMS**	**CMR**	**CPL**	**CPS**	**CPR**	**CTL**	**CTS**	**CTR**	**CZL**	**CZS**	**CZR**
19	**0.21**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	**0.22**	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
21	**0.23**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
22	**0.24**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	**0.25**	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
24	**0.26**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
25	**0.27**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
26	**0.28**	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
27	**0.29**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
28	**0.30**	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
29	**0.31**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
30	**0.32**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
31	**0.33**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1
32	**0.35**	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0
33	**0.37**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
34	**0.38**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
35	**0.39**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
36	**0.40**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
37	**0.41**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
38	**0.42**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
39	**0.43**	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	**0.44**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
41	**0.45**	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	**0.46**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
43	**0.48**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
44	**0.49**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
45	**0.50**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
46	**0.51**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
47	**0.53**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
48	**0.54**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	**0.56**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0
50	**0.58**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Sl. No	Rf	254nm																						
		CAL	CAS	CAR	CBL	CBS	CBR	CCL	CCS	CCR	CHL	CHS	CHR	CML	CMS	CMR	CPL	CPS	CPR	CTL	CTS	CTR	CZL	CZS	CZR
51	**0.60**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
52	**0.62**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
53	**0.63**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	**0.64**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	**0.65**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
56	**0.66**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
57	**0.67**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
58	**0.69**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
59	**0.70**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	**0.71**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
61	**0.72**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	**0.74**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
63	**0.75**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
64	**0.76**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
65	**0.77**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
66	**0.79**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	**0.80**	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	**0.81**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
69	**0.82**	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0
70	**0.83**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	**0.84**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
72	**0.85**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	**0.86**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
74	**0.87**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
75	**0.89**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
76	**0.91**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
77	**0.92**	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1
78	**0.93**	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	**0.95**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
80	**0.96**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
81	**1.07**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	**1.16**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
83	**1.24**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Sl. No	Rf	254nm																						
		CAL	**CAS**	**CAR**	**CBL**	**CBS**	**CBR**	**CCL**	**CCS**	**CCR**	**CHL**	**CHS**	**CHR**	**CML**	**CMS**	**CMR**	**CPL**	**CPS**	**CPR**	**CTL**	**CTS**	**CTR**	**CZL**	**CZS**	**CZR**
84	**1.28**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	**1.31**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	**1.37**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
87	**1.43**	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	**1.44**	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	**1.48**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	**1.56**	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	**1.57**	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
92	**1.60**	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
93	**1.65**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	**1.72**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	**1.66**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	**1.75**	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	**1.81**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	**1.83**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
99	**1.91**	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
100	**1.92**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
101	**1.96**	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Table. 7 Comparative analysis of R_f values (HPTLC) observed under UV 366 nm

Sl.No.	Rf	366nm																						
		CAL	CAS	CAR	CBL	CBS	CBR	CCL	CCS	CCR	CHL	CHS	CHR	CML	CMS	CMR	CPL	CPS	CPR	CTL	CTS	CTR	CZL	CZS	CZR
1	0.01	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.02	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
3	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.04	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0.07	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0.09	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
8	0.10	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
9	0.11	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0
10	0.12	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
11	0.13	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0.14	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
13	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.16	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
15	0.17	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
16	0.18	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
17	0.19	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0
18	0.20	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0
19	0.21	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
20	0.22	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0.23	1	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1
22	0.24	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
23	0.25	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
25	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

26	**0.28**	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	**0.29**	0	1	1	0	0	0	1	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
28	**0.30**	1	0	0	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0		0	0	0
29	**0.32**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
30	**0.33**	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
31	**0.35**	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	**0.36**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
33	**0.37**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
34	**0.38**	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
35	**0.39**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	**0.40**	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
37	**0.41**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
38	**0.42**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
39	**0.43**	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1
40	**0.44**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	**0.45**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	**0.46**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
43	**0.48**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
44	**0.50**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
45	**0.51**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	**0.52**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	**0.53**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	**0.54**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	**0.55**	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0
50	**0.56**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	**0.58**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
52	**0.60**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	**0.61**	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0

54	**0.62**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	**0.63**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
56	**0.64**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	**0.65**	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	**0.66**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	**0.67**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
60	**0.68**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
61	**0.70**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	**0.72**	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	**0.73**	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
64	**0.74**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
65	**0.79**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
66	**0.80**	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
67	**0.81**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
68	**0.82**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0
69	**0.83**	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
70	**0.84**	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
71	**0.85**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
72	**0.86**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
73	**0.87**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
74	**0.88**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
75	**0.91**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1
76	**0.92**	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
77	**0.93**	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	**0.94**	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1
79	**0.95**	1	0	1	0	0	0	1	1	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0
80	**0.96**	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
81	**0.97**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
82	**0.98**	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

83	**0.99**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	**1.05**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	**1.14**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	**1.17**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	**1.26**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	**1.38**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
89	**1.45**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
90	**1.48**	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
91	**1.57**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	**1.58**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	**1.60**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	**1.62**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
95	**1.66**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
96	**1.67**	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
97	**1.70**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
98	**1.75**	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
99	**1.80**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	**1.88**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	**1.91**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
102	**1.94**	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
103	**1.95**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table. 8 Comparative analysis of R_f values (HPTLC) observed under UV 550 nm

Sol. No.	Rf	550nm																						
		CAL	CAS	CAR	CBL	CBS	CBR	CCL	CCS	CCR	CHL	CHS	CHR	CML	CMS	CMR	CPL	CPS	CPR	CTL	CTS	CTR	CZL	CZS	CZR
1	0.01	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.02	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
3	0.04	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.05	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
5	0.06	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0
6	0.07	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	1
7	0.08	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
8	0.09	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0.10	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0
10	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
11	0.12	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
12	0.13	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
13	0.14	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
14	0.16	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0.17	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
16	0.18	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0.19	0	0	0	1	0	1	0	0	0	1	1	1	1	0	1	0	1	1	0	0	1	0	0	0
18	0.20	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
19	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
20	0.22	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
21	0.23	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
22	0.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
23	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
24	0.26	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
25	0.27	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

26	**0.28**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	**0.29**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
28	**0.30**	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
29	**0.31**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
30	**0.32**	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0
31	**0.33**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	**0.34**	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
33	**0.35**	1	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0
34	**0.36**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
35	**0.37**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	**0.38**	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0
37	**0.39**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
38	**0.40**	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	**0.41**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	**0.42**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
41	**0.43**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
42	**0.44**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
43	**0.45**	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
44	**0.46**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
45	**0.47**	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
46	**0.48**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	**0.49**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
48	**0.50**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
49	**0.51**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
50	**0.52**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
51	**0.53**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
52	**0.56**	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0
53	**0.57**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0

54	**0.58**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
55	**0.59**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
56	**0.60**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
57	**0.61**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
58	**0.62**	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0
59	**0.63**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	**0.64**	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
61	**0.67**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	**0.68**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
63	**0.69**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
64	**0.71**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
65	**0.72**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
66	**0.73**	0	0	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
67	**0.74**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
68	**0.76**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
69	**0.77**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	**0.78**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0
71	**0.79**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
72	**0.80**	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0
73	**0.81**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	**0.83**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
75	**0.84**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
76	**0.85**	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0
77	**0.86**	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
78	**0.87**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	**0.88**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	**0.89**	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	**0.90**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
82	**0.91**	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0

83	**0.92**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
84	**0.93**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
85	**0.94**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
86	**0.95**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0
87	**0.96**	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
88	**0.97**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
89	**0.98**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
90	**1.06**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	**1.09**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
92	**1.10**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
93	**1.13**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	**1.14**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
95	**1.15**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
96	**1.17**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
97	**1.26**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
98	**1.28**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
99	**1.30**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
100	**1.31**	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
101	**1.41**	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	**1.43**	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
103	**1.44**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4.3.2. I. Comparative HPTLC Analysis of *Croton* Species at 254nm

In the case of 254nm, bands at Rf 1.48 and 1.66 are specifically observed for *C. aromaticus* leaf. Bands at RF 0.64, 1.31 and 1.65 are specifically noticed for *C. aromaticus* stem. Bands at Rf 0.06 and 0.15 are specifically present in roots of *C. aromaticus.* A compound at Rf 1.24 unique for leaf of *C. caudatus*. Bands at Rf 0.07 and 0.24 specifically noticed for roots of *C. caudatus.* Bands at Rf 0.02 observed only for stem of *C. hirtus*. Bands at Rf 0.58, 0.65, 0.89 and 1.16 specifically observed for leaf of *C. malabaricus*. Bands at Rf 1.37 noticed only for stem of *C. malabaricus.* Bands at Rf 0.05, 0.26, 0.40 and 0.76 observed for roots of *C. malabaricus*. Bands at Rf 0.44, 0.53, 0.66 and 0.75 specifically observed for leaf of *C. persimilis*. A compound at Rf 0.87 unique for stem of *C. persimilis.* Bands at Rf 0.41, 0.48 and 0.95 observed for roots of *C. persimilis*. Bands at Rf 0.23, 1.83 noticed for leaf of *C. tiglium*. Bands at Rf 0.51, 0.74, 0.81 observed for leaf of *C. zeylanicus*. Bands at Rf 0.71 and 0.96 specifically present in stem of *C. zeylanicus*. Bands at Rf 0.69 and 0.77 specifically observed for *C. zeylanicus*.

4.3.2. II Comparative HPTLC Analysis of *Croton* Species at 366nm

A compound at Rf 0.25 unique for root of *C. bonplandianum*. Bands at Rf 0.39, 0.51 and 0.54 observed for leaf of *C. caudatus*. Bands at Rf 0.13 and 0.45 roots of *C. caudatus*. A compound at Rf 0.87 unique for leaf for *C. persimilis*. Bands at Rf 0.27, 0.36, 0.58 and1.62 noticed for leaf of *C.tiglium*.0.63 and 1.70 observed for stem of *C. tiglium*. Bands at Rf 0.32, 0.68, 0.74 and 0.81 observed for leaf of *C. zeylanicus*.

4.3.2. III Comparative HPTLC Analysis of Croton Species at 550nm

In the case of 550nm, band of at Rf 0.09, 0.48 and 0.63 observed for leaf of *C. aromaticus*. A compound at Rf 1.06 unique for stem of *C. aromaticus.* Bands at Rf 0.33, 0.81 and 0.89 noticed for root of *C. aromaticus*. Bands at Rf

0.37 and 0.77 observed for leaf of *C. caudatus*. Bands at Rf 0.16, 0.28, 0.46 and 0.87 observed for stem of *C. caudatus*. Bands at Rf 0.04, 0.41and 0.67 observed for roots of *C. caudatus*. Bands at Rf 0.51, 0.71 and 0.98 specifically for stem of *C. malabaricus*. Bands at Rf 0.36, 0.43 and 0.76 observed

for roots of *C. malabaricus*. A compound at Rf 0.61 unique for leaf of *C. persimilis*. Bands at Rf 0.24 and 0.53 observed for stem of *C. persimilis*. A compound at Rf 0.31unique for root of *C. persimilis*. Bands at Rf 0.60 and 1.10 observed for leaf of *C. tiglium*. Bands at Rf 0.93 and 1.09 observed for stem of *C. tiglium*. A compound at Rf 0.74 unique for leaf of *C. zeylanicus*.

4.4. DENDROGRAM OF *CROTON* SPECIES BASED ON HPTLC ANALYSIS

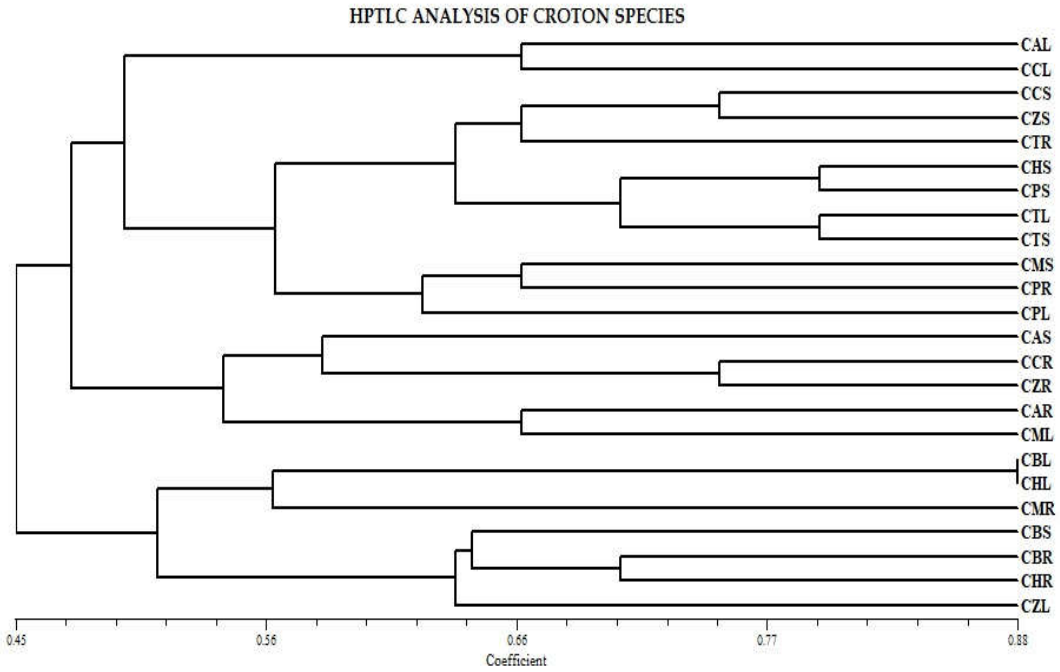


Fig:2 Dendrogram constructed by analysis of R_F values from UPGMA **cluster analysis**

CAL: *C. aromaticus* Leaf**, CAS:** *C. aromaticus* Stem**, CAR:** *C. aromaticus* Root**. CBL:** *C. bonplandianum* Leaf**, CBS:** *C. bonplandianum* Stem**, CBR:** *C.*

bonplandianum Root**. CCL:** *C. caudatus* Leaf**, CCS:** *C. caudatus* Stem**, CCR:** *C. caudatus* Root**. CHL:** *C. hirtus* Leaf**, CHS:** *C. hirtus* Stem**, CHR:** *C. hirtus* Root**. CML:** *C. malabaricus* Leaf**, CMS:** *C. malabaricus* stem**, CMR:** *C. malabaricus* Root**. CPL:** *C. persimilis* Leaf**, CPS:** *C. persimilis* Stem**, CPR:** *C. persimilis* Root**. CZL:** *C. zeylanicus* Leaf**, CZS:** *C. zeylanicus* Stem**, CZR:** *C. zeylanicus* Root**.**

The graphical representation of the results of the analysis of R_F values of solvent system using NTSYSpc2.02 for Eight *Croton* species in the form of dendrogram, is shown in Figure. Based on results from UPGMA cluster analysis, dendrogramic analyses the chemical correlation in between the leaf, stem and root of the Croton species. Root of *C. aromaticus* and leaf of *C. malabaricus* can be said to be closely related to each other, followed by stem and root of *C. bonplandianum* being the next closest one. Leaf of *C. bonplandianum* showed maximum similarity with leaf of *C. hirtus* with a coefficient correlation of 0.8%. Roots of *C. caudatus* and *C. zeylanicus* being the next closest parts. Roots of *C. bonplandianum* and *C. hirtus* being the next closest species. Followed by stem of *C.aromaticus* and leaf of *C. persimilis* can be said to be next closest species. Roots of *C.persimilis* and stem of *C. malabaricus* being the next closest one. Stem and leaf of *C. tiglium* being the next closest one. Stem of *C. persimilis* and *C. hirtus* said to be next closest pair. Roots of *C. tiglium* and stem of *C. zeylanicus* being the next closest one. Stem and leaf of *C. caudatus* said to be next closest pair. Leaf of *C. zeylanicus* is most distant with leaf of *C. aromaticus* with coefficient correlation of 0.5% and 0.49% respectively **(Fig.2).**

4.5. ETHNO BOTANICAL INFORMATION

The ethno medicinal properties of *Croton* species have a great role in traditional medico practices.

Croton bonplandianum Baill has many medicinal procedures including as insecticide. It has also anti-bacterial, anti-fungal, anti-oxidant, analgesic, nematicide, anti-coronary, hepatoprotective and wound healing activities. In

West Bengal, tribal persons utilises its root as a remedy for snake bite and the leaf extract used as a treatment for high fever. *C. bonplandianum* have been used to treat liver diseases, ring worm and dermatitis. Leaves of this plant have huge therapeutic property and are utilized for regulating blood pressure, cuts and wounds. The seeds of *C. bonplandianum* are used for the medicine of severe constipation, jaundice, abdominal dropsy and internal abscesses. The fresh plant extract is used by folk inhabitants to cure head ache. The plant is also applied by certain migratory employees for the cure of skin disorders. Generally, the plant extract is used to remedy for helminthiasis and toothache. A varied variety of diseases are caused by bacteria such as cholera, tetanus, diphtheria, tuberculosis, typhoid fever, etc. Several antibiotics isolated from plant extract have succeeded great antimicrobial activity against a vast spectrum of pathogenic bacteria. *C. bonplandianum* has genotoxic and anti-microbial activities (Das *et al.*, 2017).

Traditionally, *Croton caudatus Geiseler* applied as medicine for malaria, ardent fever, convulsions, rheumatic arthritis, and numbness, medicine for wounds, bowel complaints, dysentery, liver infection, sinusitis and gastrointestinal problems etc. (Shantabi & Jagetia , 2015).

Raw seed kernel of *C. tiglium* L. comprises of 55-57% croton oil. Oil of *C. tiglium* seeds is documented to contain phorbol esters and crotonic acid beside with the fatty acids. The dosage of 1 mL oil produces mortality in human. The elements are recorded to have gastrointestinal tract pain and consequently subjected for severe purgative action. These elements are oil soluble and hence, may be removed by cow milk during the process of Śhodhana in the present study in Shodhita Jayapala. It may be speculated that the reduction in the toxicity of *C. tiglium* seeds is due to the reduction of the level of these two compounds along with the other one (Vekariya *et al*, 2017).

4.6. DISCUSSION

The present study on morphological account on selected species of *Croton* shows some identical as well as some varied features on their morphological characters of habit, leaves, flowers etc. It can helps to recognise and identify the taxa from the field.

While observing the anatomical features, some of the charecters are common for all selected species of *Croton*, and some are varying with respect to the trichomes, depositions of secondary metabolites like tannin and calcium oxalate etc. These characters are also used to authentify the selected taxa taken for the present study. In addition to that, pharmacological studies are also support the identification and confirmation of species selected for the present analysis.

Similar observations on morphological features of *Croton mollis* were carried out by Vitarelli *et al.* (2021), they observed macro-morphological features and leaf micro-morphology under light microscopy and scanning electron microscopy. Their results demonstrated that *C. mollis* represented as morphological attributes are identical in compared to related species in an area. They also observed that five secretory structures such as extrafloral nectaries, colleters, idioblasts, glandular trichomes and laticifers are also identical in their leaves. This may help to survive this taxa in changing climatic conditions (Vitarelli *et al.,* 2021).

Likewise, the related studies were conducted on other Euphorbiaceae members like *E. aulacosperma* and *E. rhabdothosperma* by Pahlevani & Akhani, (2011). In this study, the seed morphology of such species was done by using stereomicroscopy and scanning electron microscopic studies. This can also helps to identify the taxa selected for the present analysis (Pahlevani & Akhani, 2011).

The morphology and anatomy of the flower of *Dalechampia alata*, (Euphorbiaceae) was done by Martins *et al.* (2016). The colleters present in the staminate flower of *Dalechampia* consists of a bunch of small secretory glands. Similarly, the histochemical analysis reveals, lipidic and resinuous secretions from the glands are also characteristic to identify the selected taxa for the present study.

Studies on different species of *Jatropha (*Euphorbiaceae) through SEM analysis revealed that all such species taken for present study resulted as basic paracytic stomata, excluding *J. fremontioides*, consists of anisocytic stomata. According to them, true paracytic stomata are found in subgenus *Curcas* and Brachy paracytic stomata in Subgenus *Jatropha*. Such significant characters are usually used to identify various members belongs to the family Euphorbiaceae (Dehgan 1980).

Leaf epidermal morphology of *Jatropha* species was studied by using both light and scanning electron microscopy by Olowokudejo, (1993). His observations revealed that, the adaxial and abaxial epidermal cells of most species are generally polygonal structure with either straight or curved anticlinal walls. In some species wax present in the form of either particles or plugs, while in others prominent cuticular striations are present which may be parallel or random, trichomes are uniseriate. The presence of stalked glands on leaf margins is unique to *J. gossypiijolia*. The close relationship between *J. neriijulia* and *J. atacorenis* proved with respect to some anatomical similarities. He also discussed about the importance of various taxonomic charecters which leads to the identification and clarification of species and their affinities (Olowokudejo 1993).

The leaf anatomy of different *Manihot* species were studied by da Cunha Neto *et al*. (2014), by using optical and scanning electron microscopic analysis. The characters like, number of vascular bundles, shape of petiole,

distribution of secretory idioblasts, presence of papillae and shape of midrib etc. are common features of taxonomical significance. (da Cunha Neto *et al*., 2014).

Histochemical analysis on leaf and stem of *Croton bonplandianum Baill.* and *Croton gracilipes Baill* through scanning electron and light microscopic revealed that, the presence of neutral and acidic lipids, as well as phenolic compounds were present in their latex have to be considered for taxonomical importance in the selected taxa *C. bonplandianum Baill.* and *C. gracilipes Baill* (Rosa *et al*., 2021).

Histochemical studies on *Alchornea triplinervia* (Euphorbiaceae) reveals that, the characteristic features like multi layered sclerenchymatous patches in petiole, gelatinous fibres with hygroscopic walls all along the central vascular system, thicker cuticle, abundance of tannin in the mesophyll, thicker palisade and spongy parenchyma with compactly arranged spongy parenchyma etc. can be used to identify authenticity of selected taxa for the present analysis. Such anatomical significances also co-relates with its ecological attributes (Rôças *et al*., 1997).

The preliminary phytochemical analysis on selected *Croton* species revealed that, phytochemicals are randomly present in the extracts of different plant parts like root, stem and leaves. The quantitative analysis of these phytochemicals shows a characteristic Rf values, which are unique in some extracts and at the same time they are common for all extracts too. Based up on the Rf values obtained during the analysis are also used to prepare a dengrogram **(Fig. 2)**, which gives the detailed picture of quantitative characterization of phytochemicals which are present in the selected species of *Croton*.

The HPTLC analysis on two taxa belongs to Euphorbiaceae like *Euphorbia hirta* and *Euphorbia thymifolia* shows total phenolic contents (TPC) and total flavonoid contents (TFC) was abundantly present in the methanolic and ethanolic extracts of *E. hirta* rather than *Euphorbia thymifolia*. Besides these, the secondary metabolites like Rutin, quercetin, and gallic acid were present in both plants. This phytochemical characterization is also used by the authors to authenticate the selected taxa for further analysis. (Vadalia *et al.,* 2020).

The phytochemical studies on *Phyllanthus fraternus* by Kavit *et al*., (2013), showed the alkaloids like morphine and boldine were present in chloroform leaf extract of the selected taxa. This being also support and proved the application of leaf extract in traditional medicinal practices to prepare various herbal formulations. Similarly, HPTLC analysis on *Jatropha glandulifera* L. revealed specific bioactive compounds which are present in the different parts of the selected taxa are generally used as a biomarker to standardize the taxa for further phytochemical and pharmacological investigations (Dwivedi *et al*., 2020).

Phyllanthus amarus is a significant medicinal plant and widely used in the Indian system of medicine to treat various diseases. Various types of secondary metabolites which are found in the different organs of *Phyllanthus* populations were analyzed by using NTSYS-pc program to examine phytochemical variations with respect to distinct geographical areas. The present results showed that, the population P18 shows much more phytochemical variations than P3 and P6. The present results also highlights phytochemical variations are directly correlates with both habit and habitats of many plant population (Khan *et al*., 2011).

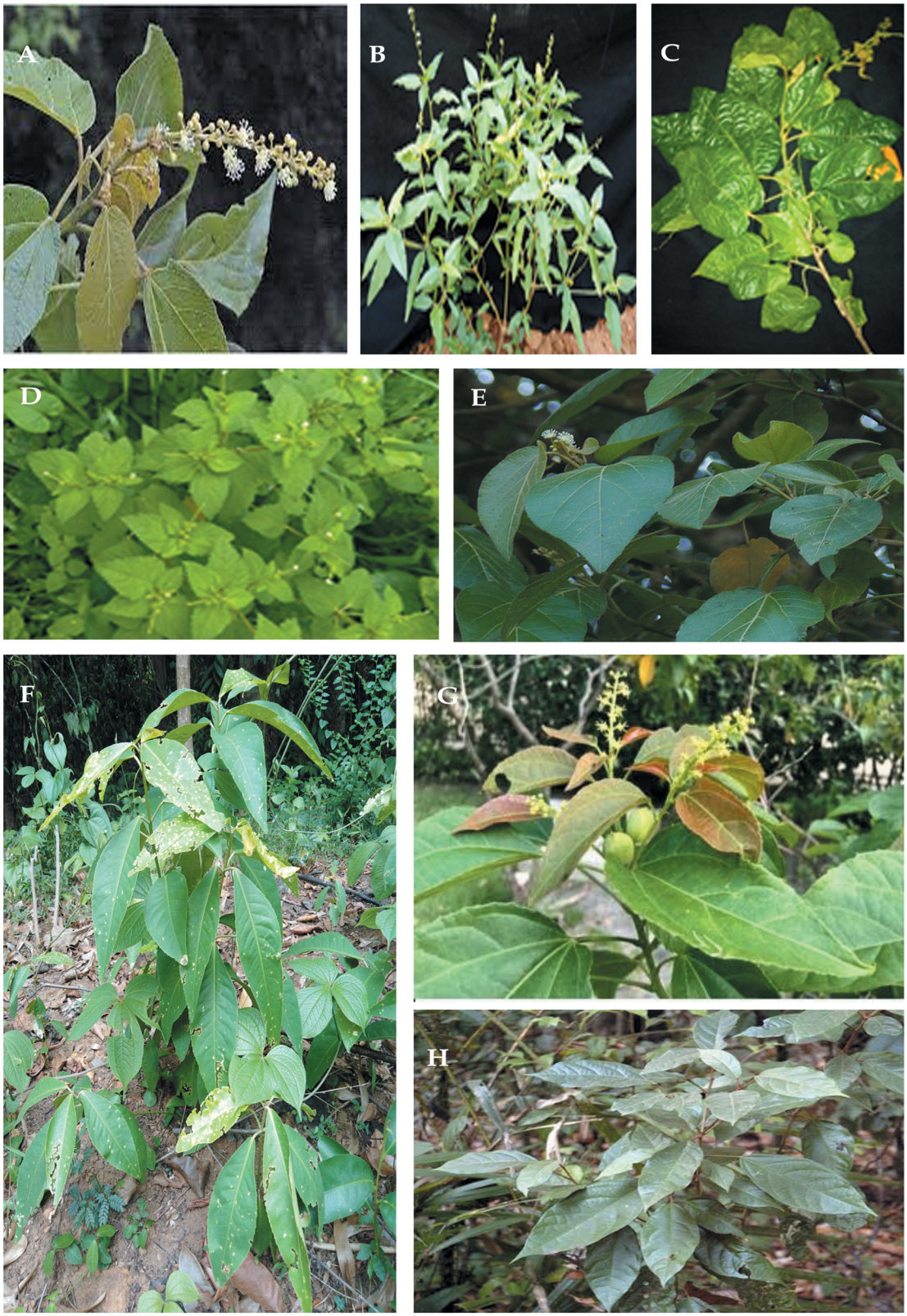


Plate 1: Taxa selected for the present study: **A**. *C.aromaticus*; **B**. *C.bonplandianum*; **C**. *C.caudatus*; **D**. *C.hirtus*; **E**. *C.malabaricus*; **F**. *C.persimilis*; **G**. *C.tiglium*; **H**. *C.zeylanicus*.

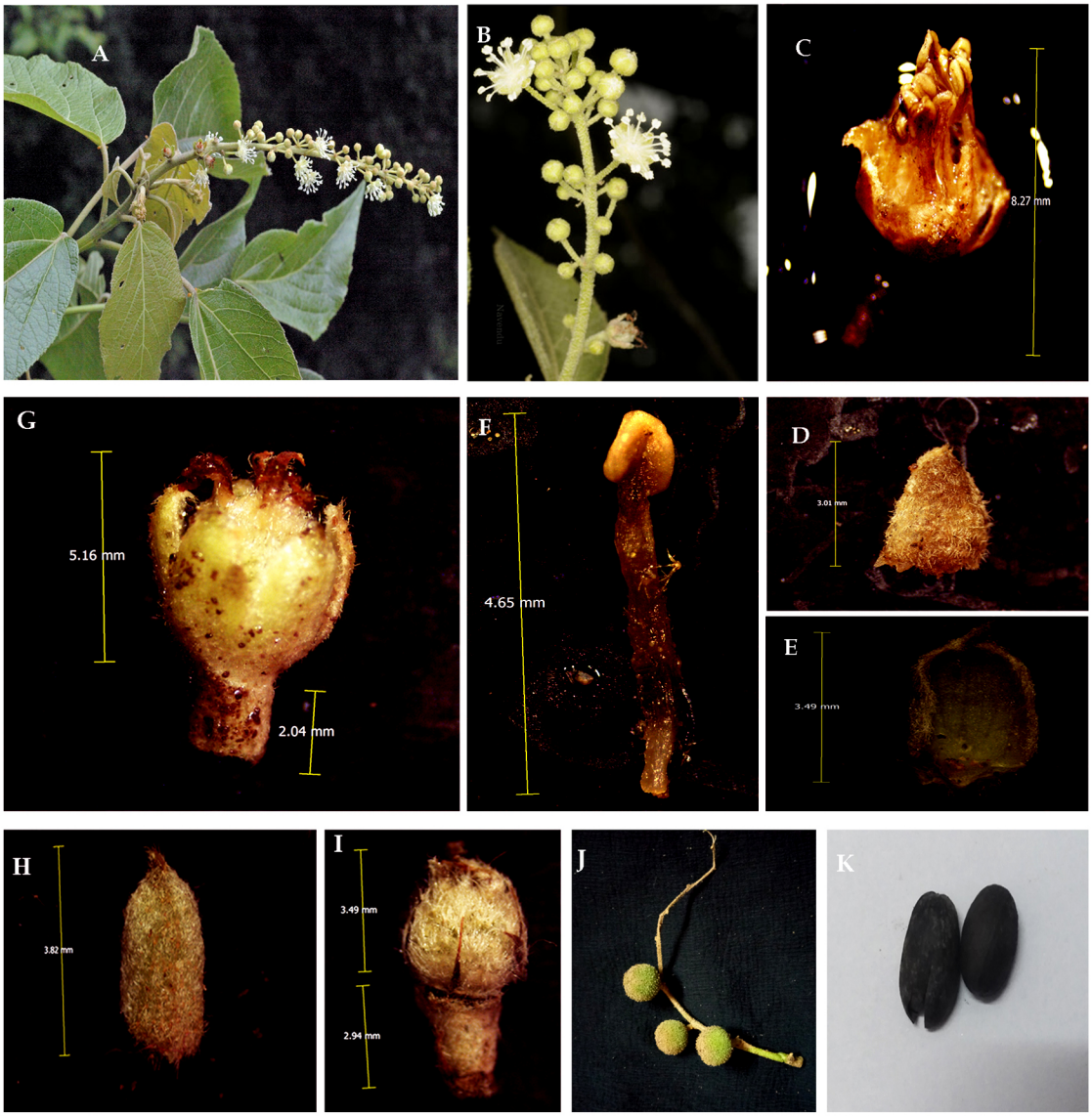


Plate 2: ***Croton aromaticus*****.**L- **A.** Habit; **B.** Inflorescence; **C.** Male Flower; **D**. Inner layer Tepal; **E.** Outer layer Tepal; **F**. Stamen; **G**. Female Flower; **H**. Tepal; **I**.Gynoecium; **J.** Mature Fruit; **K**.Seeds.

Plate.3 : ***Croton bonplandianum***..Baill-**A** . Habit; **B**. Inflorescence; **C**. Male Flower; **D**.Outer layer Tepal; **E**. Inner layer Tepal; **F**. Stamen; **G.** Female Flower,; **H**. Tepal; **I.** Gynoecium; **J.** Mature Fruit; **K**. Seeds.

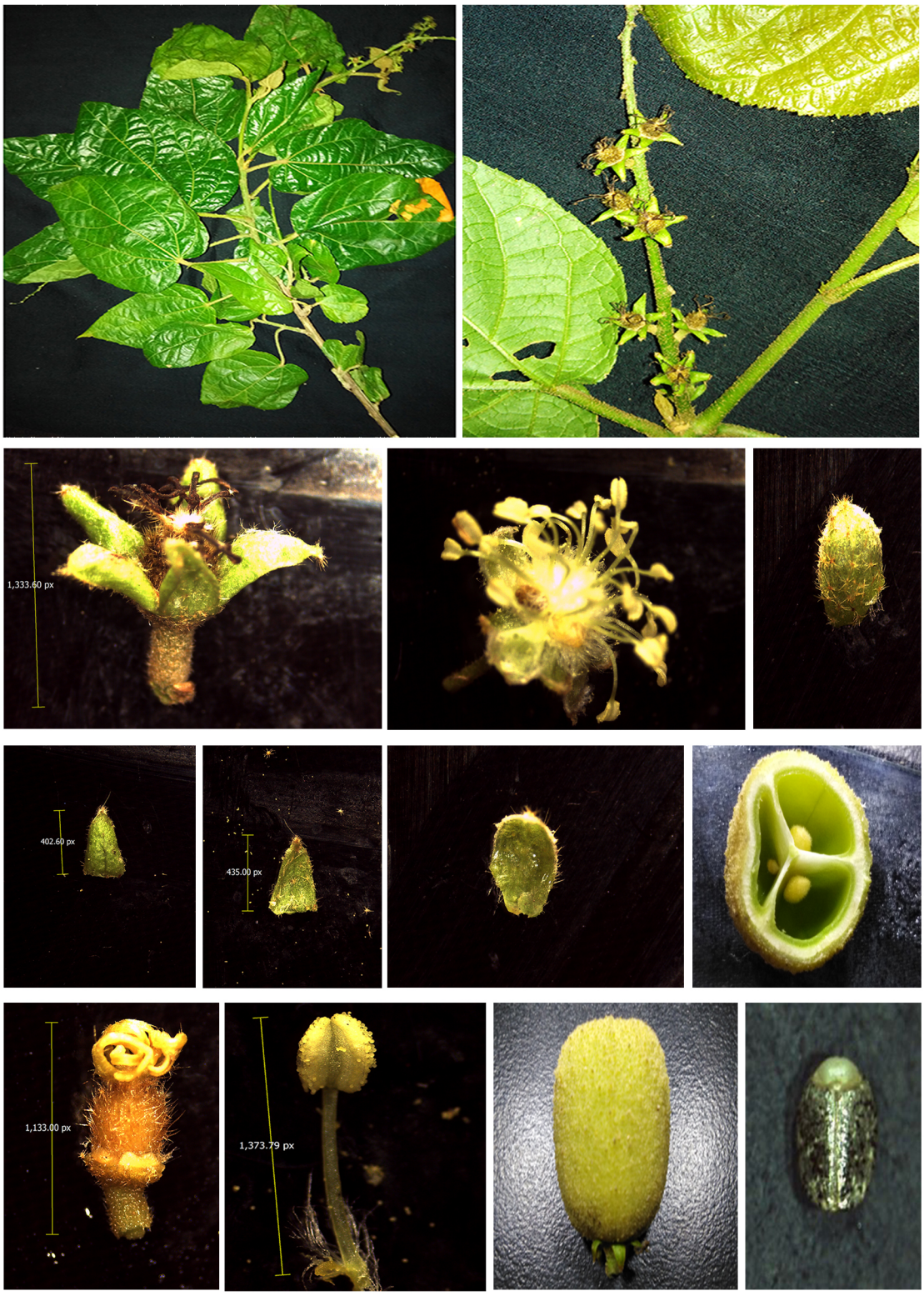


Plate 4: ***Croton. caudatus Geisel***.- **A**. Habit; **B**. Inflorescence; **C**. Female Flower; **D**. Outer layer of Tepal; **E**. Inner layer of Tepal; **F**. Gyneocium; **G**. Male Flower; **H**. Outer layer of Tepal; **I**. Innerlayer of Tepal; **J**. Stamen; **K**. Fruit; **L**. C.S. of Fruit; **M.**Seed.

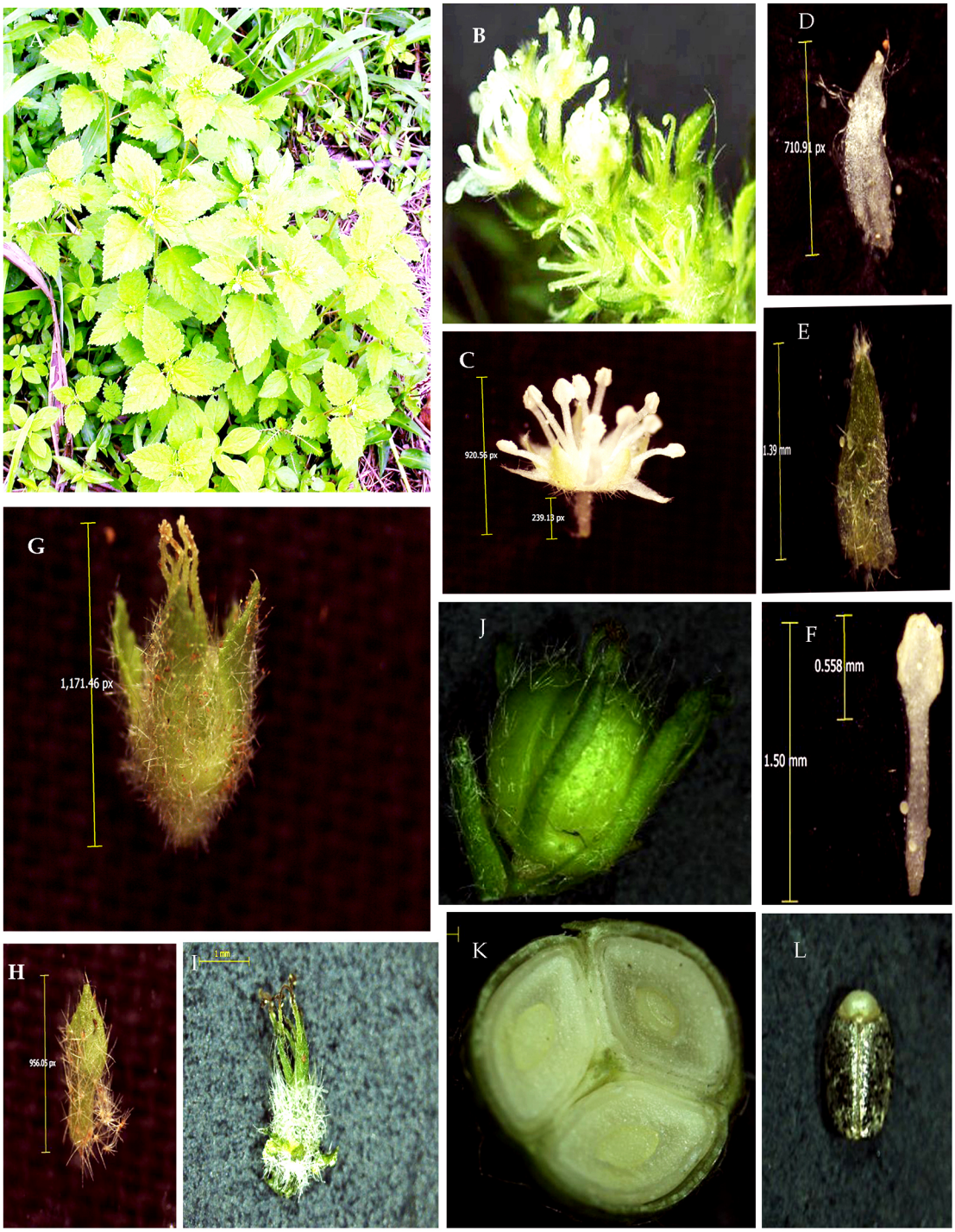


Plate. 5: ***Croton hirtus*** L ' Herit. -**A**.Habit; **B**. Inflorescence; **C**. Male Flower; **D**. Inner layer Tepal; **E**. Outer layer Tepal; **F**. Stamen; **G**. Female Flower; **H**. Tepal; **I**. Gynoecium; **J**. Mature Fruit; **K**. Seed.

Plate. 6. ***Croton malabariicus***. Bedd. -**A**. Habit; **B**. Inflorescence; **C**. Male Flower; **D**. Inner layer Tepal; **E**. Outer layer Tepal; **F**. Stamen; **G**. Female Flower; **H**. Tepal; **I**. Gynoecium; **J**. Mature Fruit; **K**. Seed.

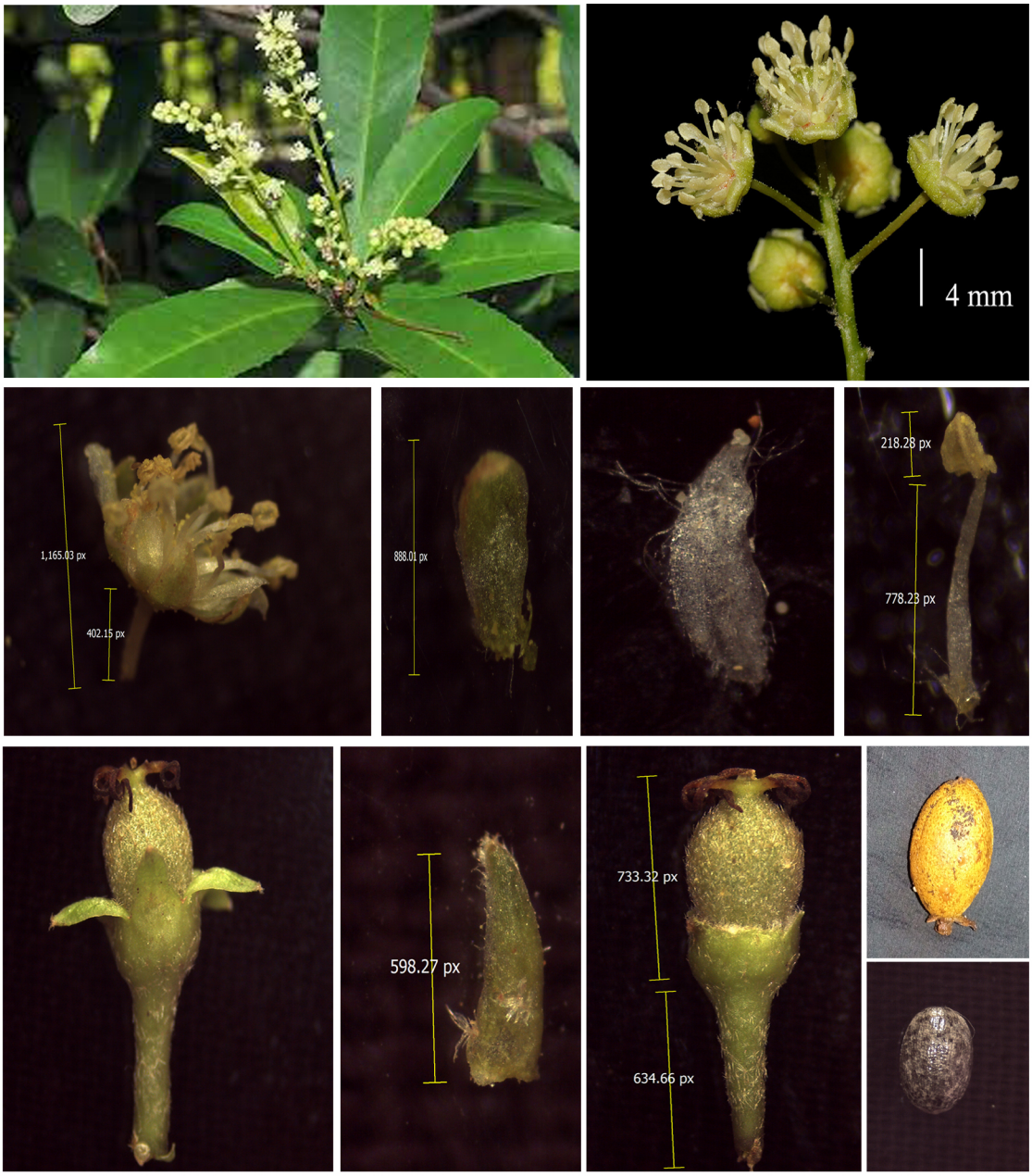


Plate 7: ***Croton. persimilis*** **Mull**.Arg - **A**. Habit; B: Inflorescence, C: Male flower; **D.** Inner layer Tepal; **E**. Outer layer Tepal; **F**. Stamen; **G**. Female flower; **H**. Tepal, **I**. Gynoecium; **J**. Mature Fruit; **K**. Seed.

Plate 8: ***Croton. tiglium*** L - **A**. Habit; **B**. Inflorescence; **C**. Male flower; **D**1 Inner layer Tepal; **E**. Outer layer Tepal; **F**. Stamen; **G**. Female flower; **H**. Tepal; **I**. Gynoecium; **J**. Mature Fruit; **K**. Seed.

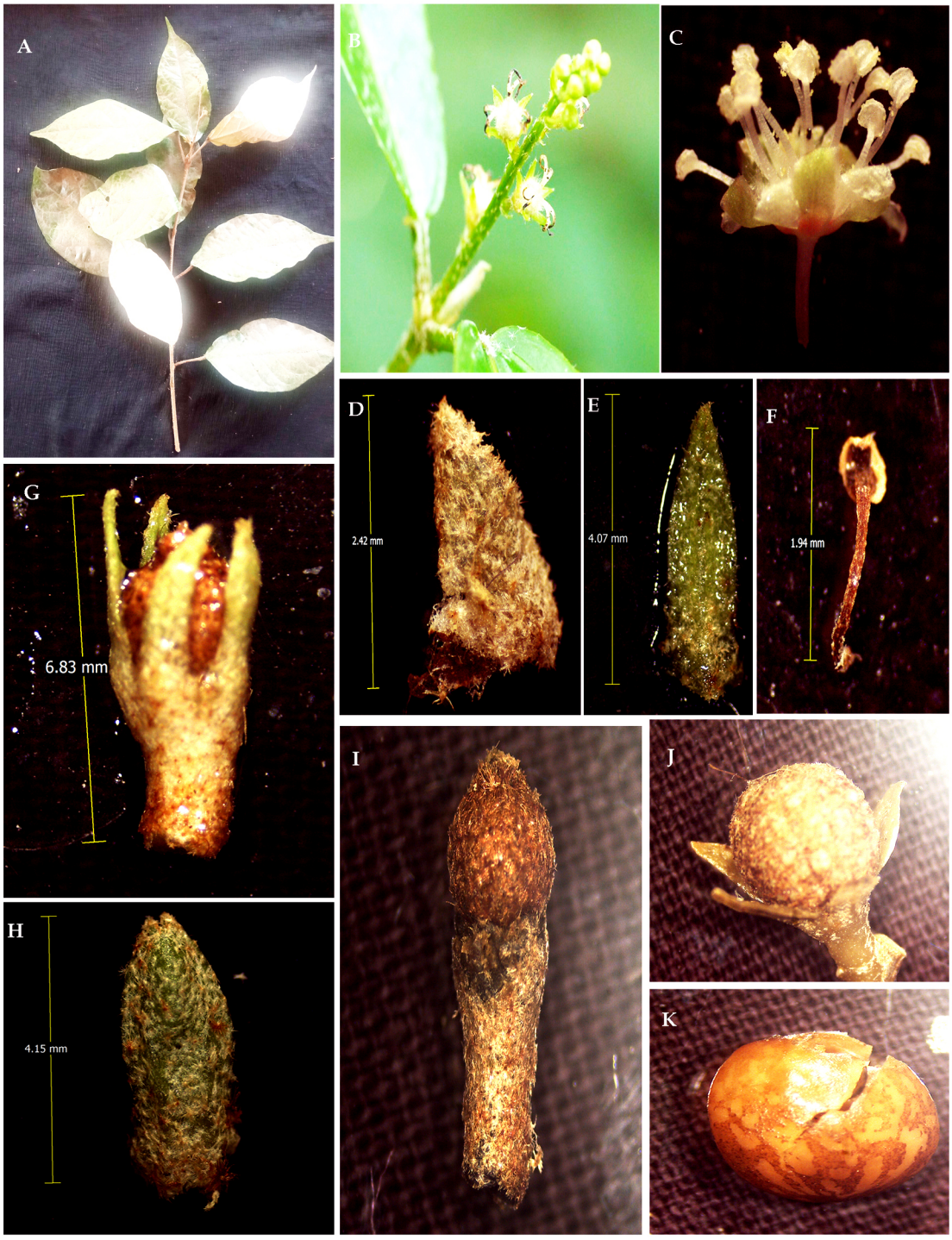


Plate 9. ***Croton. zeylanicus*** Mull.Arg - **A.** Habit; **B**. Inflorescence; **C**. Male flower; **D**. Inner layer Tepal; **E**. Outer layer Tepal; **F**. Stamen; **G**. Female flower; **H**. Tepal; **I**. Gynoecium; **J**. Mature Fruit; **K**. Seed.

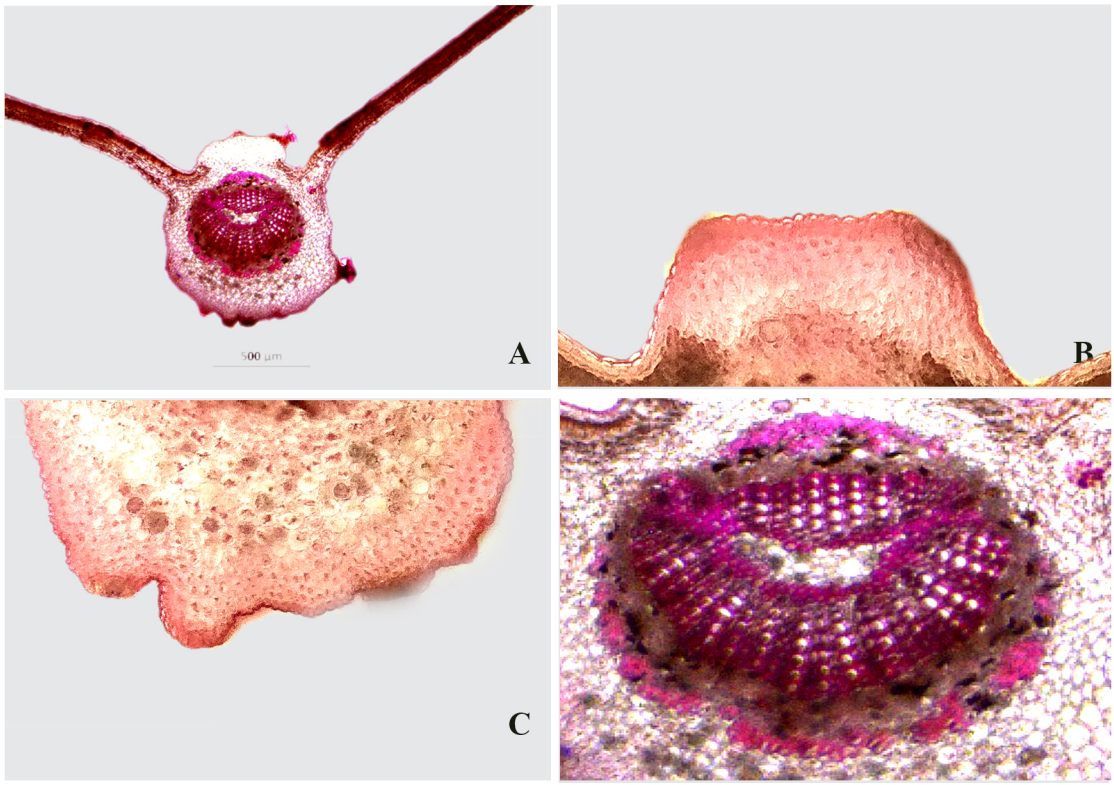


Plate 10: Anatomical Study of *C. aromaticus* Leaf- A. T.S. of leaf, **B.** Portion showing protruded upper region, **C.** Portion showing protruded lower region, **D.** Portion showing ground tissue in mid rib region.

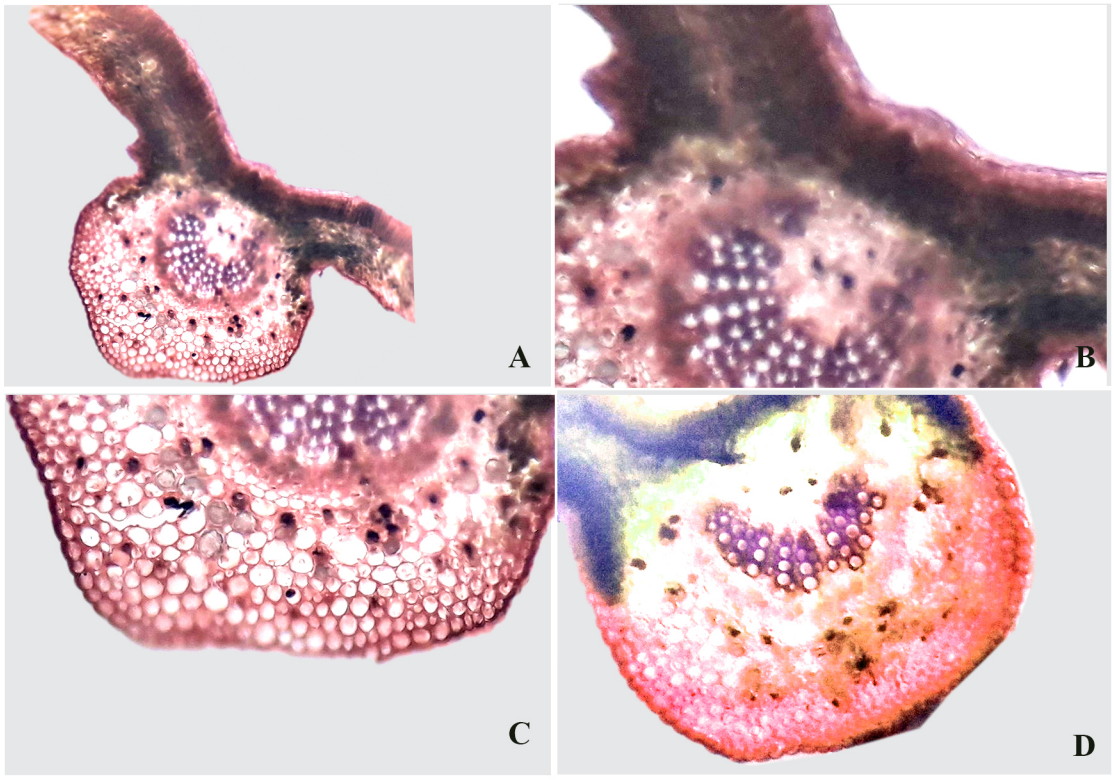


Plate 11: Anatomical Study of *C. bonplandianum Leaf*- A. T.S. of leaf; **B.** Portion showing protruded upper region; **C.** Portion showing protruded lower region; **D.** Portion showing ground tissue in mid rib region.

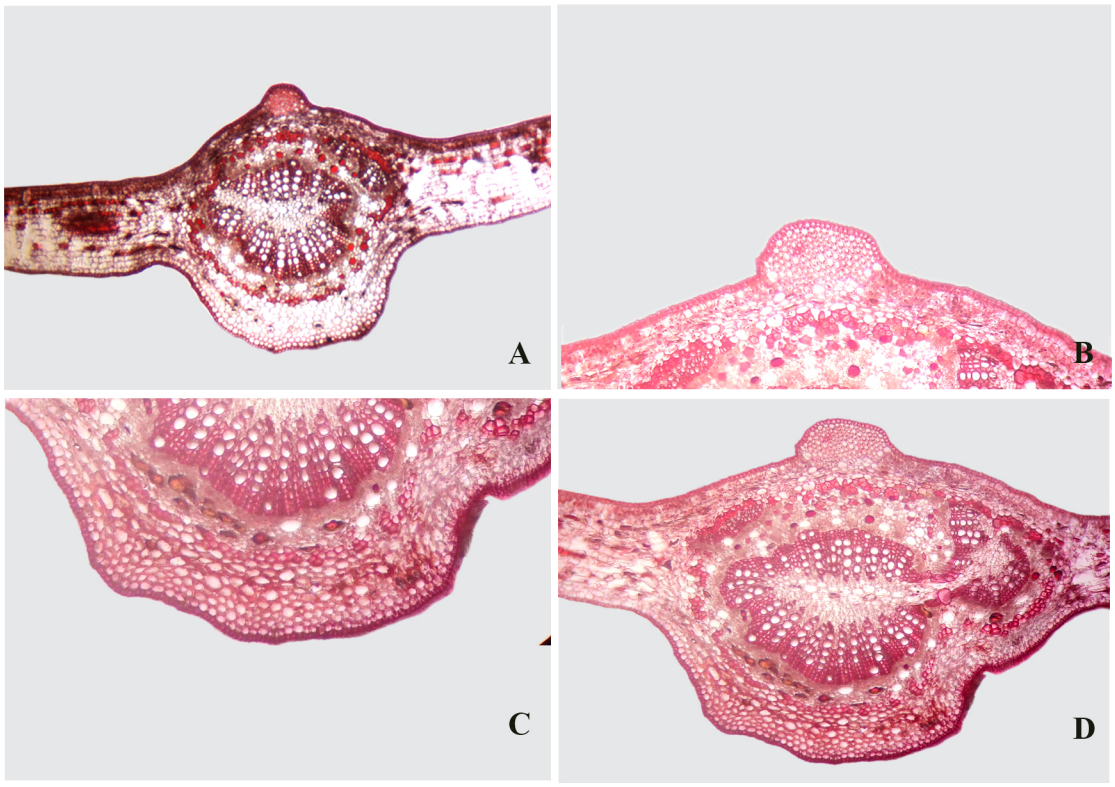


Plate 12: Anatomical Study of *C. caudatus* Leaf- A. T.S. of leaf; **B.** Portion showing protruded upper region; **C.** Portion showing protruded lower region; **D.** Portion showing ground tissue in mid rib region.

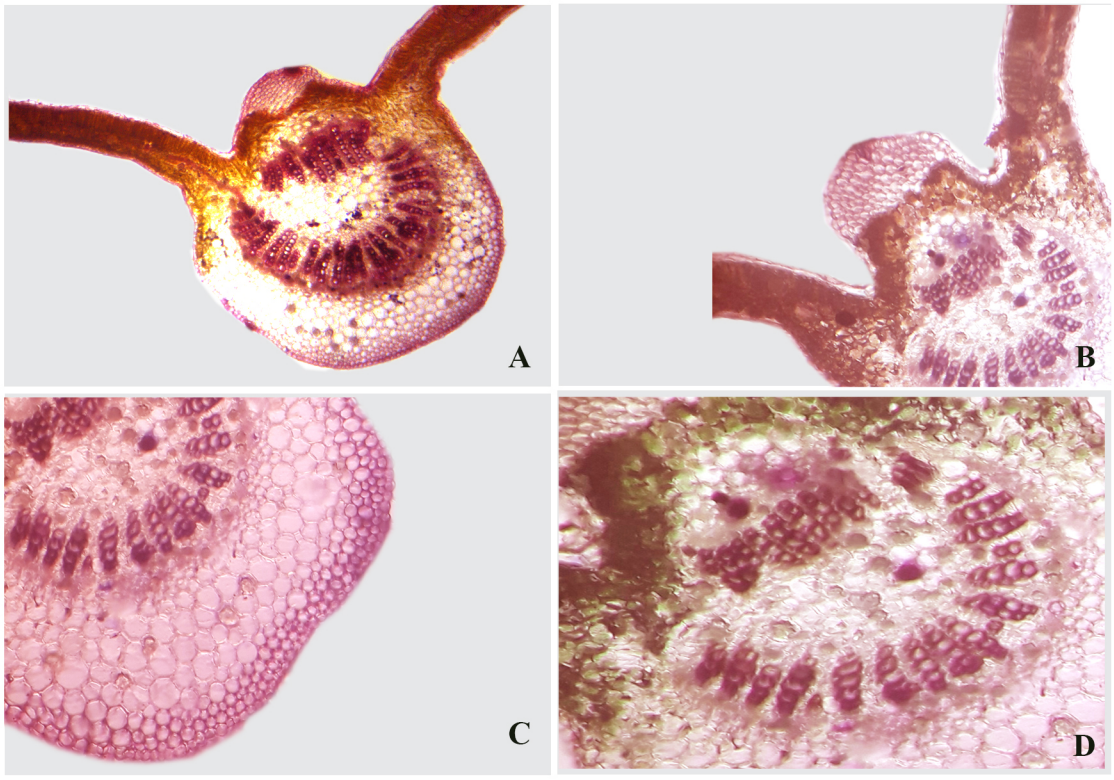


Plate 13: Anatomical Study of *C. hirtus* Leaf- A. T.S. of leaf; **B.** Portion showing protruded upper region; **C**. Portion showing protruded lower region; **D.** Portion showing ground tissue in mid rib region.

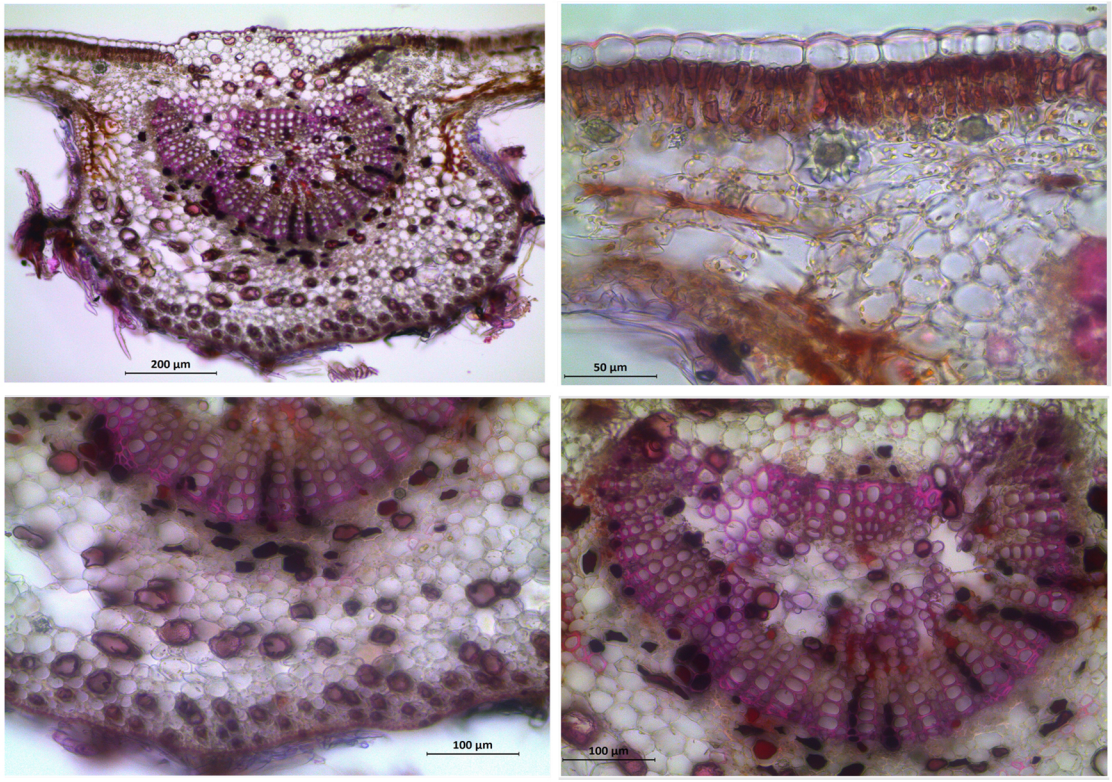


Plate 14: Anatomical Study of *C. malabaricus* Leaf- A. T.S. of leaf; **B.** Portion showing protruded upper region; **C.** Portion showing protruded lower region; **D.** Portion showing ground tissue in mid rib region.

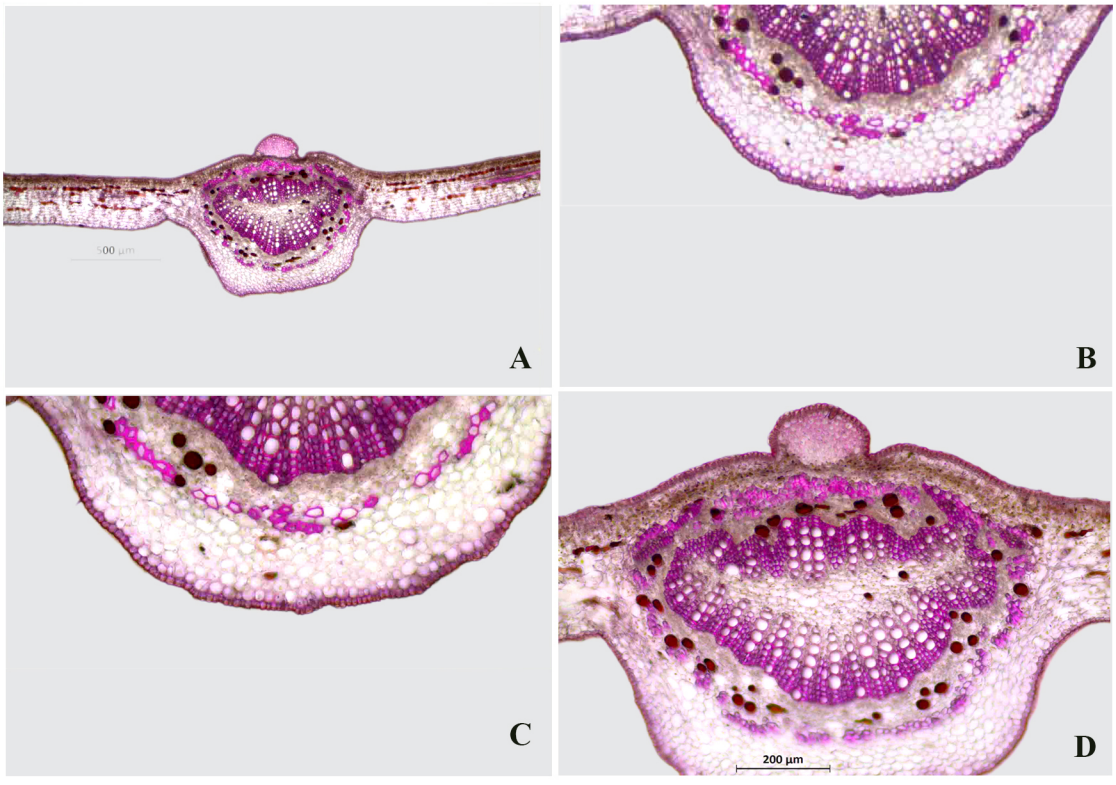


Plate 15: Anatomical Study of *C. persimilis* Leaf- A.T.S. of leaf, **B.** Portion showing protruded upper region, **C.** Portion showing protruded lower region, **D.** Portion showing ground tissue in mid rib region.

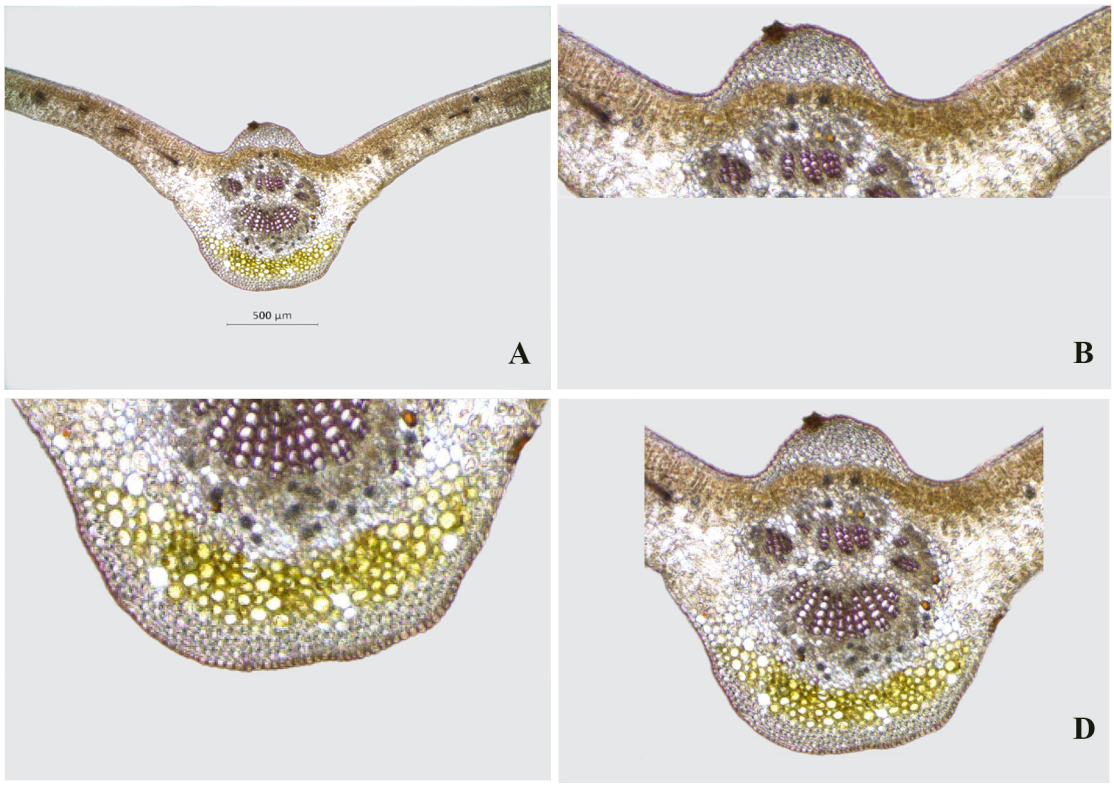


Plate 16: Anatomical Study of *C. tiglium* Leaf- A. T.S. of leaf; **B**. Portion showing protruded upper region; **C**. Portion showing protruded lower region; **D**. Portion showing ground tissue in mid rib region.

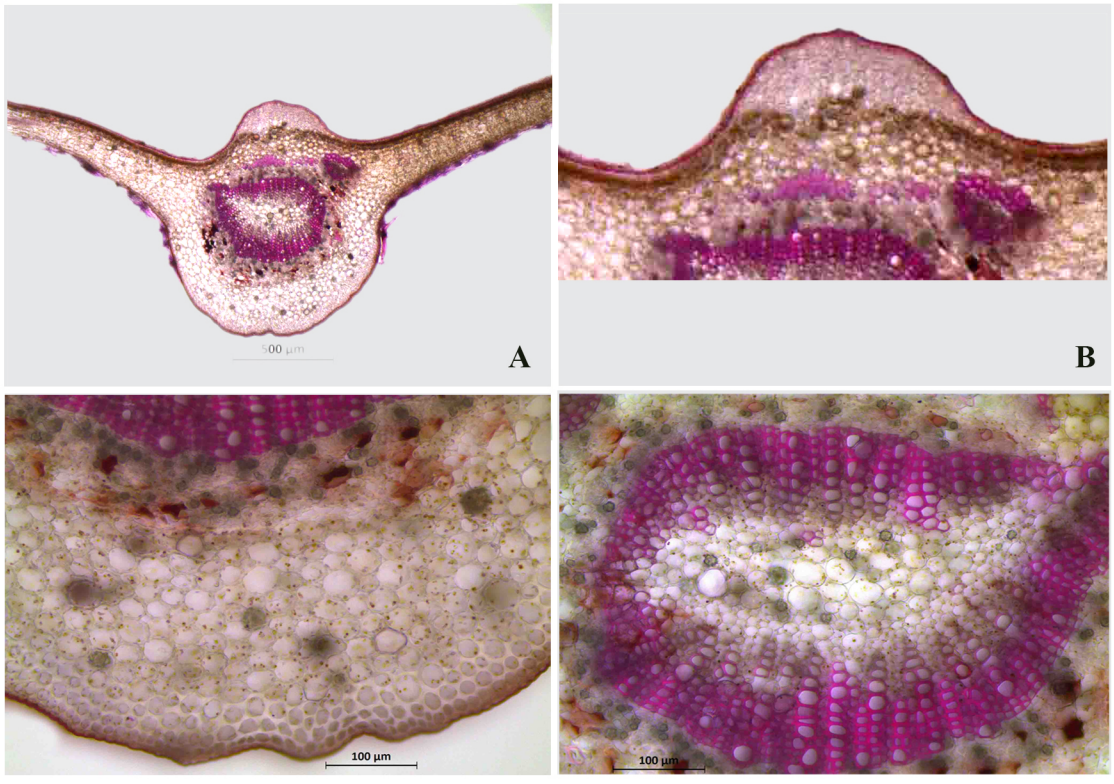


Plate 17: Anatomical Study of *C. zeylanicus* Leaf- A. T.S. of leaf; **B**. Portion showing protruded upper region; **C**. Portion showing protruded lower region; **D**. Portion showing ground tissue in mid rib region.

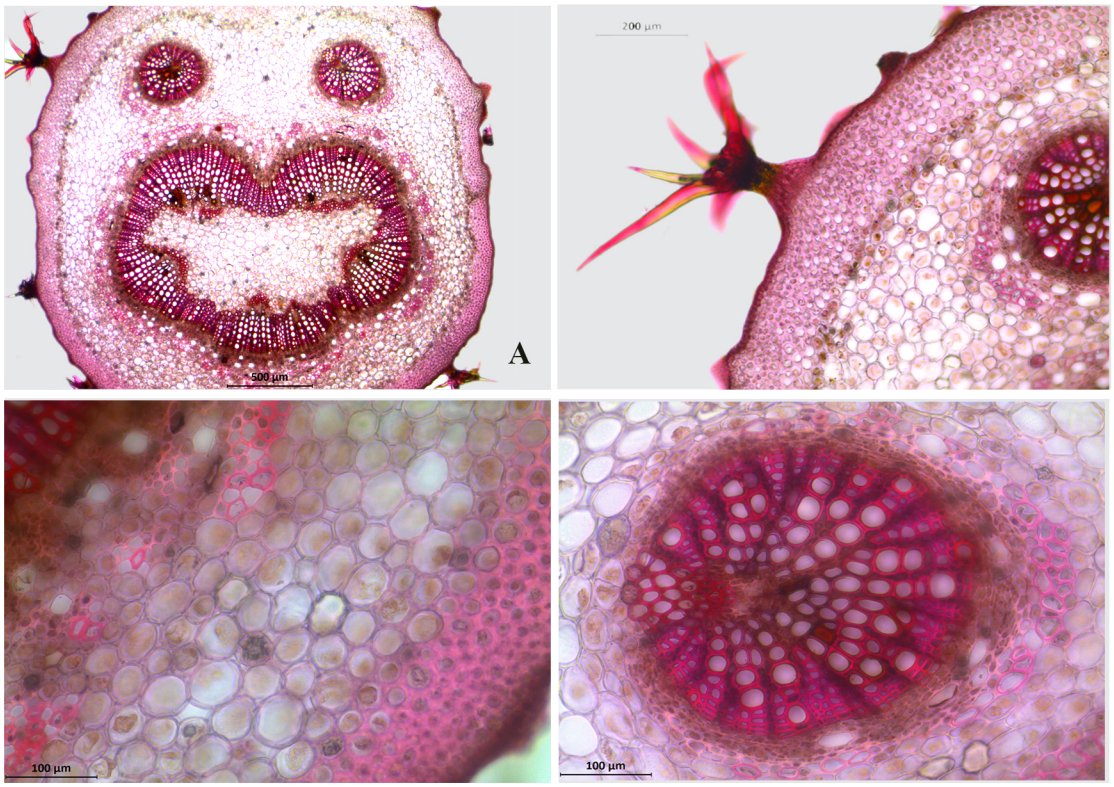


Plate 18: Anatomical Study of *C. aromaticus* Petiole- A. T.S. of petiole; **B**. Enlarged view of projection bearing trichomes; **C**. Portion showing discontinuous hypodermis;, **D**. Enlarged view of central arc of stele region.

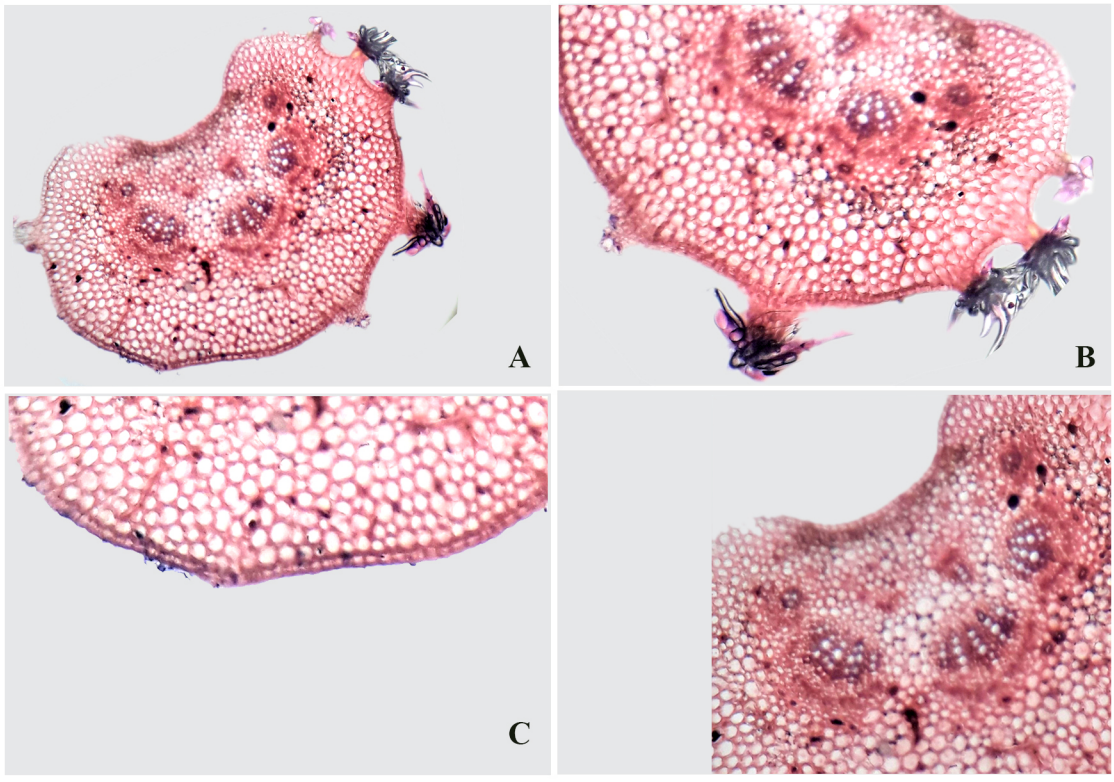


Plate 19: Anatomical Study of *C. bonplandianum Petiole*- A. T.S. of petiole; **B.** Enlarged view of projection bearing trichomes; **C.** Portion showing discontinuous hypodermis; **D.** Enlarged view of central arc of stele region.

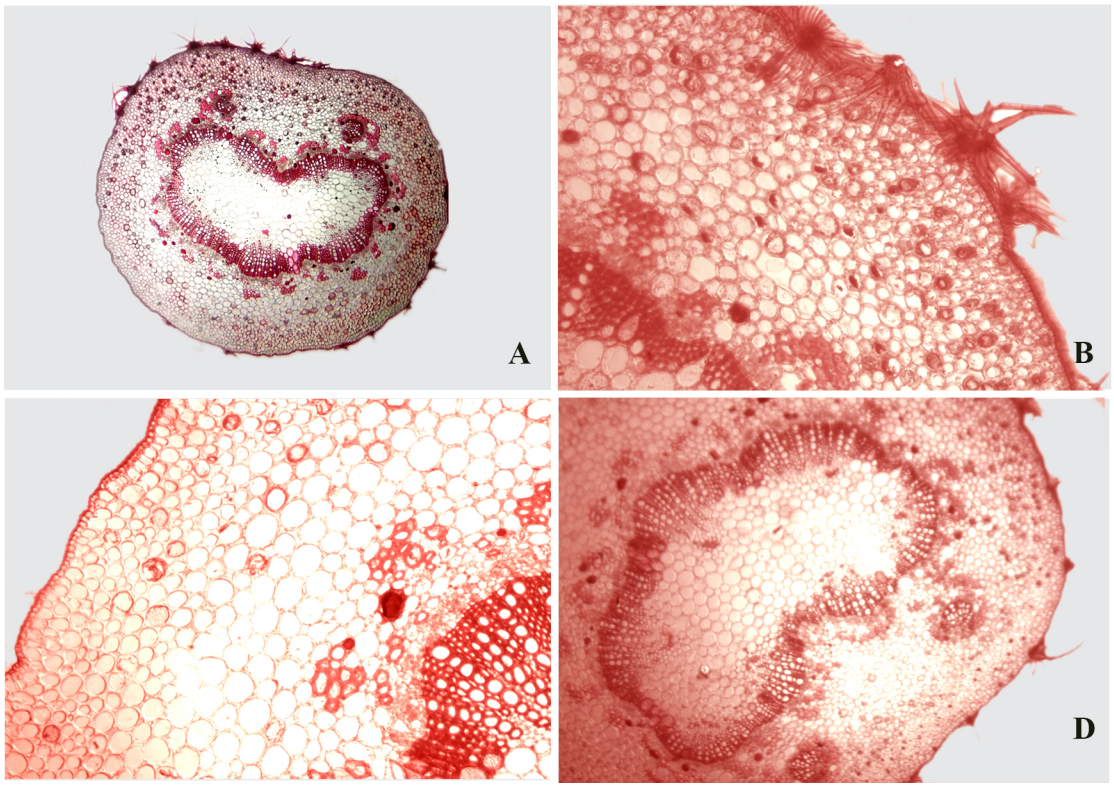


Plate 20: Anatomical Study of *C. caudatus* Petiole- A. T.S. of petiole; **B**. Enlarged view of projection bearing trichomes; **C**. Portion showing discontinuous hypodermis,; **D**. Enlarged view of central arc of stele region.

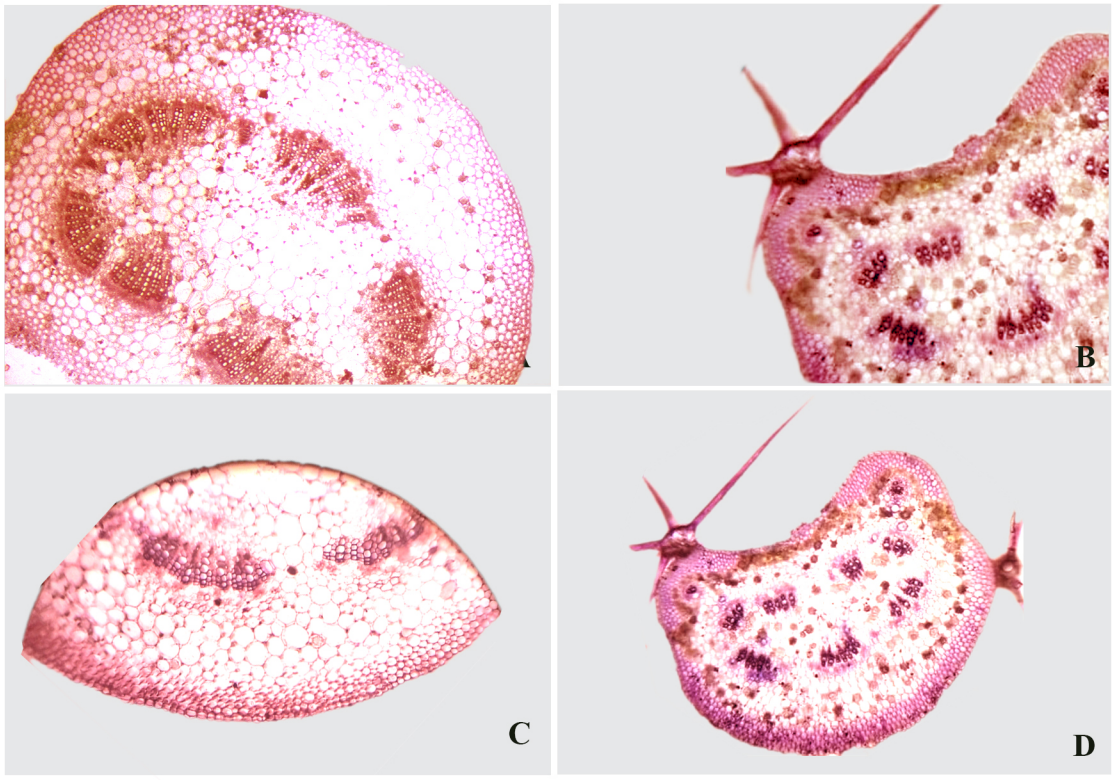


Plate 21: Anatomical Study of *C. hirtus* Petiole - A. T.S. of petiole; **B**. Enlarged view of projection bearing trichomes; **D**. Portion showing discontinuous hypodermis; **E**. Enlarged view of central arc of stele region.

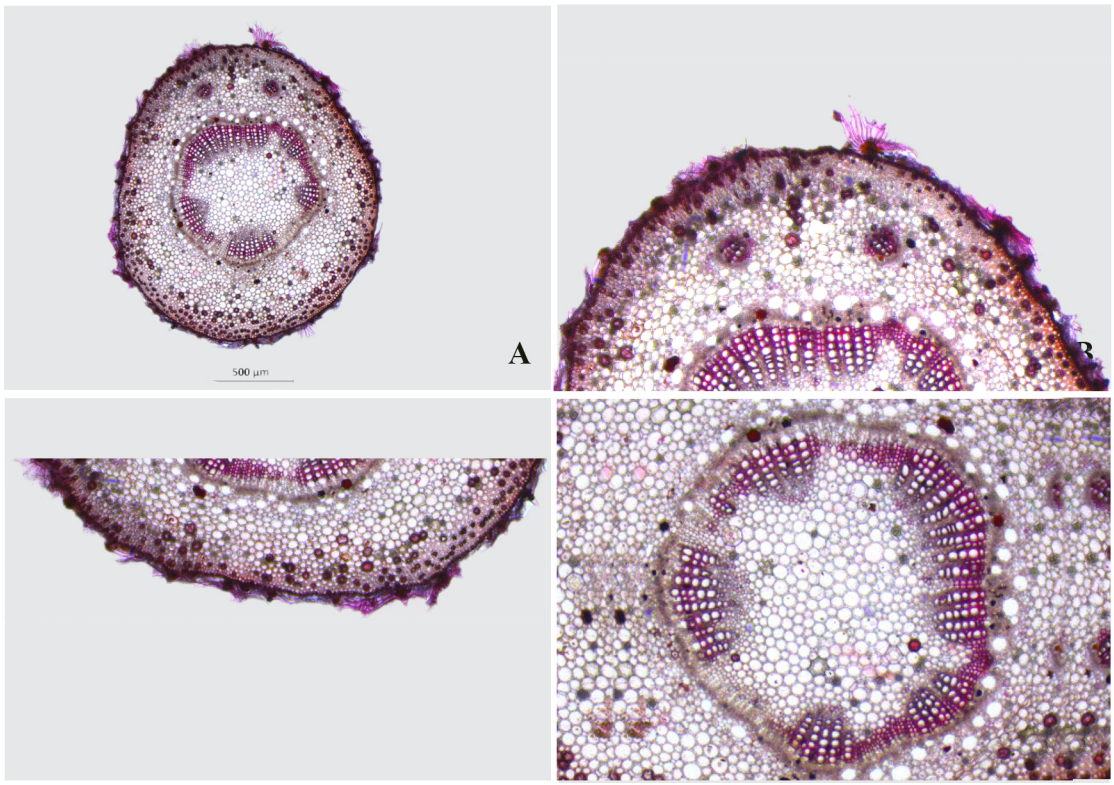


Plate 22: Anatomical Study of *C. malabaricus* Petiole- A. T.S. of petiole; **B**. Enlarged view of projection bearing trichomes; **B**. Portion showing discontinuous hypodermis; **D.** Enlarged view of central arc of stele region.

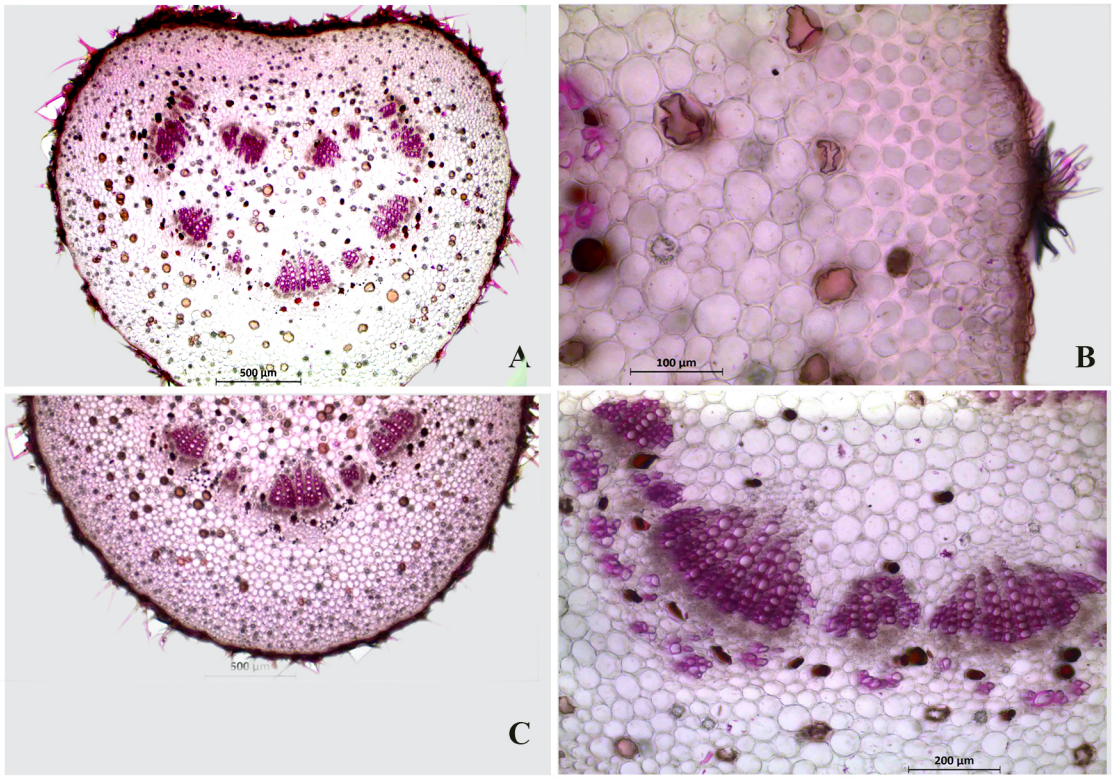


Plate 23: Anatomical Study of *C. persimilis* Petiole - A.T.S. of petiole; **B.** Enlarged view of projection bearing trichomes; **C.** Portion showing discontinuous hypodermis; **D**.Enlarged view of central arc of stele region.

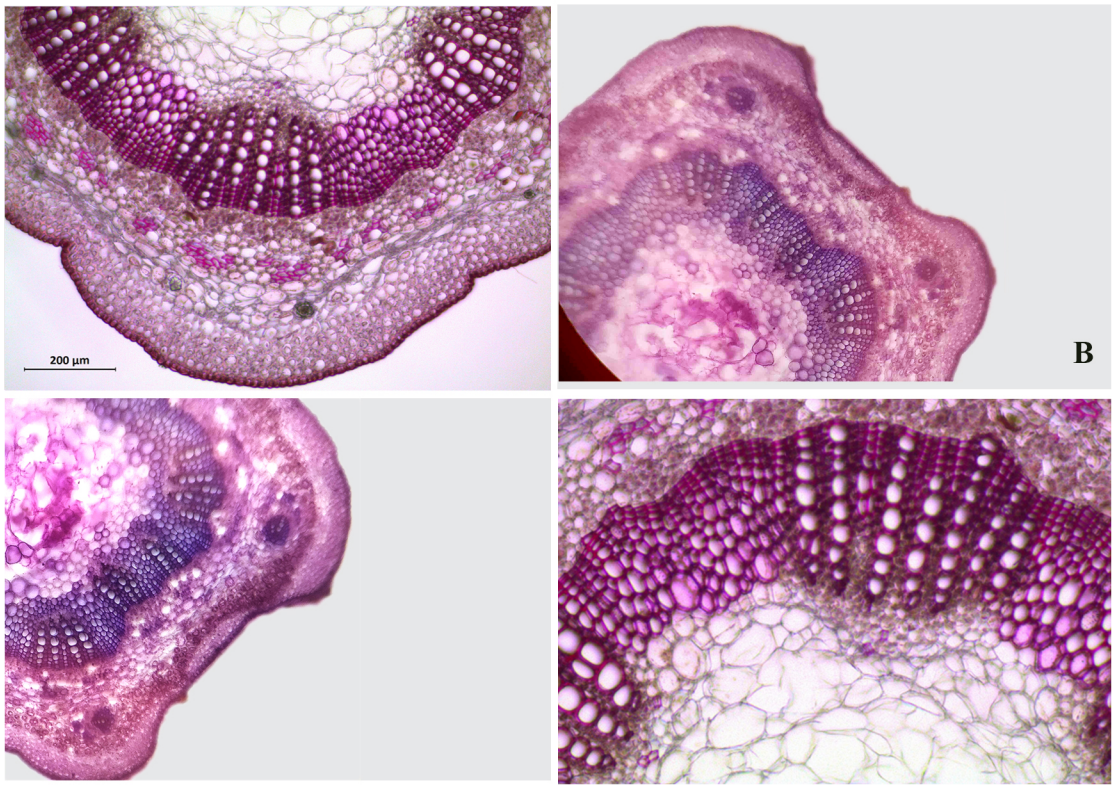


Plate 24: Anatomical Study of *C. tiglium* Petiole - **A**. T.S. of petiole; **B**. Enlarged view of projection; **C**. Portion showing discontinuous hypodermis; **D**. Enlarged view of central arc of stele region.

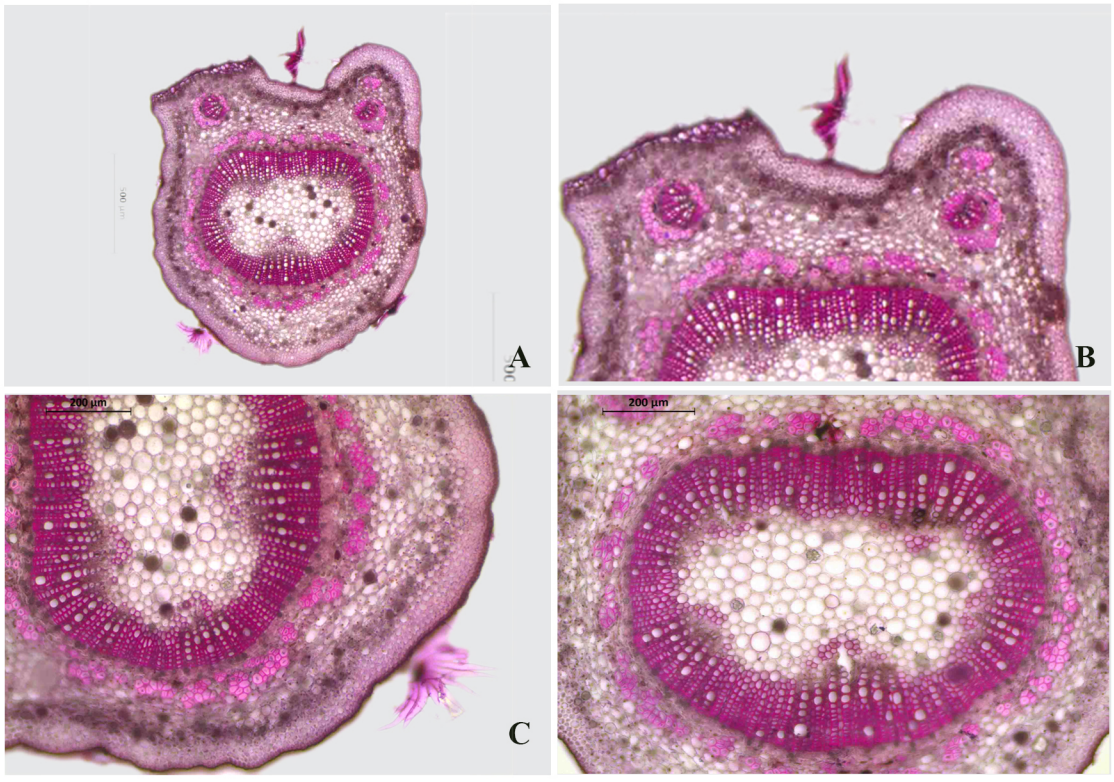


Plate 25: Anatomical Study of *C. zeylanicus* Petiole - **A**. T.S. of petiole; **B**. Enlarged view of projection bearing trichomes; **C**. Portion showing discontinuous hypodermis,; **D**. Enlarged view of central arc of stele region.

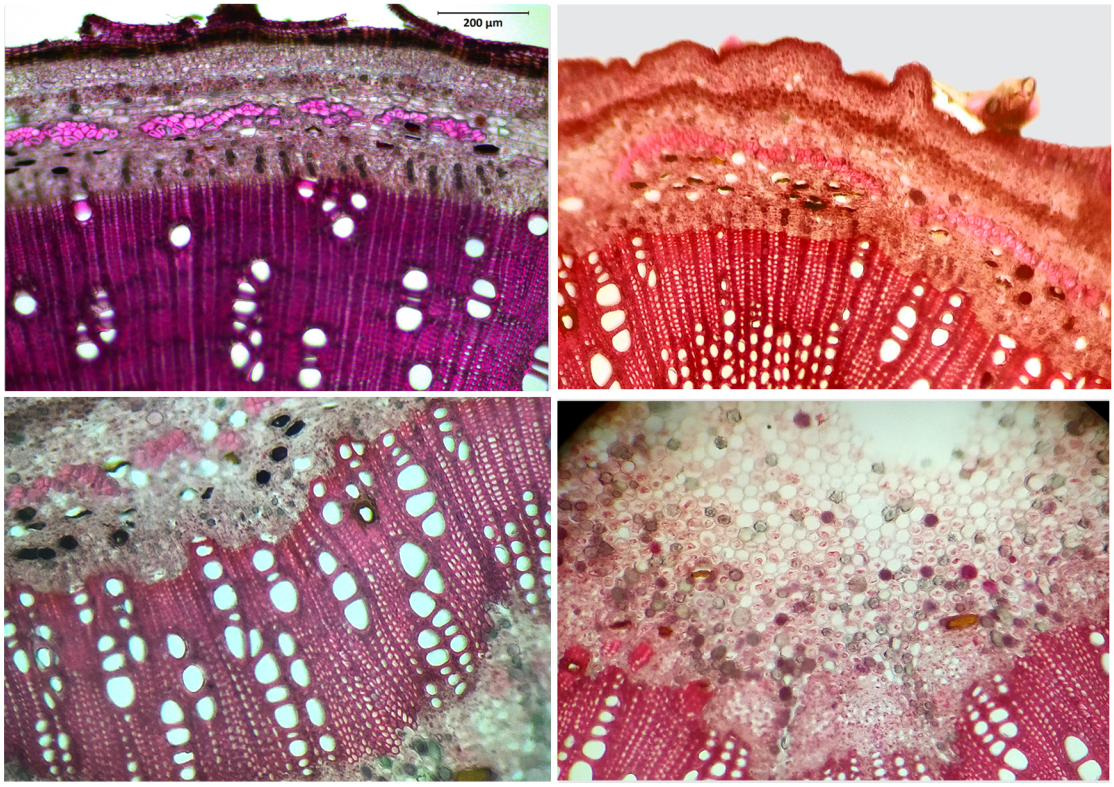


Plate 26: Anatomical Study of *C. aromaticus* Stem - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

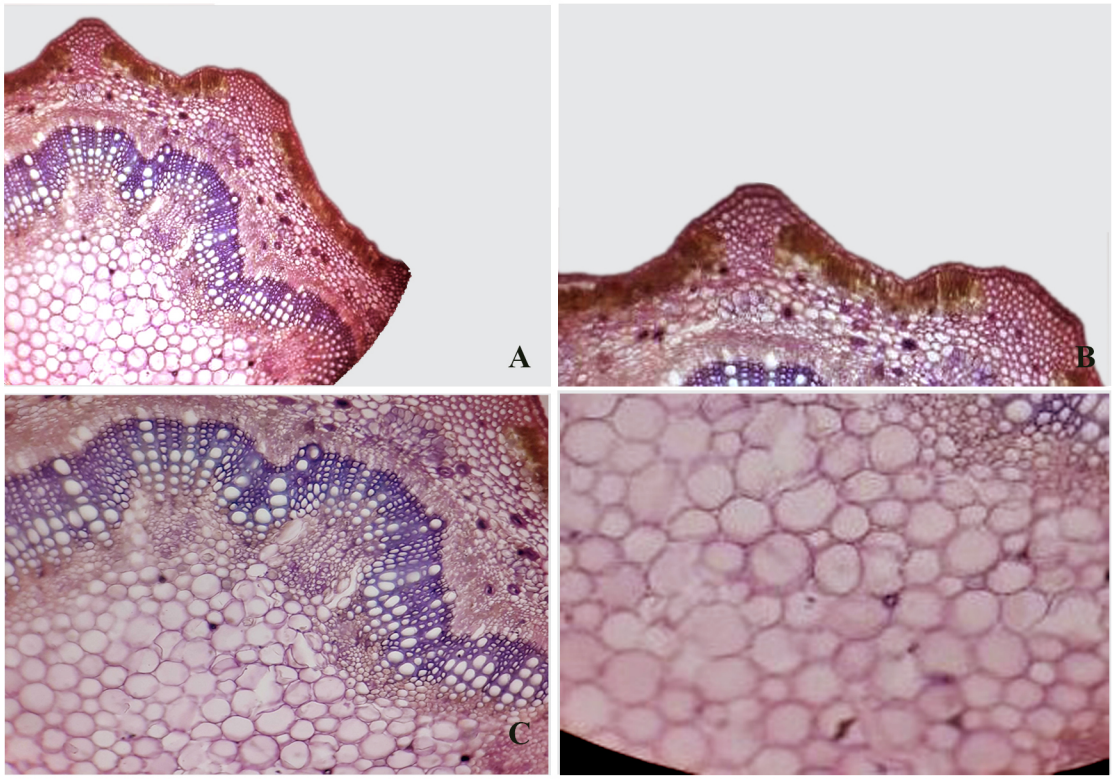


Plate 27: Anatomical Study of *C.bonplandianum* Stem - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

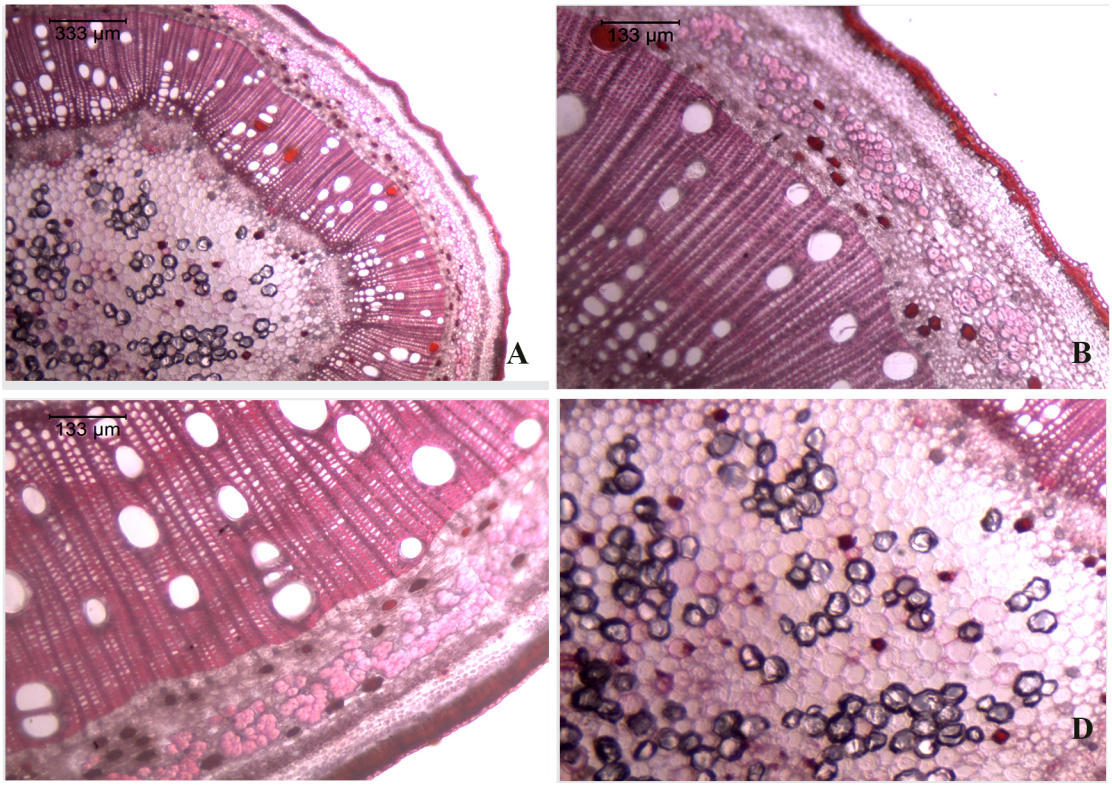


Plate 28: Anatomical Study of *C. caudatus* Stem - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

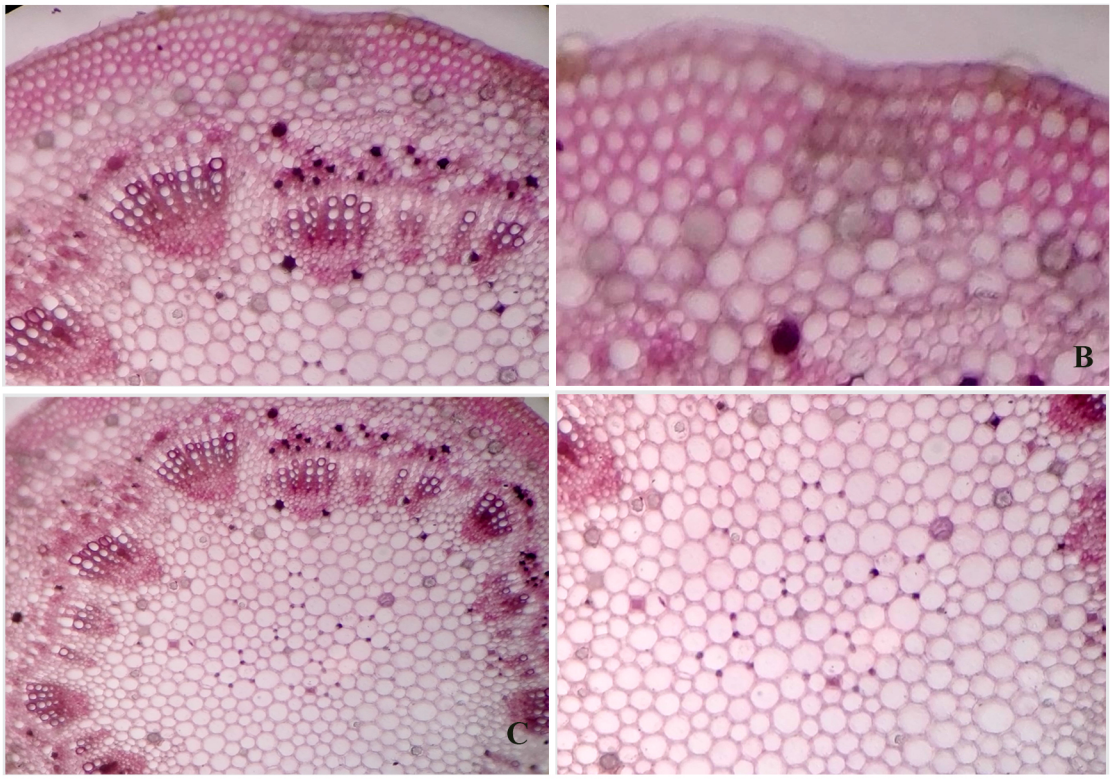


Plate 29: Anatomical Study of C. hirtus Stem- A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; ; **D**. Enlarged view of pith region.

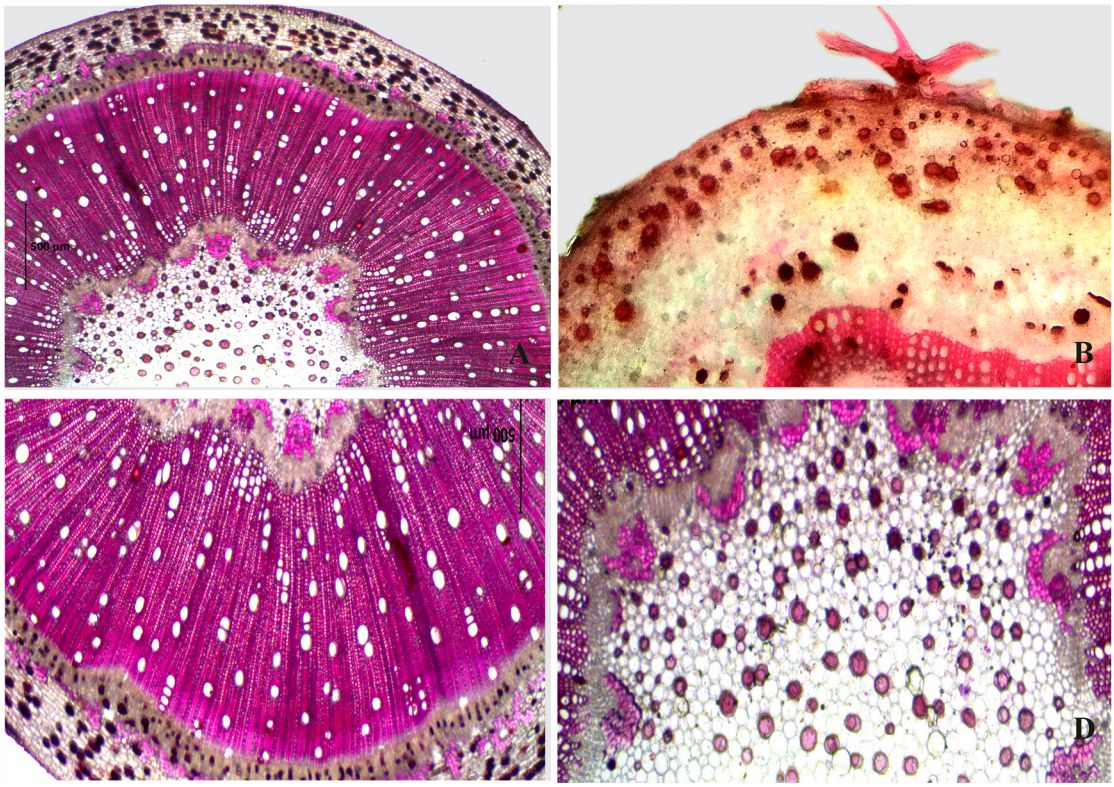


Plate 30: Anatomical Study of *C. malabaricus* Stem - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

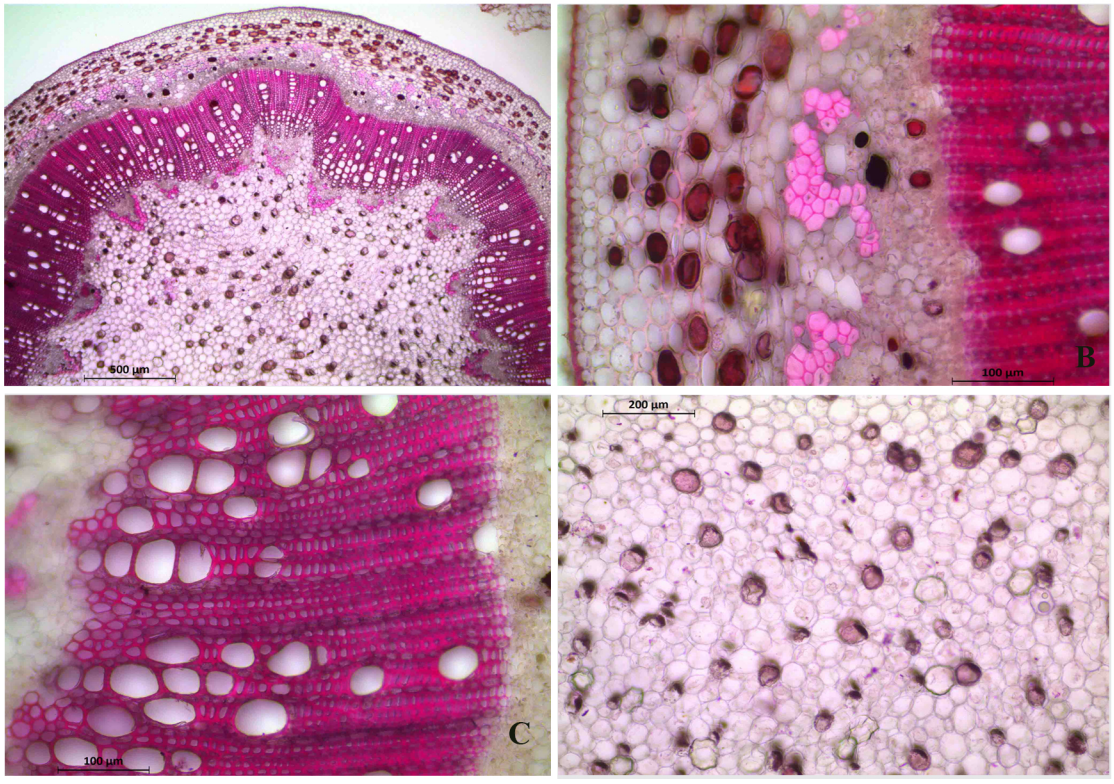


Plate 31: Anatomical Study of *C. persimilis* Stem- A. T.S. of stem; **B.** Enlarged view of cortical region; **C.** Enlarged view of xylem region; **D**. Enlarged view of pith region.

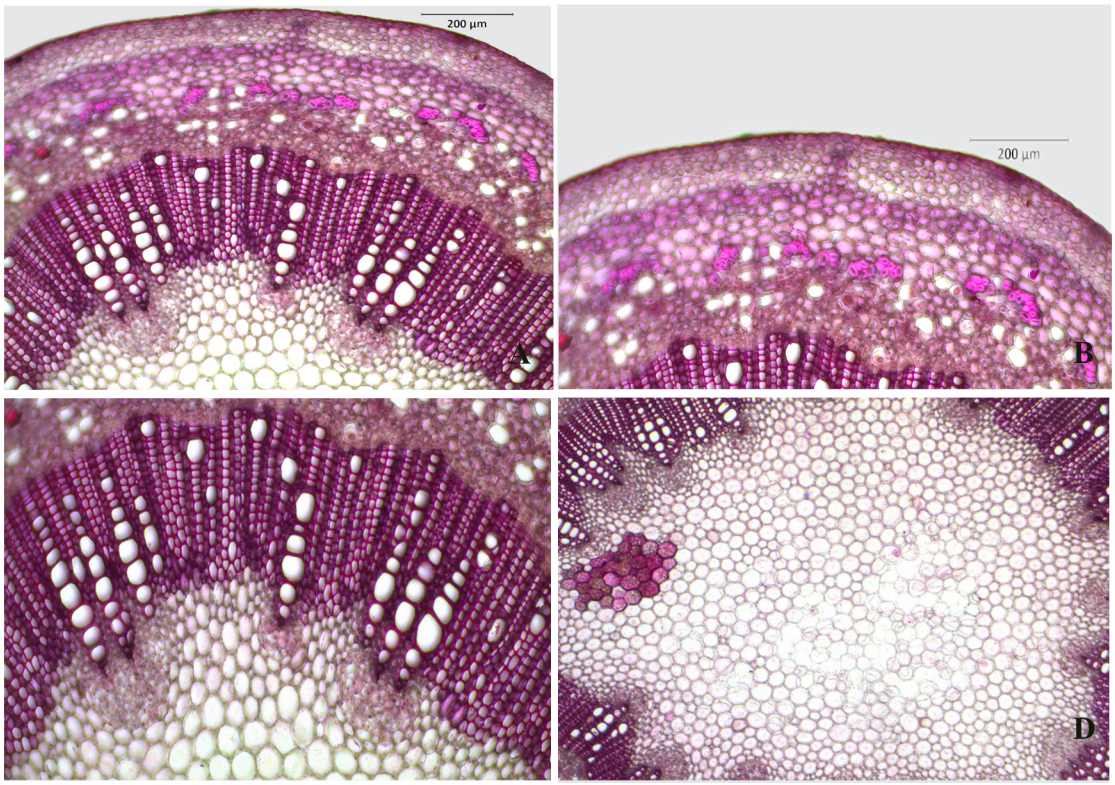


Plate 32: Anatomical Study of *C. tiglium* Stem - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

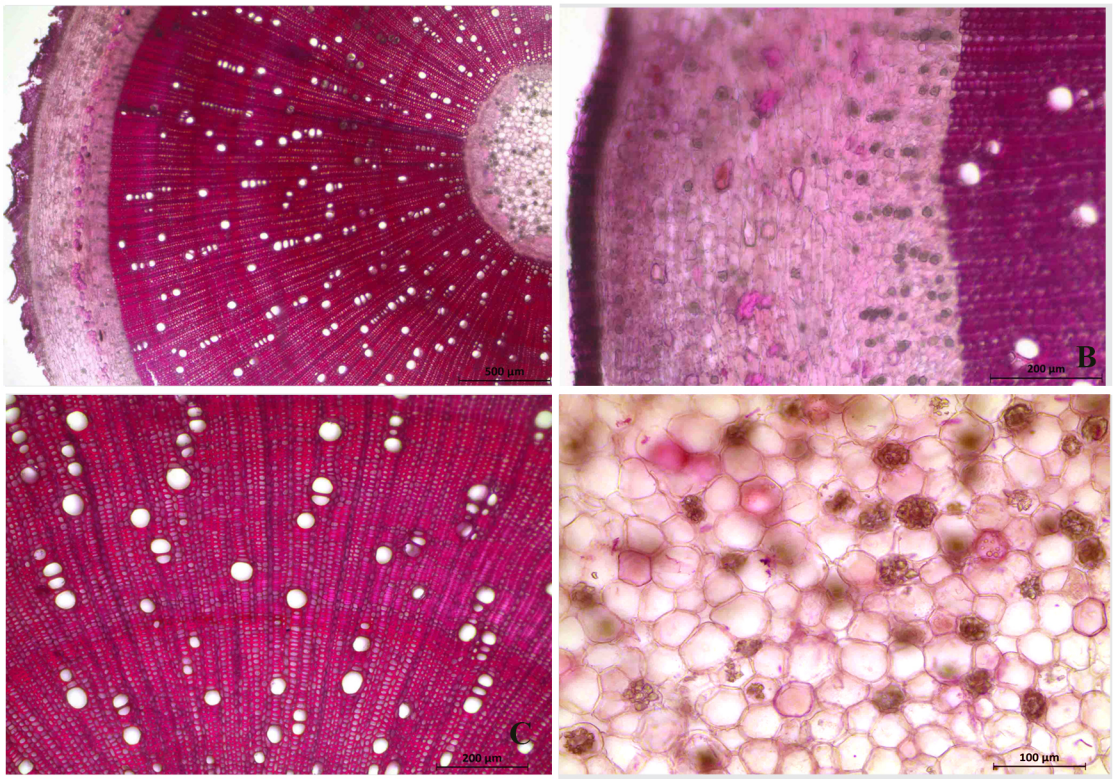


Plate 33: Anatomical Study of *C. zeylanicus* Stem- A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

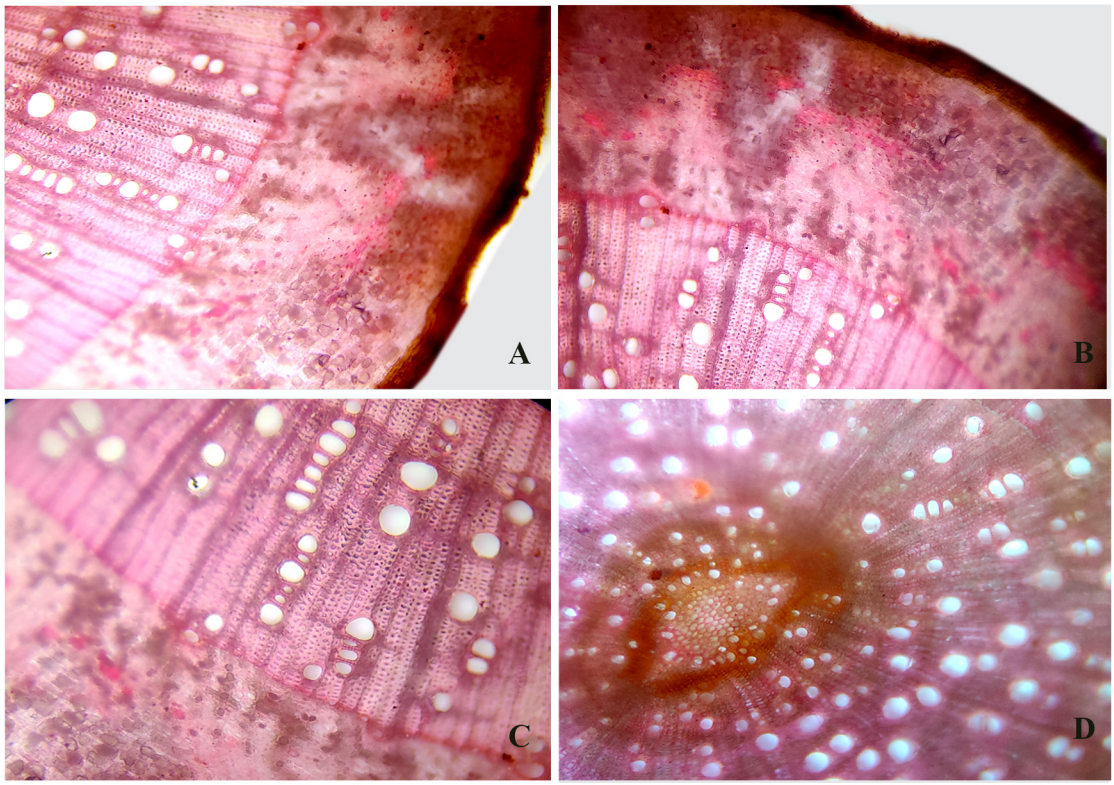


Plate 34: Anatomical Study of *C. aromaticus* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

Plate 35: Anatomical Study of *C.bonplandianum* Root- A. T.S. of root; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

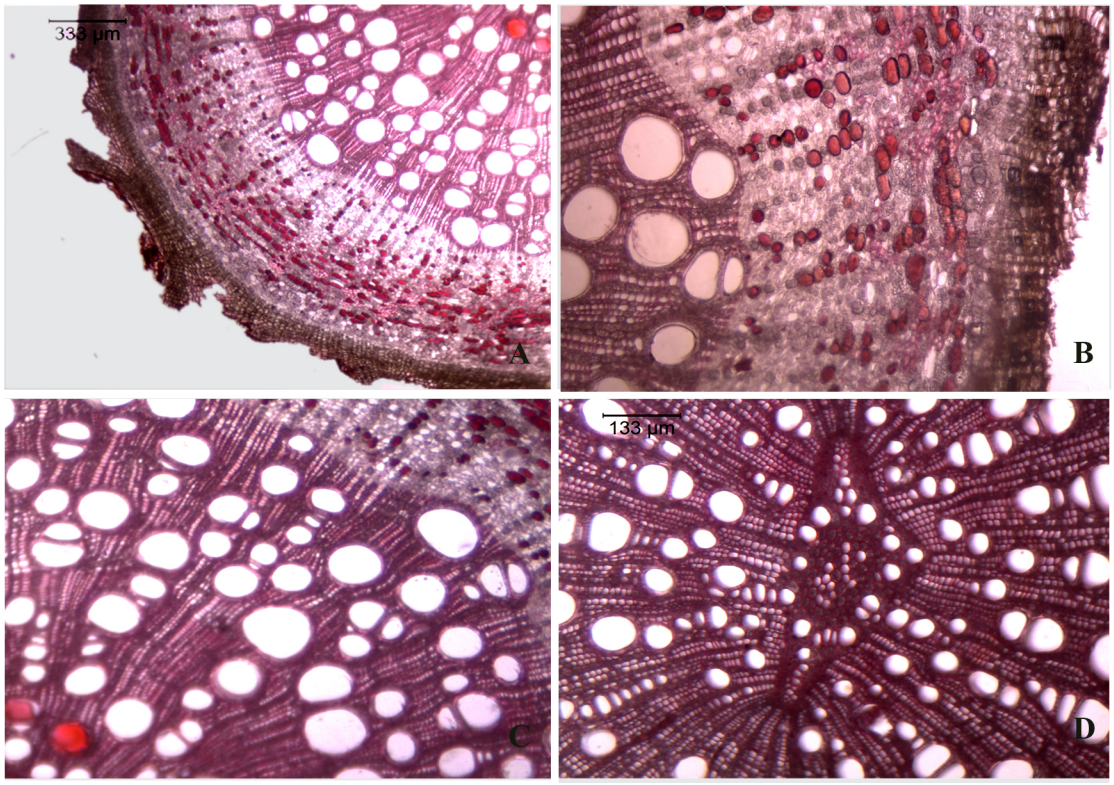


Plate 36: Anatomical Study of *C. caudatus* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

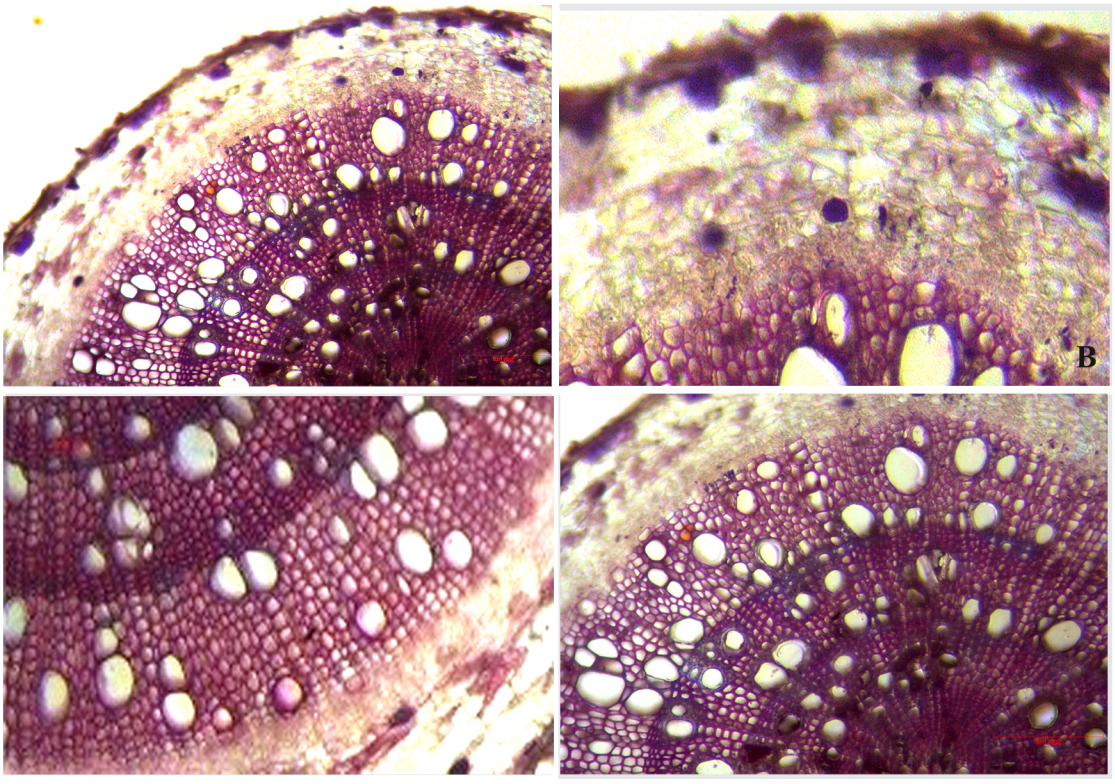


Plate 37: Anatomical Study of *C. hirtus* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**: Enlarged view of xylem region; **D**. Enlarged view of pith region.

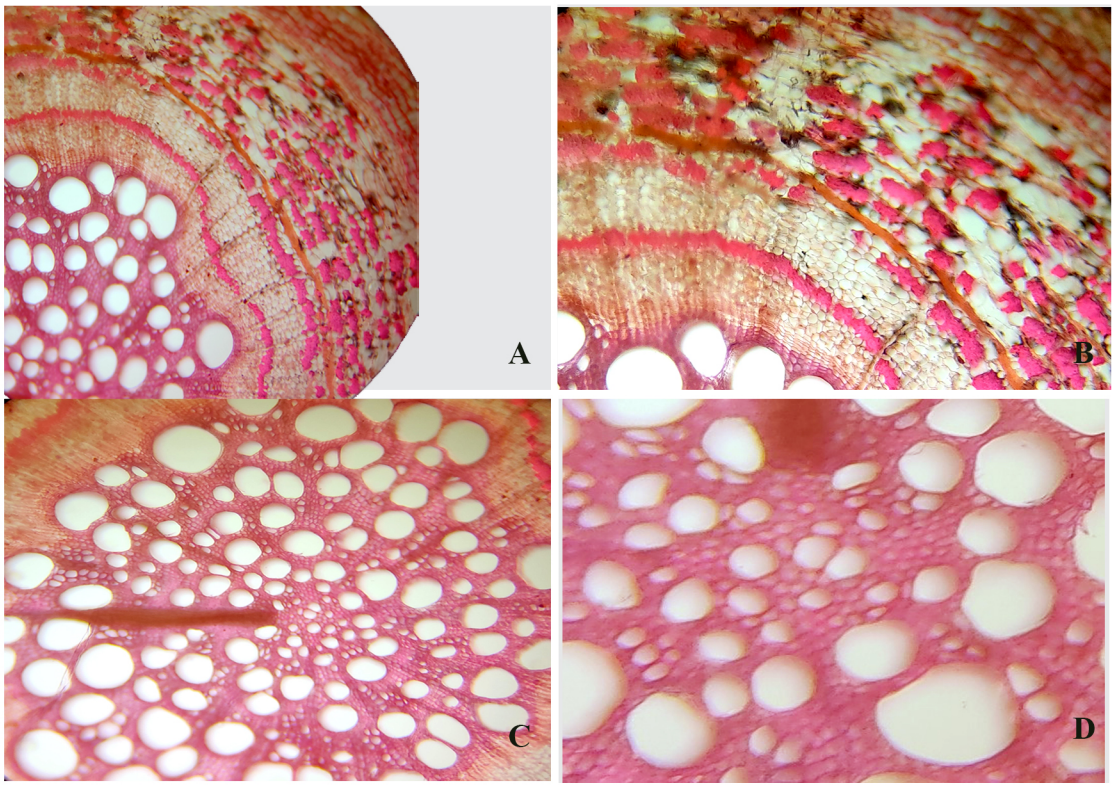


Plate 38: Anatomical Study of *C. malabaricus* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

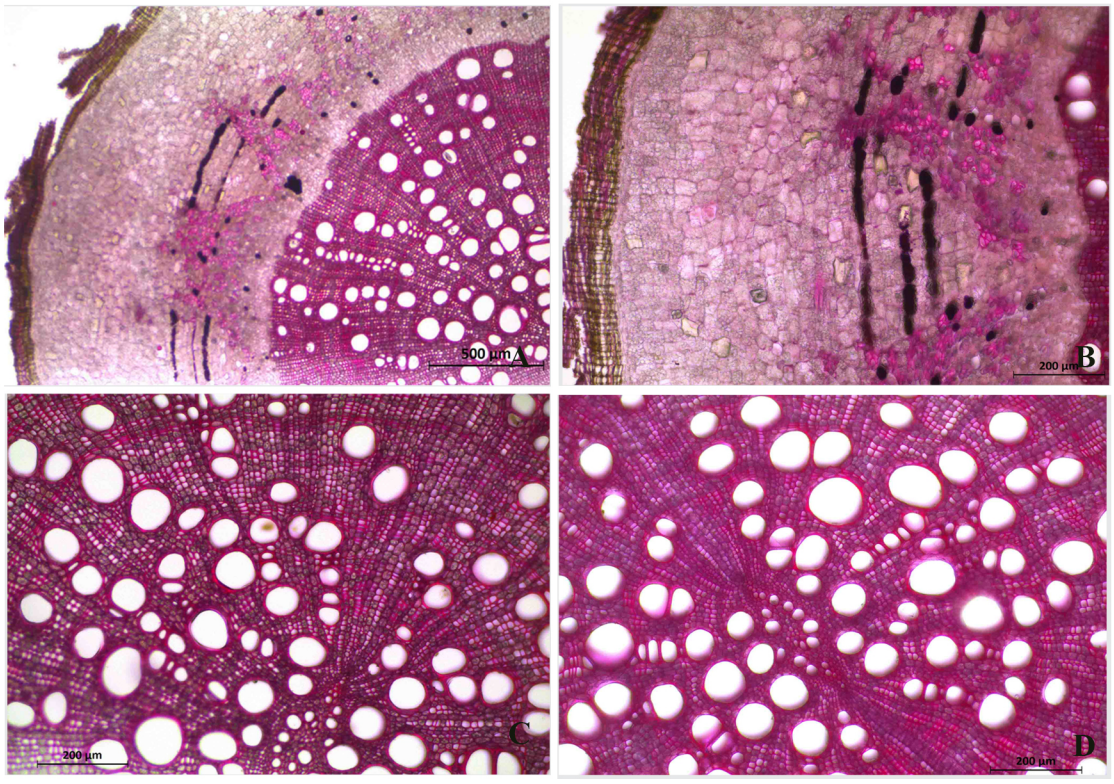


Plate 39: Anatomical Study of *C. persimilis* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region.

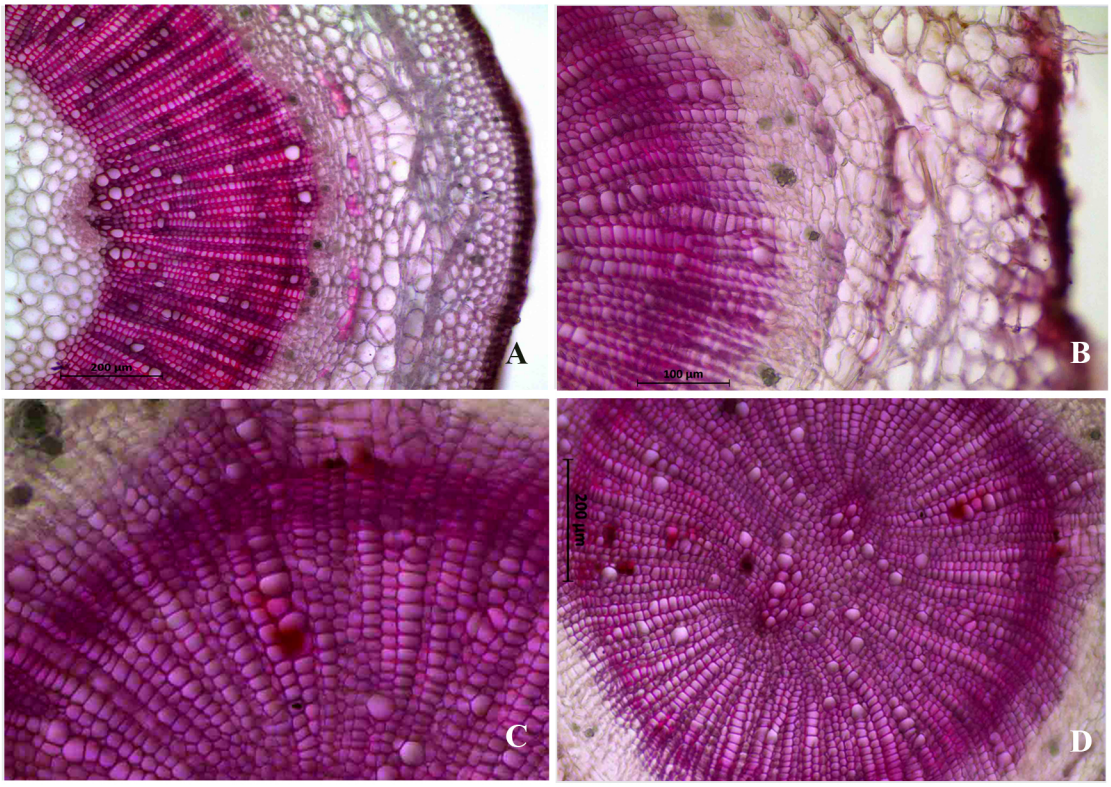


Plate 40: Anatomical Study of *C. tiglium* Root - A. T.S. of root; **B**. Enlarged view of cortical region; **C**: Enlarged view of xylem region; **D**. Enlarged view of pith region.

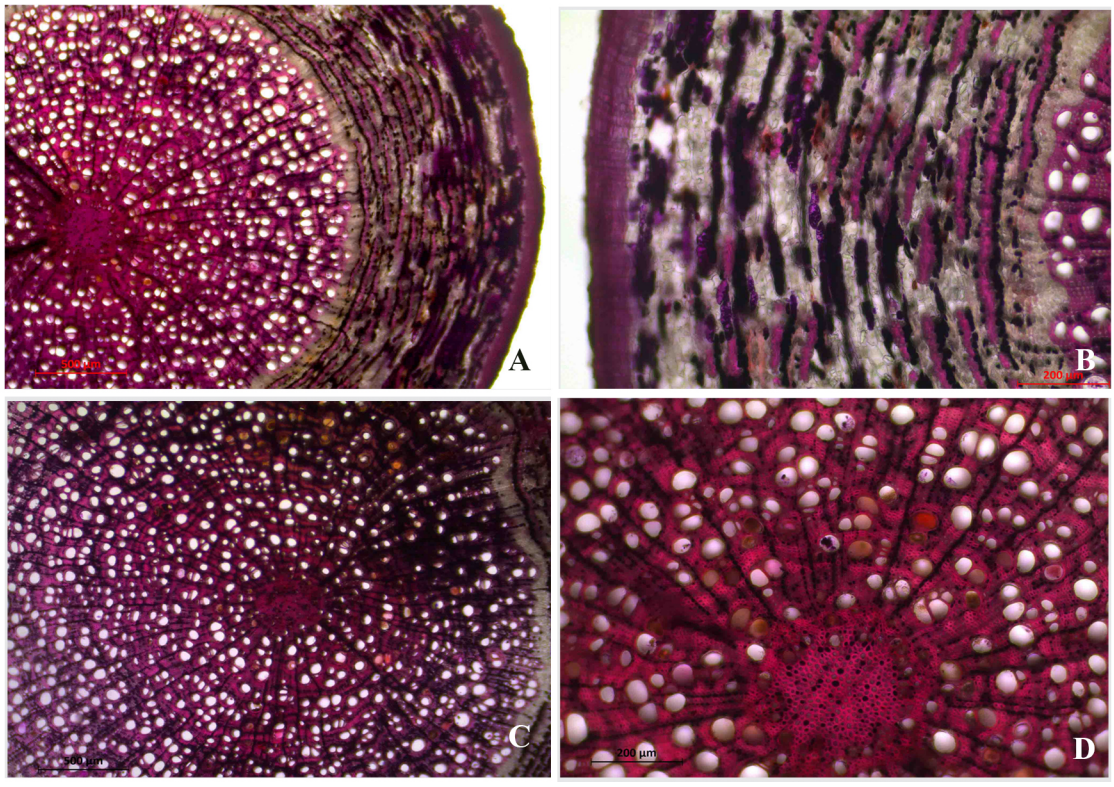


Plate 41: Anatomical Study of *C. zeylanicus* Root - A. T.S. of stem; **B**. Enlarged view of cortical region; **C**. Enlarged view of xylem region; **D**. Enlarged view of pith region

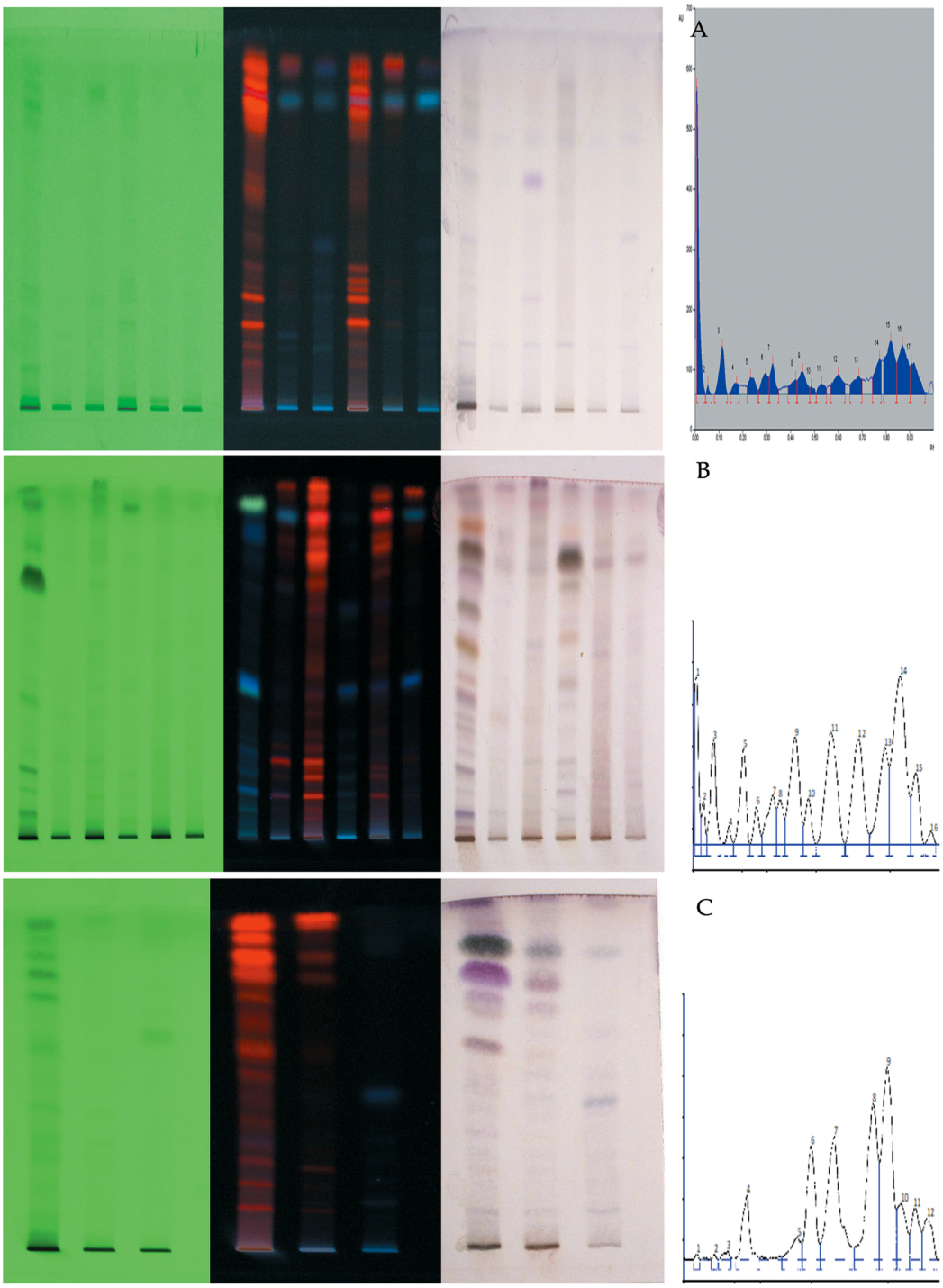


Plate 42: HPTLC profile of *C.bonplandianum,C.hirtus, C.caudatus, C.persimilis* & *C.zeylanicus*. A: HPTLC of *C.hirtus* & *C.bonplandianum* under 254nm,366nm,550nm.Track 1:Leaf extract of *C.hirtus*,Track 2: Stem extract of *C.hirtus*, Track 3:Root extracts of *C.hirtus*,Track4:Leaf extract of *C.bonplandianum*,Track 5: Stem extract of *C.bonplandianum*,Track 6:Root extract of *C.bonplandianum*. B: HPTLC Profiling of *C.caudatus* & *C.persimilis* under 254nm,366nm & 550nm. Track 1: Root extract of *C.caudatus*, Track 2: Stem extract of *C.caudatus*,Track 3: Leaf extraxct of *C.caudatus*,Track 4:Root extract of *C.persimilis*,Track 5: Stem extracts of *C.persimilis*, Track 6:Leaf extracts of *C.persimilis* C:.HPTLC Profiling of *C.zeylanicus* under 254nm,366nm & 550nm. Track 1: Leaf extract of *C.zeylanicus*, Track 2:Stem extract of *C.zeylanicus*,Track 3: Root extract of *C.zeylanicus*.

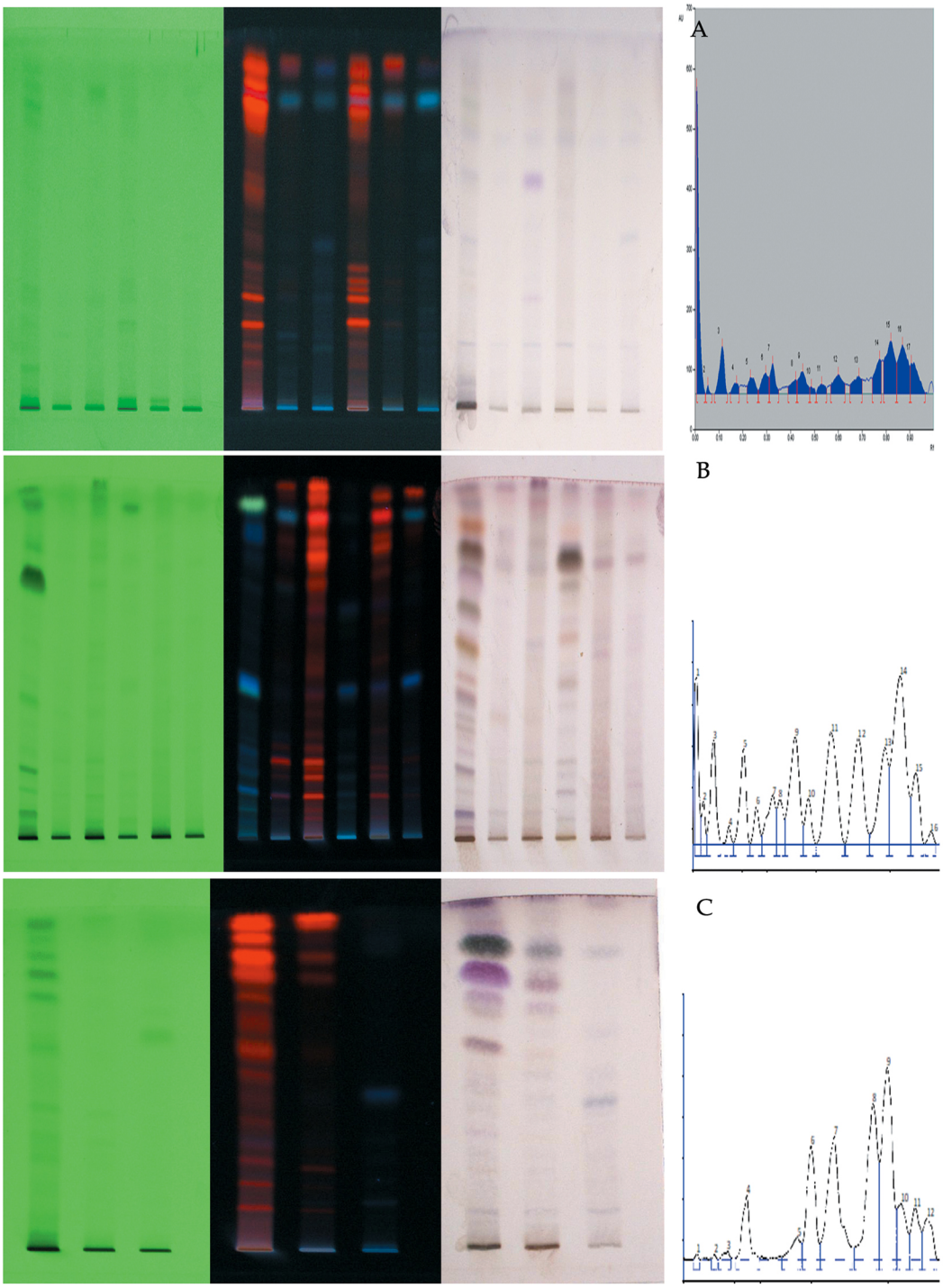


Plate 42: HPTLC profile of *C.bonplandianum,C.hirtus, C.caudatus, C.persimilis* & *C.zeylanicus*. A: HPTLC of *C.hirtus* & *C.bonplandianum* under 254nm,366nm,550nm.Track 1:Leaf extract of *C.hirtus*,Track 2: Stem extract of *C.hirtus*, Track 3:Root extracts of *C.hirtus*,Track4:Leaf extract of *C.bonplandianum*,Track 5: Stem extract of *C.bonplandianum*,Track 6:Root extract of *C.bonplandianum*. B: HPTLC Profiling of *C.caudatus* & *C.persimilis* under 254nm,366nm & 550nm. Track 1: Root extract of *C.caudatus*, Track 2: Stem extract of *C.caudatus*,Track 3: Leaf extraxct of *C.caudatus*,Track 4:Root extract of *C.persimilis*,Track 5: Stem extracts of *C.persimilis*, Track 6:Leaf extracts of *C.persimilis* C:.HPTLC Profiling of *C.zeylanicus* under 254nm,366nm & 550nm. Track 1: Leaf extract of *C.zeylanicus*, Track 2:Stem extract of *C.zeylanicus*,Track 3: Root extract of *C.zeylanicus*.

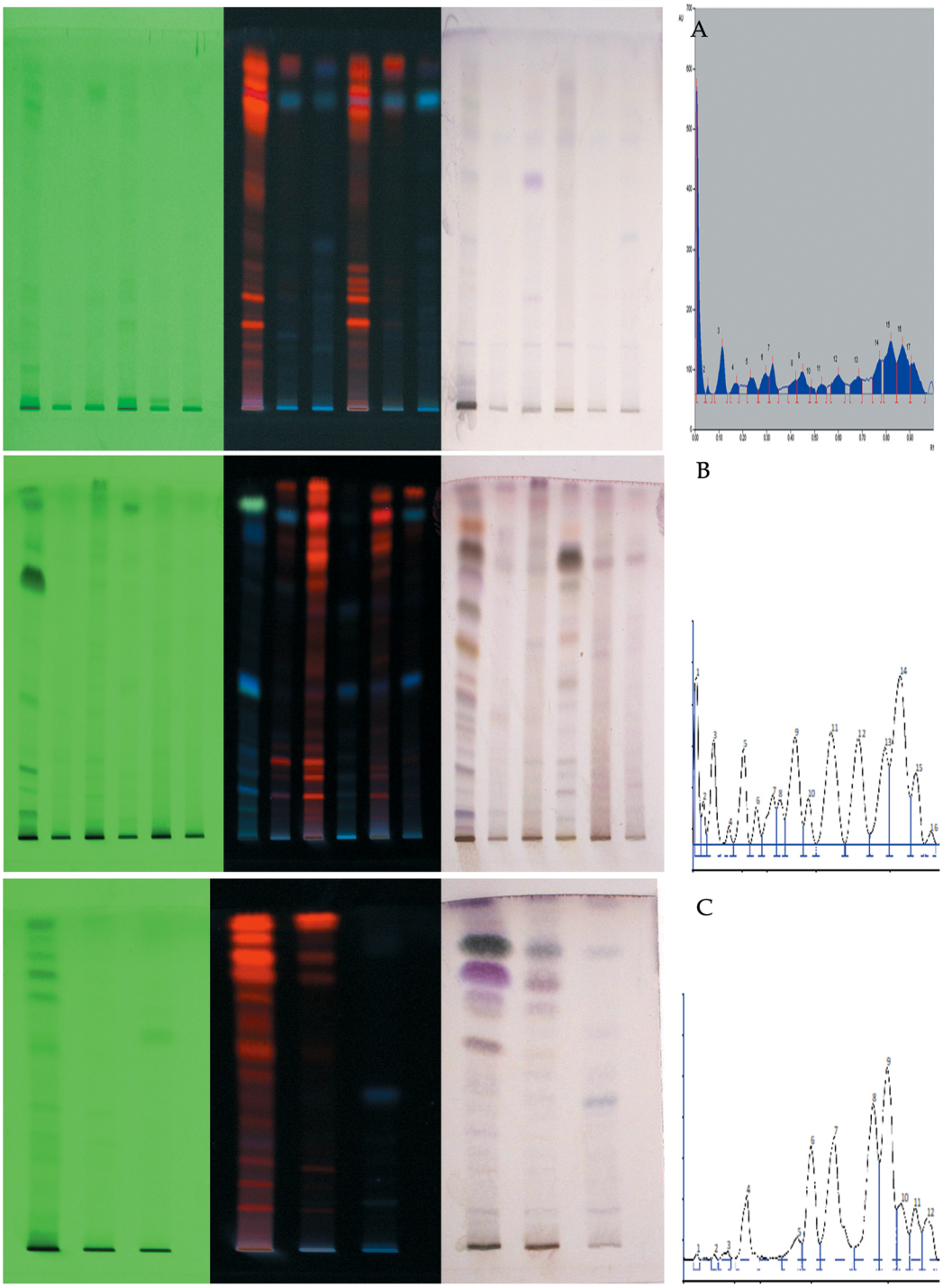


Plate 42: HPTLC profile of *C.bonplandianum,C.hirtus, C.caudatus, C.persimilis* & *C.zeylanicus*. A: HPTLC of *C.hirtus* & *C.bonplandianum* under 254nm,366nm,550nm.Track 1:Leaf extract of *C.hirtus*,Track 2: Stem extract of *C.hirtus*, Track 3:Root extracts of *C.hirtus*,Track4:Leaf extract of *C.bonplandianum*,Track 5: Stem extract of *C.bonplandianum*,Track 6:Root extract of *C.bonplandianum*. B: HPTLC Profiling of *C.caudatus* & *C.persimilis* under 254nm,366nm & 550nm. Track 1: Root extract of *C.caudatus*, Track 2: Stem extract of *C.caudatus*,Track 3: Leaf extraxct of *C.caudatus*,Track 4:Root extract of *C.persimilis*,Track 5: Stem extracts of *C.persimilis*, Track 6:Leaf extracts of *C.persimilis* C:.HPTLC Profiling of *C.zeylanicus* under 254nm,366nm & 550nm. Track 1: Leaf extract of *C.zeylanicus*, Track 2:Stem extract of *C.zeylanicus*,Track 3: Root extract of *C.zeylanicus*.

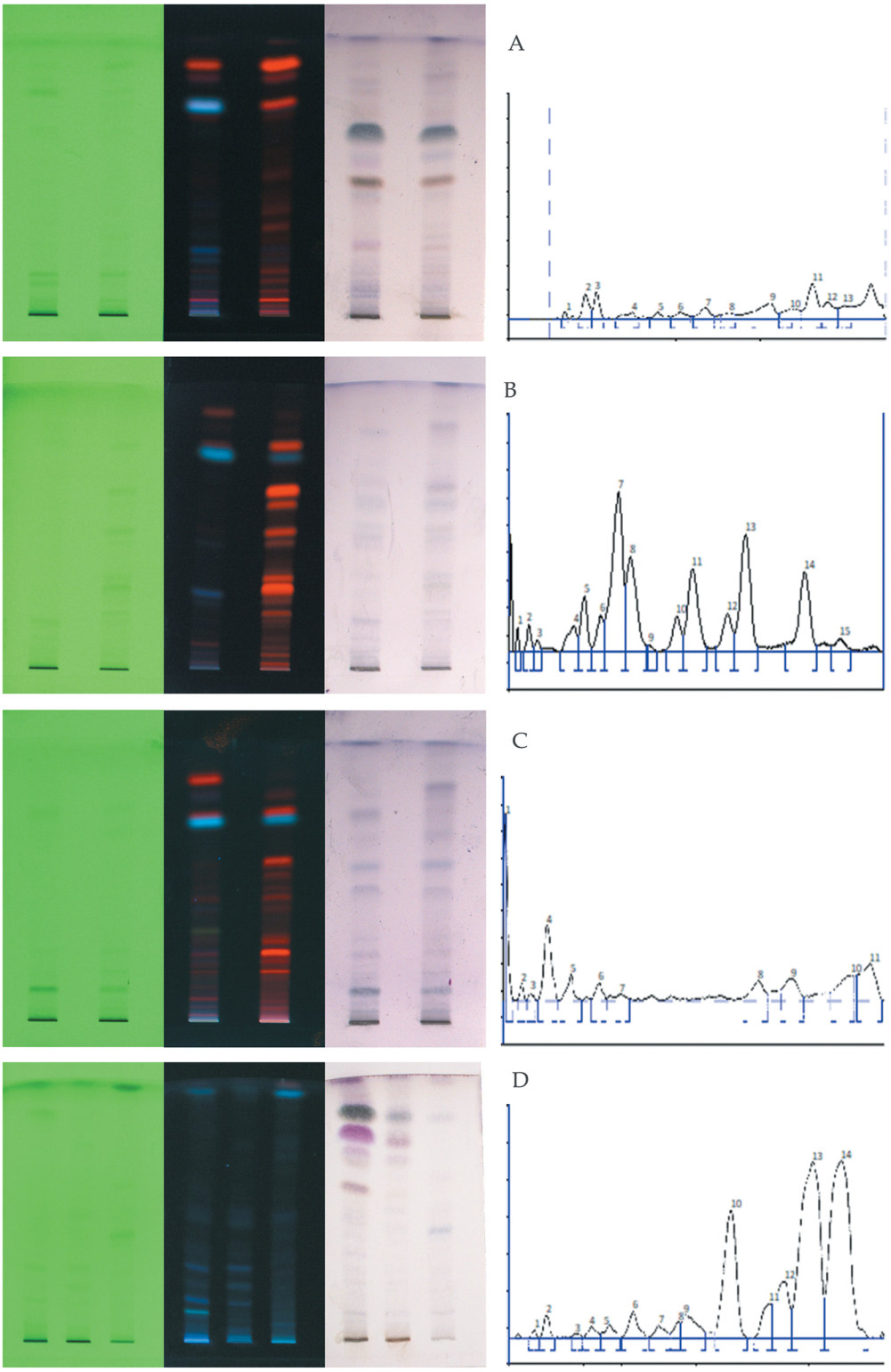


Plate 43: **HPTLC profile *C.aromaticus, C.malabaricus* & *C.tiglium***. A: HPTLC profiling of *C.aromaticus* under 254nm,366nm & 550nm. Track 1:stem,Track 2:Leaf B: HPTLC profiling of *C.malabaricus* under 254nm, 366nm & 550nm Track 1:stem,Track 2:Leaf C: HPTLC profiling of *C.tiglium* under 254nm,366nm& 550nm Track 1:stem,Track 2:Leaf D:HPTLC profiling of roots of *C.aromaticus*,*C.malabaricus* & *C.tiglium* under 254nm,366nm&550nm.Track 1: Root Extract of *C.aromaticus*, Track 2: Root extract of C.malabaricus &Track 3: Root extract of *C.tiglium*..

CHAPTER 5
SUMMARY AND CONCLUSION

Plants are one of the major source of crude drug, which has been transformed from the ancient civilization of human being and which is a significant element of the preventive medicine. Synthetic drugs are present in plants. Medicines, bioactive compounds, antioxidants, and some healthy supplements formed from plants. Plants containing phytoconstituent, having medicinal values. To improve the immunity of human beings and animals, the medicinal value of plants have a great role.

The present study have documented 8 species of *Croton* (Euphorbiaceae) from the study area. In order to distinguish the species with the help of dichotomous indented keys. The morphological features of Croton species shows some similarities and variations. While observing these species also shows its diversity in habit or growth form from small herbs to trees and its habitat such as road sides, grasslands to evergreen forest. Similarly, the structural variations of leaves also helps to identify these taxa from the field. The inflorescence, floral structures shows some similarities in almost all species, which is confused to categorizing each other. In this context, the present investigation helps to specify its morphological uniqueness and it leads to the proper identification of selected taxa for the present study.

During the examination of anatomical features, some of the characters are general for all selected species of Croton, whereas some of them are identical. These unique characters help for the identification and classification of selected taxa. During the pharmacological investigations these identical characters may help to identify the correct taxa and also helps to check the adulterants by using some unknown plants rather than the correct ones.

The preliminary phytochemical analysis documented based on the phyto chemicals present in the selected species of genus *Croton.* The separate

extracts of plant were analysed under the various tests provided by the experts. In the case of root extracts of *Croton*, *C. caudatus* and *C. persimilis* consisting of highest amount of compounds, whereas the lowest amount of compounds present in *C. bonplandianum* and *C. hirtus*. Likewise, large amount of phytocompounds present in the stem extracts of *C. caudatus*, whereas lowest quality of phytochemicals present in C. zeylanicus. In leaf extracts, phytochemicals abundantly found in *C. caudatus* and *C. persimilis*, whereas a few amount of phytochemicals present in *C. zeylanicus*.

The quantitative phytochemical analysis subjected by the HPTLC techniques. The Rf values observed under 254nm, 366nm and550nm.diffrent types of bands, Rf values obtained. Some of the Rf values showing unique bands, some of them are combined with respect to each other and some other bands are common for the extracts of selected species of *Croton*. On the basis of HPTLC data, a dendrogram plotted using NTSYSpc2.02 UPGMA cluster analysis. These data revealed that the closest chemical correlation between leaf, stem and roots of selected species of *Croton*. The dendrogram shows that, Root of *C. aromaticus* and leaf of *C. malabaricus* can be said to be closely related to each other, followed by stem and root of *C. bonplandianum* being the next closest one. Leaf of *C. bonplandianum* showed maximum similarity with leaf of *C. hirtus.* Leaf of *C. zeylanicus* is most distant with leaf of *C. aromaticus* with coefficient correlation. The ethnobotanical information of Croton species revealed that its great role in the crude drug in traditional medicinal practices by these taxa.

The present study will be useful to distinguish the identity of selected species of *Croton* by detailing its morphological, anatomical, qualitative and quantitative characters along with dendrogramic illustration. Such studies are also helps to validate the authenticity of genuine source plants, which are used to prepare various herbal formulations in different Ayurvedic and other pharmaceutical industries.

RECOMMENDATIONS

Beyond this, some future perspectives based on the present study are also proposed, they are

- To validate the selected taxa by incorporating molecular data and also correlate it with phylogenetic basis.
- Further pharmacological studies are also recommended on such selected taxa through an appropriate clinical trials.
- An extensive phytochemical studies are also suggested to elucidate characteristic phyto-compounds which are present in different plant extracts.
- The present analysis are also extend to validate the genuineness of original source plants which used in the preparation of various herbal-formulations.

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COMPARATIVE STUDIES ON MORPHOLOGY, ANATOMY AND PHYTOCHEMISTRY OF SELECTED SPECIES OF *CROTON* L. (EUPHORBIACEAE)

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Abstract

The study on two *Croton* species of Euphorbiaceae was carried out to differentiate their morphological, anatomical and phytochemical characteristics. The present study reveals their morphological, anatomical and phytochemical individualities. It also highlights its great value for future studies to disclose their potential medicinal values for human welfare.

Key words : *C. bonplandianus*, *C. hirtus*, Morphology, Anatomy, Phytochemistry.

Introduction

All plants produce chemical constituents, part of their normal metabolic activities (Tyler *et al.,* 1981, Rosenthal *et al.,* 1979). Plants are furnished with various phytochemical molecules such as terpenoids, phenolic acids, vitamins, lignins, stilbenes, tannins, amines, betalains, flavonoids, quinones, coumarins, alkaloids, and other metabolites (Kirankumar & Deenadayalan, 2017). *Croton* (Euphorbiaceae) is one of the largest genera of flowering plant of family Euphorbiaceae, with between 1200 and 1300 species of herbs, shrubs, trees, and occasionally lianas that are ecologically prominent and important elements of secondary vegetation in the tropics and subtropics worldwide (Webster, 1993; Govaerts *et al.,* 2000). The name "*Croton*" is a Greek word referring to thick smooth seeds, a common feature of most *Croton* plants which belongs to the family Euphorbiaceae (Palgrave, 1990 & 2002; Mabberley, 2009).

Croton bonplandianus Baill. (Euphorbiaceae), commonly known as *Bantulsi* is a perennial herb, one of the exotic weeds, found in waste lands and road side areas in India, Bangladesh and all other countries of South Asia (Chakrabarty & Balakrishnan, 1992). Flowering and fruiting time of this plant is from September to December (Reddy, 1995). *Croton hirtus* is a shrub belonging to genus *Croton* and family Euphorbiaceae. In Kerala *Croton hirtus* is distributed in Alappuzha, Thrissur, Palakkad, Kozhikode, Ernakulam, Kollam and Malappuram. In India, it was first reported from Tirunelveli Hills of Western Ghats (Ramachandran *et al.,* 1992). Later it is reported to be common throughout the coastal regions of Kerala (Preetha&Binojkumar, 2006). Recently recorded this invasive species from Dindigul hills and reported as an addition to the flora of Eastern Ghats (Kottaimuthu *et al.,* 2008).

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Materials and Methods

Collection of Materials

The plants used for the present study are *Croton bonplandianum* Baill. were collected from Maruthamalai hills of Coimbatore District, Tamil Nadu. Similarly *Croton hirtus* L. *Herit*. collected from Pattambi and Kozhichena of Malapuram District, Kerala.

Morphological Study

Morphological characterizations of different parts were carried out. Details of habit, stem, stipule, leaves, pubescent, venation pattern, petiole, inflorescence, bract, flower, calyx, sepal, androecium, gynoecium, ovary, placentation, seed, etc. were recorded for each species.

Morphological characters were analysed by using LABOMED CSM2 and photographs were taken by using Leica EZ4Hd.

Morphological characters are identified by the help of flora's, such as Flora of British India (Hooker, 1896), Flora of India vol: 23 (Balakrishnan *et al.,* 2012), Flora of The Presidency of Madras (Gamble, 2008), A hand book of Coimbatore (Somasundaram, 1963), An excursion Flora of Central Tamilnadu (Matthew, 1991), Flora of Palani Hills (South India) (Matthew, 1999), Flora of Coimbatore (Chandrabose & Nair, 1987), Flora of Andhra Pradesh (India) (Pullaiah & Moulali, 1997), Flora of Karnataka Analysis (Sharma *et al.,* 1984), Flora of Udupi (Bhat, 2003), Flora of Coorg (Murthy & Yoganarasimhan, 1990), Flora of Agasthyamala (Mohanan & Sivadasan, 2002), Flora of Alappuzha (Sunil & Sivadasan, 2009), Flora of Trissur (Sasidharan & Sivarajan, 1996), Flora of Palghat (Vajravellu, 1990), Flora of Calicut (Manilal & Sivarajan, 1982), Flora of Cannonore (Ramachandran & Nair, 1988).

Anatomical Study

Anatomical studies were done for identifying and comparing the species *Croton bonplandianus* Baill and *Croton hirtus* L. Her. The cross sections of leaf, petiole, stem, and root of selected species were taken by hand sectioning. The thin sections were stained in Saffranine and Toludine blue (SaiPrasanna & Karpagam, 2015). The stained materials were mounted using glycerine and observed under compound microscope-LABOMED CXLPLUS.

Preparation of extract for phytochemical analysis

The methanolic extract of leaf, stem and root of selected *Croton* species were prepared for the investigation. The collected material was subjected to cleaning, drying and powdering. The extraction was done using Reflex condenser. 10g of each powdered material of leaves, stem and root were taken in the RB flask containing methanol (200ml). The setup at boiling temperature was kept for about three hours. The extracts thus obtained were filtered and concentrated to 30ml in a water bath. The extract that obtained is diluted and used for preliminary phytochemical tests. Concentrated extract were subjected to HPTLC and HPLC analysis.

It is a distillation technique involving the condensation of vapours and the return of this condensate to the system from which it originated. It is used in industrial and laboratory distillations. It is also used in chemistry to supply energy to reactions over a long period of time. The term reflux is widely used in industries that utilize large scale distillation column and fractionators such as petroleum refineries, petrochemical and chemical plants and natural gas possessing plants. A liquid reaction mixture is placed in a vessel open at the top. This vessel is connected to a condenser, such that any vapours given off are cooled back to the liquid and fall back into the reaction vessel. The vessel is then heated vigorously for the course of the reaction. The purpose is to thermally accelerate the reaction by conducting it at an elevated temperature. The advantage of this technique is that it can be left for a long period of time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapour is immediately condensed in the condenser.

Phytochemical Studies

The stored extract was diluted and used for the various phytochemical studies. A preliminary phytochemical analysis done to determine the presence of phytochemical components such as alkaloids, carbohydrates, reducing sugars, flavonoids, saponins, tannins, steroids, proteins, glycosides, phenols, amino acids and terpenoids.

Tests for Alkaloids

1) Mayer's test: To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids (Tiwari *et al.,* 2011).
2) Hager's test: To a few ml of plant sample extract, two drops of Hager's reagent are added along the sides of test tube. Appearance of yellow precipitate indicates the presence of alkaloids (Tiwari *et al.,* 2011).
3) Wagner's test: A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive (Tiwari *et al.,* 2011).

Tests for Carbohydrates

1) Molish's test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol was added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates. (Banu & Cathrine, 2015).

Tests for Reducing sugars

1) Fehling's test: Fehling A and Fehling B reagents are mixed and few drops of extract was added and boiled. A brick red coloured precipitate of cuprous oxide forms, if reducing sugars present (Joseph *et al.,* 2013).
2) Benedict's test: 0.5ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes

and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar (Rishikesh *et al.,* 2013).

Tests for Flavonoids

1) Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids (Jaradat *et al.,* 2015).
2) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids (Tiwari *et al.,* 2011).

Tests for Saponins

1) Foam test: The stock solution (1 ml) was taken in a test tube and diluted with 20 ml of distilled water. It was shaken by hand for 15 minutes. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins (Hossain *et al.,* 2013).

Tests for Tannins

1) Braymer's test: 5ml solution of the extract was taken in a test tube. Then 1ml of 5% Ferric Chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins (Rishikesh *et al.,* 2013).

Tests for Steroids

1) Salkowski tests: To 2 ml of extract, add 2ml chloroform and 2 ml concentrated H_2SO_4 and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of steroids (Joseph *et al.,* 2013).

Tests for Proteins

1) Millon's test: 5mL of Millon's reagent is added to 3mL of aqueous solution of extract sample. The appearance of white precipitate which slowly turns to pink or red when warmed gently indicates the presence of proteins (Morsy, 2014).

Tests for Glycosides

1) Keller Killiani's test: To the test solution, 2ml of glacial acetic acid containing a few drops of $FeCl_3$ solution was added. 1ml of conc. H2SO4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxy sugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer (Singh & Bag, 2013).

Tests for Phenols

1) Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol (Santhi & Sengottuvel, 2016).
2) Lead acetate test: 10mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of phenol (Santhi & Sengottuvel, 2016).

Tests For Amino acids

1) Ninhydrin test: 2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour (Prasad *et al.,* 2015).

Tests for Terpenoids

1) Copper Acetate test: Extracts is dissolved in water and treated with a few drops of copper acetate solution. Formation emerald green color indicates the presence of diterpenes (Morsy, 2014).

HPTLC Studies

HPTLC is a sophisticated and automated form of TLC. HPTLC aluminium plate precoated with silica gel 60 F 254 was used as stationary phase. Mobile phases employed in this study were prepared by mixing toluene, ethyl acetate and methanol in the ratio 7:3:1 respectively. 10 µl of the samples were applied on precoated plate using Camag automatic TLC sampler 4. Densitometric scanning of the plates was done by using Camag TLC scanner at 254 nm and 366 nm.

High Performence Liquid Chromatography (HPLC)

This solution is then injected into a column that contains resin that will interact with the sample. HPLC analysis was carried out using Shimadzu High Performance Liquid Chromatographic system equipped with LC-10-ATVP pump, SPD M10AVP Photo Diode Array Detector in combination with CLASS-VP 6.12 SP5 integration software. Gradient elution was performed with methanol (solvent A) and 0.1 % formic acid in water (solvent B) in a gradient flow of solvent B concentration; 0.01 min 90%; 10 min 80%; 15 min 70%; 20min 60%, 25-30min 90%. The total run time was optimized to 30 minutes. Injection volume was 20 µl. The flow rate was maintained to 0.8 ml/min. The PDA signal was recorded at 254 nm.

Results

Morphological Description of Selected Plant Species

Morphological features of vegetative as well as reproductive parts were carried out. Results showed the different species exhibited both similarities and dissimilarities in morphology.

Systematic position of *Croton Hirtus L 'Herit.*

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: *Croton hirtus* L.

Croton hirtus L. Herit., Strip. Nov. 17, t. 9.1785; Hook.f. Fl.Brit. India. 5: 242. 1896; Webster in Ann. Missouri Bot. Gard. 54: 262. 1967; Ramachandran *et al.,* in Indian For. 15 (2): 183. 1992; Sunil & Sivadasan, Fl. Alappuzha Dist. 625. 2009; Balakr. & Chakrab *et al.,* in Bull. Bot. Surv. India 23: 228. 2012.

Aromatic herbs, annual, erect up to 30cm, dichotomously branched monoecious; stem terete, pubescent with watery latex; The leaves 5.5 – 6.2× 3.5-4.7cm simple, alternate, stipulate, petiolate; Petiole 2.5-3.1cm cauline, green, stellate hairs; stipules 5-7×1-2mm, 2, filiform, free, opposite, tomentose; lamina ovate-lanceolate, base round with a pair of glands, 3-5 palmately nerved at base and pinnately nerved at lamina, serrate, acute, chartaceous, stellate hairs,green on both sides;Inflorescence 5-10cm, terminal raceme, white, densely hirsute; Male Flowers 2-4 mm, above, unisexual, actinomorphic, bracteate and pedicellate; Pedicel 0.6-1 mm long, cauline, white, stellate hairs with glands; bract 1-2mm, linear, green, hairy, fringed with 2-5 capitate glands, Perianth biseriate, tepel 10, outer layer 1-2mm, 5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.5 -1mm, 5, fused, white, stellate hairs, Stamens 0.9-1mm, 10, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flowers 2.5-5mm, below, unisexual, actinomorphic, hypogynous, bracteate and pedicellate; pedicel 0.5 mm long, cauline, stellate hairs, green, bract 1-2mm, linear, green, hairy, Perianth uniseriate, tepel 0.5-1 mm, 5, fused, obovate, green, persistent, stellate hairy, ovary 1mm, ovoid, syncarpous with free stigma, superior, axile, green, densely hirsute, style 3, 2mm long, bifid; capsule3-6mm long, ovoid, trilocular, green, hirsute; seeds 3-4mm, 3, black and cream patches, glaberous (Plates 1,2,3,4 &5).

Fl. & Fr.: Throughout the year.

Habit: Herb

Habitat: Wastelands

Distribution

Weed of waste places, Plantations and roadsides of Kerala, Tamil Nadu.

Notes: Also known as *Croton glandulosus*, annual herb, native to West Indies and Central and South America, which has become aggressive weed in Tropical Asia and Africa.

Systematic position of *Croton Bonplandianus* Baill.

Class: Dicotyledons

Sub Class: Monochlamydeae

Series: Unisexuales

Family: Euphorbiaceae

Genus: *Croton* L.

Species: *Croton bonplandianus* Baill.

Croton bonplandianus Baill., Adansonia 4: 339. 1864. *Croton sparsiflorus* Morong, Ann. New York Acad. Sci. 7:221. 1893; Mani. & Sivar., Fl. Calicut 266.1982; Rani in Matthew, Fl. Tam. Carnatic 3: 1420. 1983; Ramachandran and Nair, Fl. Cannanore. 272. 1988; Gamble, Fl. Pres. Madras 1316. 1925; Croizat, J. Bombay Nat. Hist. Soc. 41: 573. 1940; Vajravellu, E. Fl. Palghat Dist. 426. 1990; Mohanan & Henry, Fl. Thiruvananthapuram 411. 1994; Subramanian, Fl. Thenmala 326. 1995; Sivar. &Mathew, Fl. Nilambur 613. 1996; Sasi. &Sivar., Fl. Pl. Trissur For. 399. 1996; Dassanayake, A Revised handbook to the Fl. Ceylon 11: 90. 1997; Pattithanam, A Pocket Fl. Siramalai Hills, South India 228-229. 2001; Suryanarayana& Rao, Fl. Nellore Dist. Andhra Pradesh 477. 2002; Mohanan & Sivad., Fl. Agasthyamala 604. 2002; Bhat, Fl. Udupi 560. 2003; Anil Kumar, Sivad. & Ravi, Fl. Pathanamthitta 441. 2005**;** Sunil &Sivadasan, Fl. Alappuzha 624-625. 2009; Balakrishnan*et al.,* Fl. India 23: 228. 2012.

Aromatic shrub, perennial, erect up to 80 cm, dichotomously branched, monoecious; Stem floccose, green, watery latex, tender parts warty; Leaves 2.5-5 × 1-2.5 cm, simple, alternate, exstipulate, petiolate; Petiole 1.3- 1 cm, cauline, floccose, green; lamina lance - ovate, base obtuse, venation cladodromous, serrulate, acute adaxial side dark green and glabrous, abaxial side light green and pubescent; Inflorescence 8-15 cm, terminal raceme, white, pubescent; Male flower 3-2 cm, above, unisexual, actinomorphic, bracteate and pedicillate; Pedicel 1-2mm, cauline, white, floccose; Bract 1 -2mm, green, hairy, triangular, green; Perianth biseriate, tepels 10, Outer layer 1-2 mm, 5, fused, toothed, green, pubescent; Inner layer 2mm, 5, fused, pubescent, white; Stamens 0.5-1.5 mm, 15, basifixed, white; Anther globose, longitudinal dehiscence, light yellow; Female flower 3-2

cm long, below, few in number, unisexual, actinomorphic, hypogynous, bracteate and pedicillate, each flower with a gland at the base of pedicel; Pedicel - 1mm long, cauline, green, floccose; Bract 1mm, triangular, hairy, green; Perianth 1-2 mm, one seriate, tepals 5, green, pubescent, lanceolate; Ovary superior, green, pubescent, sub globose, 3 loculed, placentation axile; Style short, 3, white; Stigma 3, each forked into 6 lobes, brown; Capsules 6 × 4mm, epicarp warty, trigonous, green, floccose; Seeds 3, 4 × 3 mm, greyish black, shiny (Plates 6,7,8,9 &10).

Fl. & Fr.: Throughout the year.

Habit: Herb

Habitat: Terrestrial

Distribution: Found in waste lands and road side areas in India, Bangladesh and all other countries of South Asia

Notes: Commonly known as *Bantulsi*, perennial herb, one of the exotic weeds, native to South America, widely used in folk medicine and traditional ayurvedic medicine. Since it has a characteristic aroma also used as a mosquito repellent. The plant *C. bonplandianus* is treat liver disorders, skin diseases including ring worm infection, to cure the swelling of body, bronchitis and asthma. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abcesses (Singh *et al.,* 2015).

In *C. bonplandianus* leaf, at the mid rib lower epidermis convex and upper epidermis slightly concave, trichomes are short, adaxial hypodermis absent and abaxial hypodermis is parenchymatous, vascular bundle single and presence of xylem in U shape. While in *C. hirtus* leaf, both upper and lower epidermis is convex, trichomes are long, adaxial hypodermis few layered and collenchymatous, abaxial hypodermis multilayered and collenchymatous followed by parenchymatous and having 2 vascular bundle, arranged oppositely.

In *C. bonplandianus* petiole, trichome is short, cortex undifferentiated and parenchymatous, vascular bundles 4 – 5, in which abaxial bundles are much larger than adaxial bundles. While in *C. hirtus* petiole, trichome is long, cortex differentiated which is collenchymatous followed by parenchyma cells, vascular bundles 6 – 7.

In *C. bonplandianus* stem, outline is wavy, trichomes are short, outer cortex made up of alternate patches of chlorenchyma and parenchyma cells, inner cortex parenchymatous in which sclerenchymatous patches present. While in *C. hirtus*stem, outline is circular, trichomes are long, outer cortex collenchymatous and inner cortex parenchymatous and pith shows the presence of calcium oxalate crystals.

In *Croton bonplandianus* leaf, phytochemical constituents such as alkaloids, carbohydrates and terpenoids were present. Whereas reducing sugar, flavanoids, saponin, tannin, steroids, glycosides, proteins, phenols and amino acids were absent.

In *Croton bonplandianus* stem extract phytochemical constituents such as alkaloid, carbohydrate, flavonoids, steroids, glycosides and terpenoids were present. Whereas reducing sugar, saponins, tannins, proteins, phenols and amino acids were absent.

In *Croton bonplandianus* root extract, phytochemical constituents such as alkaloid, carbohydrates, steroids, glycosides and terpenoids were present. Whereas reducing sugar, flavonoids, saponins, tannins, proteins, phenols and amino acids were absent.

In *Croton hirtus* leaf extract, phytochemical constituents such as alkaloid, carbohydrates, steroids, glycosides and terpenoids are present. Whereas reducing sugar, flavonoids, saponins, tannins, proteins, phenols and amino acids are absent.

In *Croton hirtus* stem extract, phytochemical constituents such as alkaloid, carbohydrate, steroids, glycosides and terpenoids were present. Whereas reducing sugar, flavonoids, saponins, tannins, protein, phenols and amino acids were absent.

In *Croton hirtus* root extract, phytochemical constituents such as alkaloids, carbohydrates, steroids, glycosides and terpenoids were present. Whereas reducing sugar, flavonoids, saponins, tannins, protein, phenols and amino acids were absent.

Leaf

UV 254nm: Under UV 254 nm compounds at Rf 0.01, 0.22, 0.30, 0.72, 0.79, 0.85 and 0.92 were present in both *C. hirtus* and *C. bonplandianus*. Band at Rf 0.12 was very specific for *C. hirtus.* (Plate 5)

UV 366nm: Under UV 366 nm compounds at Rf 0.01, 0.04, 0.12, 0.18, 0.23, 0.30, 0.35, 0.38, 0.55, 0.60, 0.82, 0.84, 0.86 and 0.92 were present in both *C. hirtus* and *C. bonplandianus*. Band at Rf 0.46 was specific for *C. hirtus* and band at Rf 0.65 was specific for *C. bonplandianus*. (Plate 5)

ANS 550nm: Under ANS 550 nm compounds at Rf 0.01, 0.19, 0.62, 0.73, 0.80 and 0.92 were present in both *C. hirtus* and *C. bonplandianus*. Band at Rf 0.23 very specific for *C. hirtus* and bands at Rf 0.05, 0.32, 0.35 and 0.45 were specific for *C. bonplandianus.* (Plate 5)

Stem

UV 254nm: Under 254nm compounds at Rf 0.82 and 0.92 were present in both *C. hirtus* and *C.*

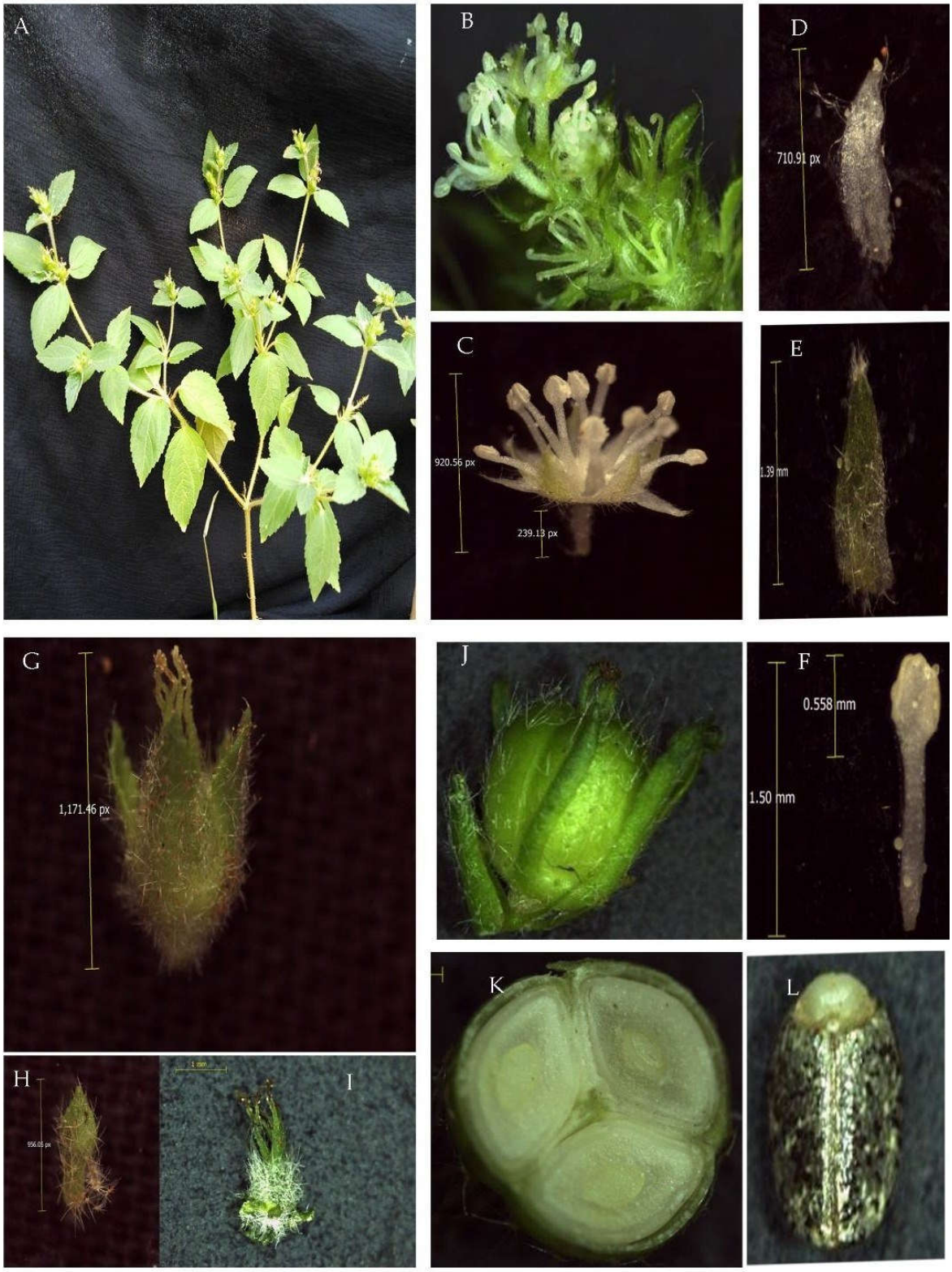

Plate.1: ***Croton hirtus*** - **A**: Habit, **B**:Inflorescence, **C**:Male flower, **D**: inner layer tepal,**E**:Outer layer tepal, **F**: Stamen,**G**:Female flower,**H**:tepal, **I**: Gynoecium, **J** : Mature fruit,**K** : C.S. of Fruit,**L**:Seed.

Plate.2 : ***Croton bonplandianus***. **A** : Habit, **B**: Inflorescence, C: Male flower, D:Outer layer tepal,E: Inner layer tepal,F: Stamen, G: Female flower, **H**: tepal, I: Gynoecium, J: Mature fruit, K:Seeds.

Table 1: Anatomical comparison of leaves of *C. bonplandianus* and *C. hirtus.*

	Croton bonpladianus	***Croton hirtus***
Epidermis·	Single layered, parenchymatous, thick walled, compactly packed, Cuticular. In the mid rib, lower epidermis convex and upper epidermis slightly concave.	Single layered, parenchymatous, thick walled, compactly packed, Cuticular. In the mid region both upper and lower epidermis convex.
Stomata·	Paracytic, surrounded by 2 subsidary cell. Stomatal pore elliptical in outline.	Paracytic, surrounded by 2 wavy subsidarycell. Stomatal pore elliptical in outline.
Trichome	Stalked, glandular, unicellular,short, branched at base and arises from lower epidermis.	Stalked, glandular, unicellular, long,branched at base and arises from both lower and upper epidermis.
Hypodermis	Present at abaxial side. Parenchymatous, spherical in shape, thin walled arranged with inter cellular space.	Adaxial hypodermis few layered, collenchymatous. Abaxial hypodermis multilayered, collenchymas cells followed by parenchyma cells.
Vascular bundle	Single, conjoint, collateral, endarch and closed.	2 bundles arranged oppositely, conjoint, collateral, endarch and closed.
Xylem	Arranged in U shape, made up of x. vessels, x. parenchyma and x. tracheids. Protoxylem oriented towards center and metaxylem towards periphery.	Made up of x. vessels, x. parenchyma and x. tracheids. Protoxylem oriented towards center and metaxylem towards periphery.
Phloem	Seen just below the xylem, made up of small, irregularly shaped and compactly packed cells.	Seen just below the xylem, made up of small, irregularly shaped and compactly packed cells.

Table 2: Anatomical comparison of Petioles of *C. bonplandianus* and *C. hirtus.*

	Croton bonpladianus	***Croton hirtus***
Epidermis	Single layered, parenchymatous, barrel shaped, thick walled, compactly packed and cuticular.	Single layered, parenchymatous, barrel shaped, thick walled, compactly packed and cuticular.
Trichome	Stalked glandular, unicellular,short and branched at base.	Stalked, glandular, unicellular, long and branched at base.
Cortex	7- 8 layered, spherical parenchymatous, thin walled, unequel sized and arranged without inter cellular space.	Differentiated- outer cortex 2-3 layered, collenchymatous arranged compactly. Inner cortex 5-6 layered, parenchymatous, spherical in shape, thin walled and compactly packed.
Vascular bundle	4-5 vascular bundle, triangular in outline, abaxial bundles are much larger than adaxial bundles, bundles are conjoint, collateral, endarch and closed.	6-7 vascular bundle, conjoint, collateral, endarch and closed.
Xylem	Made up of x. vessels and x. parenchyma.protoxylem oriented towards center and metaxylem towards periphery.	Made up of x. vessels and x. parenchyma. protoxylem oriented towards center and metaxylem towards periphery.
Phloem	Seen just below the xylem, made up of small, irregularly shaped and compactly packed cells.	Seen just below the xylem, made up of small, irregularly shaped and compactly packed cells.

bonplandianus. Band at Rf 0.01 was specific for *C. bonplandianus* and band at Rf 0.02 was specific for *C. hirtus*. (Plate 5)

UV 366nm: Under UV 366nm compounds at Rf 0.30, 0.84 and 0.92 were present in both *C. hirtus* and *C.bonplandianus*. Characteristic blue bands at Rf 0.01 and 0.18 were specific for *C. hirtus*. Characteristic bands at Rf 0.23, 0.33, 0.80 and a brown colored band at Rf 0.01 were specific for *C. bonplandianus*. (Plate 5)

ANS 550nm: Under ANS 550nm compounds at Rf 0.01, 0.19 and 0.73 were present in both *C. hirtus* and *C. bonplandianus*. Band at Rf 0.62 was specific for *C. hirtus.* (Plate 5)

Root

UV 254nm: Under UV 254 nm compounds at Rf 0.30, 0.82 and 0.92 were present in both *C. hirtus* and *C. bonplandianus*. Bands at Rf 0.01 and 0.43 were specific for *C. bonplandianus*. Bands at Rf 0.03 and 0.25 were specific for *C. hirtus*. (Plate 5)

UV 366nm: Under UV 366nm compounds at Rf 0.01, 0.18, 0.20, 0.38, 0.43, 0.84 and 0.92 were present in both *C. hirtus* and *C. bonplandianus*. Band at Rf 0.30 was specific for *C. hirtus* and bands at Rf 0.23 and 0.25 were specific for *C. bonplandianus.* (Plate 5)

ANS 550nm: Under ANS 550nm compounds at Rf

Table 3: Anatomical comparison of Stems of *C. bonplandianus* and *C. hirtus.*

	Croton bonpladianus	***Croton hirtus***
Epidermis	Single layered, parenchymatous, barrel shaped, thick walled, compactly packed. Cuticular. Outline wavy.	Single layered, parenchymatous, barrel shaped, thick walled, compactly packed. Cuticular. Outline circular.
Trichome	Stalked, glandular, unicellular, short, branched at base.	Stalked, glandular, unicellular, long, branched at base.
Cortex	Differentiated. Outer cortex- 4-5 layered, made up of alternate patches of chlorenchyma and parenchyma cells.Inner cortex- 5-6 layered, made up of polygonal parenchyma. Sclerenchymatous patches present.	Differentiated. Outer cortex- 3-4 layered, collenchymatous. Inner cortex- 5-6 layered, parenchymatous, polygonal in shape, arranged compactly.
Vascular bundle	Conjoint, bicollateral, endarch and open. Arranged as a wavy manner.	Conjoint, bicollateral, endarch and open. Arranged as a ring.
Xylem	Made up of x. vessels, x. parenchyma and x. tracheids. Protoxylem oriented towards pith and metaxylem towards periphery. Growth ring forms after secondary growth.	Made up of x. vessels, x. parenchyma and x. tracheids. Protoxylem oriented towards pith and metaxylem towards periphery. Growth ring forms after secondary growth.
Phloem	Present on upper and lower side of xylem. Made up of p. parenchyma and sieve cells.	Present on upper and lower side of xylem. Made up of p. parenchyma and sieve cells.
Pith	Massive, parenchymatous, spherical cells, thin walled arranged with inter cellular space.	Massive, parenchymatous, spherical cells, thin walled arranged with inter cellular space. Calcium oxalate crystals present.

Table 4: Anatomical comparison of Roots of *C. bonplandianus* and *C. hirtus.*

	Croton bonpladianus	***Croton hirtus***
Periderm	Ruptured, forming wide fissures, circular in outline.	Ruptured, circular in outline.
Cortex	5-6 layered, made up of parenchyma and sclerenchyma cells, arranged with inter cellular space.	4-5 layered, made up of elongated barrel shaped arenchyma cells, packed without inter cellular space, in between sclerenchyma cells present.
Vascular bundle	Conjoint, collateral, exarch and open.	Conjoint, collateral, exarch and open.
Xylem	Made upof x. tracheids, x. parenchyma and x. vessels. Medullary ray biseriate. Protoxylem oriented towards periphery and metaxylem towards center.	Made upof x. tracheids and x. vessels. Medullary ray uniseriate.Protoxylem oriented towards periphery and metaxylem towards center.
Phloem	Seen just above the xylem, made up of p. parenchyma and sieve cells.	Seen just above the xylem, made up of p. parenchyma and sieve cells.
Pith	Absent.	Reduced, made up of parenchyma and loosely packed with inter cellular space.

0.01, 0.19, 0.72 and 0.80 were present in both *C. hirtus* and *C. bonplandianus*. Bands at Rf 0.30, 0.35 and 0.62 were specific for *C. hirtus*. A characteristic violet band at Rf 0.45 was very specific for *C. bonplandianus*. (Plate5)

HPLC analysis of *C. hirtus* leaf extract showed the presence of total 42 compounds while HPLC analysis of *C. bonplandianus* leaf extract showed the presence of total 33 compounds. Among this, in *C. hirtus* leaf, maximum quantity (14.121% and 11.543%) indicated by area percentage was for the compounds at retention time 23.797 and 25.792 respectively. Whereas the maximum quantity of compound in *C. bonplandianus* leaf extract (12.919%) was for compound at retention time 1.899.

HPLC analysis of *C. hirtus* stem extract showed the presence of total 33 compounds while *C. bonplandianus* stem extract showed the presence of total 28 compounds. Among this, in *C. hirtus* stem maximum quantity (12.341%, 12.090% and 16.180%) indicated by area percentage was for the compounds at retention time 1.856, 23.899 and 24.565. Whereas the maximum quantity of compounds in *C. bonplandianus* stem extract (15.014%, 14.769% and 12.990%) was for compounds at retention time 1.899, 24.704 and 22.069 respectively.

HPLC analysis of *C. hirtus* root extract showed the

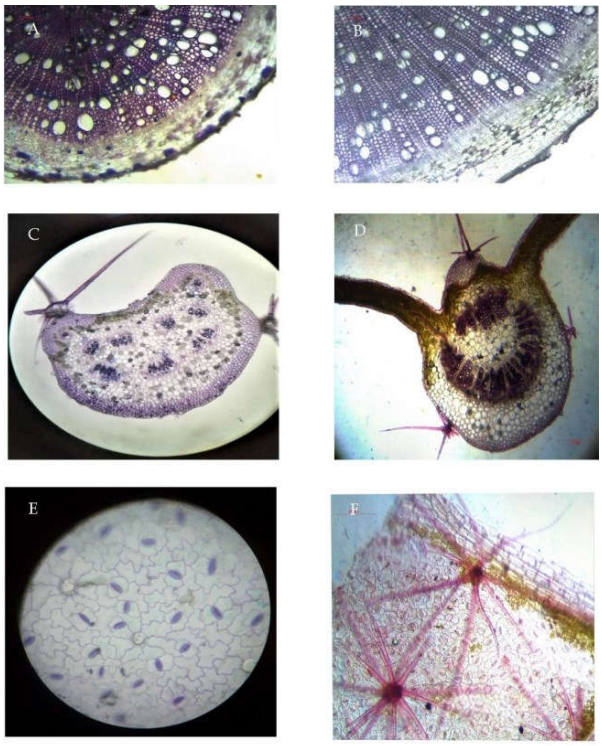

Plate.3: Anatomy of *Croton hirtus* - A:C.S.of Root, B: C.S.OF Stem, C:C.S.of Petiole,D: C.S. of Leaf,E: Paracytic type of Stomata,F:Trichome.

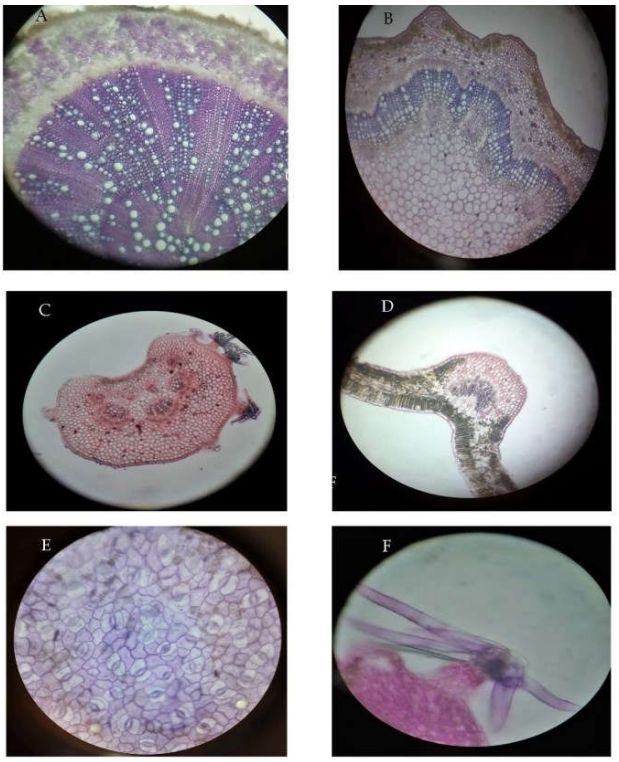

Plate.4:Anatomy of *Croton bonplandianus* - A:C.S.of Root, B: C.S.OF Stem, C:C.S.of Petiole,D: C.S. of Leaf,E: Paracytic type of Stomata,F:Trichome.

Table 5: Preliminary phytochemical tests for *Croton bonplandianus* leaf extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1	Alkaloids	Mayer's testHager's testWagner's test	+++	___
2	Carbohydrates	Molish test	+	_
3	Reducing sugar	Fehling's testBenedict's test	__	++
4	Flavanoids	Alkaline reagent testLead acetate test	__	++
5	Saponin	Foam test	_	+
6	Tannin	Braymer's test	_	+
7	Steroids	Salkowski's test	_	+
8	Proteins	Million's test	_	+
9	Glycosides	Keller killani's test	_	+
10	Phenols	Ferric chloride testLead acetate test	__	++
11	Amino acids	Ninhydrin test	_	+
12	Terpenoids	Copper acetate test	+	_

presence of total 33 compounds while *C. bonplandianus* root showed the presence of 41 compounds. Among this, in *C. hirtus* root maximum quantity (16.618% and 20.919%) indicated by area percentage was for compounds at retention time 24.011 and 24.576. Whereas the maximum quantity of compounds in *C. bonplandianus* root extract (11.656% and 13.900%) was for compounds at retention time 1.899, 24.619 respectively.

The comparative HPTLC and HPLC analysis showed clear differences and similarities between *C. bonplandianus* and *C. hirtus*. HPTLC analysis of leaf extracts of *C. hirtus* and *C. bonplandianus* showed greater similarities among the phytochemical constituents. However some unique bands were seen in *C. hirtus* in all three different wavelengths. Four unique bands were specific to *C. bonplandianus* in 550 nm. Stem extracts of *C. hirtus* and *C. bonplandianus* showed least number of bands. Specific coloured bands are seen in 366nm of both species. Two distinct bands are seen in 254 nm of the root extracts of *C. hirtus* and *C. bonplandianus* respectively. Three bands at Rf 0.30, 0.35 and 0.62 were specific to *C. hirtus* and a characteristic violet band at Rf 0.45 was very specific for *C. bonplandianus* in root extracts at 550 nm.

The HPLC profiles were developed for Methanol extracts of different parts, such as leaf, stem and root of *C. bonplandianus* and *C. hirtus*(Tables 14, 15, 16, 17, 18 and 19 and Figures 1, 2, 3, 4, 5 and 6). Maximum

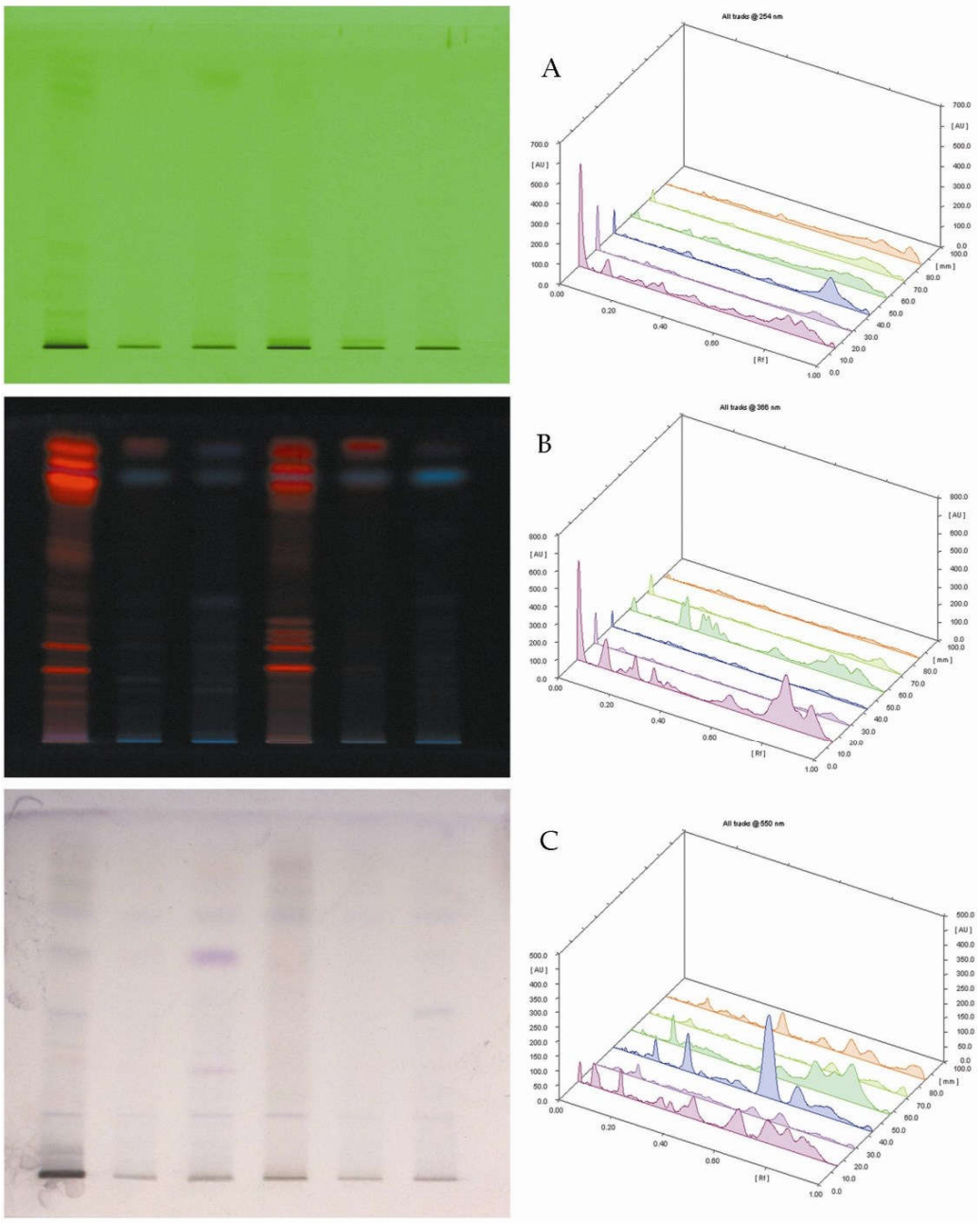

Plate 5: HPTLC profile of root, stem & leaf of ***C.hirtus*** & ***C.bonplandianus***. **A**: HPTLC chromatogram& densitometric scanning image under 254nm. **B:** HPTLC chromatogram & densitometric scanning image under 366nm, **C:** HPTLC chromatogram & densitometric image under 550nm. Track 1: Leaf Extract of C.hirtus, Track 2: Stem extract of C.hirtus, Track 3: Root Extract of C.hirtus, Track 4: Leaf Extract of C.bonplandianus, Track 5: Stem Extract of C.bonplandianus, Track 6: Root Extract of C.bonplandianus.

Table 6: Preliminary phytochemical tests for *Croton bonplandianus* stem extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1	Alkaloid	Mayer's testHager's testWagner's test	+++	___
2	Carbohydrate	Molish test	+	_
3	Reducing sugar	Fehling's testBenedict's test	__	++
4	Flavonoids	Alkaline reagent testLead acetate test	++	__
5	Saponins	Foam test	_	+
6	Tannins	Braymer's test	_	+
7	Steroids	Salkowski's test	+	_
8	Proteins	Millon's test	_	+
9	Glycosides	Keller killani's test	+	_
10	Phenols	Ferric chloride testLead acetate test	__	++
11	Amino acids	Ninhydrin test	_	+
12	Terpenoids	Copper acetate test	+	_

Table 7: Preliminary phytochemical tests for *Croton bonplandianus* root extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1.	Alkaloids	Mayer's testHager's testWagner's test	+++	___
2.	Carbohydrates	Molish test	+	_
3.	Reducing sugar	Fehling's testBenedict's test	__	++
4.	Flavonoids	Alkaline reagent testLead acetate test	__	++
5.	Saponins	Foam test	_	+
6.	Tannins	Braymer's test	_	+
7.	Steroids	Salkowski's test	+	_
8.	Proteins	Millon's test	_	+
9.	Glycosides	Keller killani's test	+	_
10.	Phenols	Ferric chloride testLead acetate test	__	++
11.	Amino acids	Ninhydrin test	_	+
12.	Terpenoids	Copper acetate test	+	_

Table 8: Preliminary phytochemical tests for *Croton hirtus* leaf extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1.	Alkaloid	Mayer's testHager's testWagner's test	+++	___
2.	Carbohydrates	Molish test	+	_
3.	Reducing sugar	Fehling's testBenedict's test	__	++
4.	Flavonoids	Alkaline reagent testLead acetate test	__	++
5.	Saponins	Foam test	_	+
6.	Tannins	Braymer's test	_	+
7.	Steroids	Salkowski's test	+	_
8.	Proteins	Millon's test	_	+
9.	Glycosides	Keller killani's test	+	_
10.	Phenols	Ferric chloride testLead acetate test	__	++
11.	Amino acids	Ninhydrin test	_	+
12.	Terpenoids	Copper acetate test	+	_

Table 9: Preliminary phytochemical test for *Croton hirtus* stem extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1	Alkaloids	Mayer's testHager's testWagner's test	+++	___
2	Carbohydrates	Molish test	+	_
3	Reducing sugar	Fehling's testBenedict's test	__	++
4	Flavonoids	Alkaline reagent testLead acetate test	__	++
5	Saponins	Foam test	_	+
6	Tannins	Braymer's test	_	+
7	Steroids	Salkowski's test	+	_
8	Protein	Millons test	_	+
9	Glycosides	Keller killani's test	+	_
10	Phenols	Ferric chloride testLead acetate test	__	++
11	Amino acids	Ninhydrin test	_	+
12	Terpenoids	Copper acetate test	+	_

Table 10: Preliminary phytochemical tests of *Croton hirtus* root extract.

S. No.	Phytochemical constituents	Name of test	Present	Absent
1	Alkaloids	Mayer's testHager's testWagner's test	+++	___
2	Carbohydrates	Molish test	+	_
3	Reducing sugar	Fehling's testBenedict's test	__	++
4	Flavonoids	Alkaline reagent testLead acetate test	__	++
5	Saponins	Foam test	_	+
6	Tannins	Braymer's test	_	+
7	Steroids	Salkowski's test	+	_
8	Protein	Millon's test	_	+
9	Glycosides	Keller killani's test	+	_
10	Phenols	Ferric chloride testLead acetate test	__	++
11	Amino acids	Ninhydrin test	_	+
12	Terpenoids	Copper acetate test	+	_

number of compounds (42) was present in leaf extracts of *C. hirtus* and the least was in *C. bonplandianus* stem extract (28). The maximum quantity (20.919%) indicated by area percentage was for compound at retention time 24.576 in root extracts of *C. hirtus* The comparative HPLC analysis showed that the compound at retention time 1.899 was present in *C. bonplandianus* leaf, stem and root. As well as, retention time 3.712 compound was present in both *C. bonplandianus* and *C. hirtus* root extracts. The compound present at retention time 25.067 was seen in leaf extracts of both *C. bonplandianus* and *C. hirtus*.

Phytochemical constituents such as alkaloids, carbohydrates and terpenoids were uniformly present in the stem, leaves and root of *C. bonplandianus* whereas *C. hirtus* was characterised by the presence of alkaloids, carbohydrates, steroids, glycosides and terpenoids in leaf, stem, and root. HPLC analysis also shows variation in terms of phytoconstituents. Maximum number of compounds (42) indicated by peaks was present in leaf extracts of *C. hirtus* and the least was in *C. bonplandianus* stem extract (28).

Conclusion

The present study is an attempt has been made to differentiate C. *bonplandianus* and *C. hirtus* in morphological, anatomical and phytochemical aspects. Considerable differences were observed between species in their morphological, anatomical and phytochemical characters. Morphological comparison revealed substantial variation in trichomes, leaves, style, etc. Anatomical comparison of different parts revealed variation in vascular bundle, xylem, pith, medullary ray, etc. Preliminary phytochemical tests shows clear difference between these two species. Phytochemical constituents such as alkaloids, carbohydrates and terpenoids were uniformly present in the stem, leaves and root of *C.*

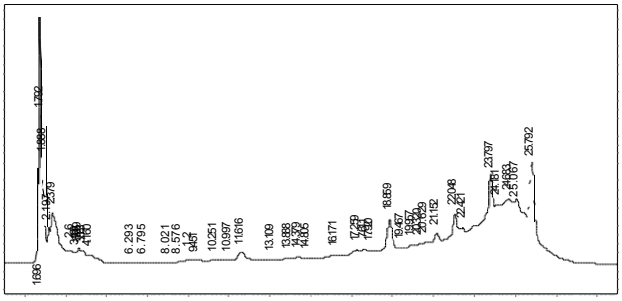

Fig. 1: HPLC chromatogram of the leaf extract of *C. hirtus.*

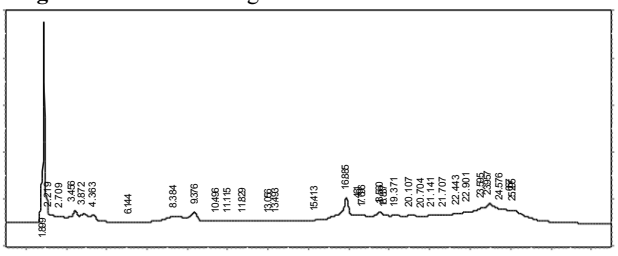

Fig. 2: HPLC chromatogram of the leaf extract of *C. bonplandianus*

Table 11: Comparitive HPTLC analysis of the leaf extracts of *C. hirtus and C. bonplandianus.*

Rf	*C. hirtus*	*C. bonplandianus*
UV 254nm		
0.01 (brown)	1	1
0.12 (brown)	1	0
0.22 (brown)	1	1
0.30 (brown)	1	1
0.72 (brown)	1	1
0.79 (brown)	1	1
0.85 (brown)	1	1
0.92 (brown)	1	1
UV 366nm		
0.01 (pink)	1	1
0.04 (light red)	1	1
0.12 (light red)	1	1
0.18 (light red)	1	1
0.23 (fluorescent red)	1	1
0.30 (fluorescent red)	1	1
0.35 (light red)	1	1
0.38 (light red)	1	1
0.46 (light red)	1	0
0.55 (light red)	1	1
0.60 (light red)	1	1
0.65 (light red)	0	1
0.82 (fluorescent red)	1	1
0.84 (light blue)	1	1
0.86 (fluorescent red)	1	1
0.92 (fluorescent red)	1	1
ANS 550nm		
0.01 (brown)	1	1
0.05 (violet)	1	0
0.19 (violet)	1	1
0.23 (violet)	0	1
0.32 (violet)	1	0
0.35 (violet)	1	0
0.45 (violet)	1	0
0.62 (violet)	1	1
0.73 (violet)	1	1
0.80 (violet)	1	1
0.92 (violet)	1	1

bonplandianus whereas *C. hirtus* was characterised by the presence of alkaloids, carbohydrates, steroids, glycosides and terpenoids in leaf, stem, and root.

The comparative HPTLC studies showed greater similarities between the two species. However, differences were observed in terms of number and density of bands between *C. bonplandianus* and *C. hirtus*.

Table 12: Comparitive HPTLC analysis of the Stem extracts of *C. hirtus and C. bonplandianus.*

Rf	*C. hirtus*	*C. bonplandianus*
UV 254nm		
0.01 (brown)	0	1
0.02 (brown)	1	0
0.82 (brown)	1	1
0.92 (brown)	1	1
UV 366nm		
0.01 (blue)	1	0
0.01 (brown)	0	1
0.18 (light blue)	1	0
0.23 (light red)	0	1
0.30 (blue)	1	0
0.30 (violet)	0	1
0.33 (light red)	0	1
0.80 (red)	0	1
0.84 (blue)	1	1
0.92 (fluorescent red)	1	1
ANS 550nm		
0.01 (violet)	1	1
0.19 (violet)	1	0
0.19 (light blue)	0	1
0.62 (light blue)	1	0
0.73 (light blue)	1	1

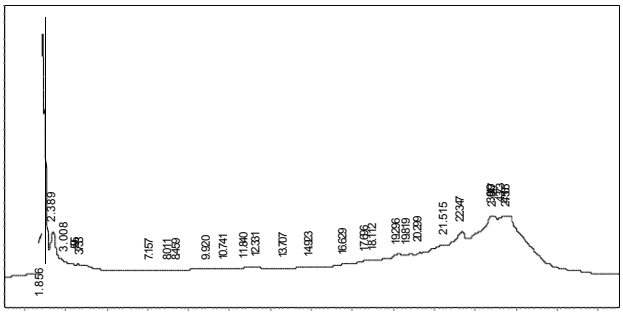

Fig. 3: HPLC chromatogram of the stem extract of *C. hirtus.*

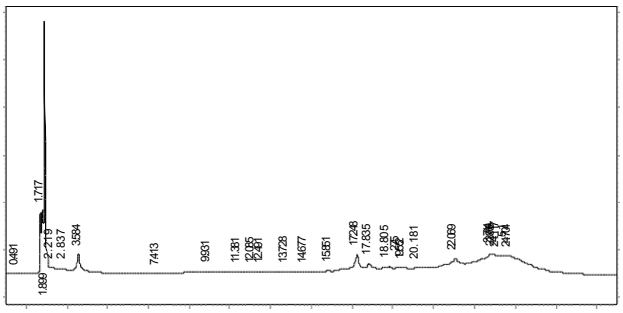

Fig. 4: HPLC chromatogram of the stem extract of *C. bonplandianus*

Table 13: Comparitive HPTLC analysis of the root extracts of *C. hirtus and C. bonplandianus.*

Rf	*C. hirtus*	*C. bonplandianus*
UV 254nm		
0.01 (brown)	0	1
0.03 (brown)	1	0
0.25 (brown)	1	0
0.30 (brown)	1	1
0.43 (brown)	0	1
0.82 (brown)	1	1
0.92 (brown)	1	1
UV 366nm		
0.01 (blue)	1	1
0.18 (blue)	1	1
0.20 (blue)	1	1
0.23 (light red)	0	1
0.25 (blue)	0	1
0.30 (fluorescent blue)	1	0
0.38 (blue)	1	1
0.43 (blue)	1	1
0.84 (light blue)	1	1
0.94 (blue)	1	1
ANS 550nm		
0.01 (brown)	1	1
0.19 (light blue)	1	1
0.30 (violet)	1	0
0.35 (violet)	1	0
0.45 (violet)	0	1
0.62 (violet)	1	0
0.73 (light blue)	1	1
0.80 (violet)	1	1

Maximum number of bands was observed in leaves than stem and root. Stem extracts of *C. hirtus* and *C. bonplandianus* showed least number of bands. Characteristic distinct coloured bands were seen in different wavelengths of *C. bonplandianus* and *C. hirtus* showed the difference in phytoconstituents of both species. Two bands specific to *C. hirtus* and two bands unique to *C. bonplandianus* was present under 254 nm of the root extracts of both species. Further analysis of these unique compounds will result in the identification of the marker compounds for these two different species belonging to the same genus. HPLC analysis also shows variation in terms of phytoconstituents. Maximum number of compounds (42) indicated by peaks was present in leaf extracts of *C. hirtus* and the least was in *C. bonplandianus* stem extract (28). The maximum quantity (20.919%) indicated by area percentage was for compound at retention time 24.576 in root extracts of *C.*

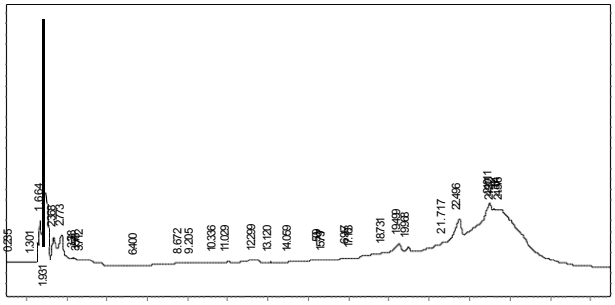

Fig. 5: HPLC chromatogram of the root extract of *C. hirtus.*

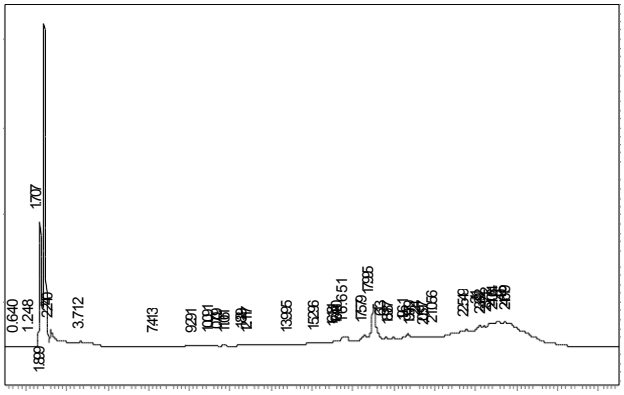

Fig. 6: HPLC chromatogram of the root extract of *C. bonplandianus*

hirtus. The comparative HPLC analysis of leaf, stem and root extracts showed that the compound at retention time 1.899 was a unique compound of *C. bonplandianus* and can be used a marker compound. The present study also highlights its great value for future studies to disclose their potential medicinal values for human welfare.

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Research Article

COMPARATIVE CHARACTERIZATION ON MORPHOLOGY, ANATOMY AND PHYTOCHEMISTRY OF THREE SPECIES OF CROTON L. (EUPHORBIACEAE)

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ABSTRACT

Aims: The Comparative analysis of three species of *Croton; Croton aromaticus*, *C. malabaricus* and *C. tiglium* were carried out based on morphology, anatomy and phytochemistry.

Methods and Material: Morphological characterizations of different plant parts were carried out and the details of plant parts characterized by using LABOMED CSM2stereo microscope and photographs were taken by using Leica EZ4Hd camera. Anatomical studies were done for identifying and comparing the selected species of Croton based on sectioning and staining method also used compound microscope-LABOMED CXLPLUS. Phytochemical characterization of plant parts carried out plant extraction method, preliminary phytochemical analysis and also HPTLC.

Results: Family Euphorbiaceae is one of the most complex, heterogeneous, large and diverse family of herbs, shrubs and trees. Croton is one of the largest genera in the family Euphorbiaceae. Comparative analysis of three species of *Croton; Croton aromaticus*, *C. malabaricus* and *C. tiglium* were carried out based on morphology, anatomy and phytochemistry. *C. tiglium* is morphologically distinct from the rest of two by the absence of trichomes in it. Anatomy was almost similar in these three species. But most of the phytochemical constituents present in the leaves of these three species than its stem. Based on the HPTLC analysis most of the phytochemical constituents are present in *C. aromaticus* and least number of components are present in *C. malabaricus.* **Conclusion:** This study also highlights its great value for future studies to disclose their potential medicinal values for human welfare.

Conclusions: By analysing the morphology, anatomy and phytochemistry of selected Croton species leads to interpret the ethanomedicinal value which is helpful for the society to reveal the active principle behind it for pharmacological applications in the near future.

INTRODUCTION

Plants produce thousand types of chemicals. Some of the organic compounds like carbohydrates, fats, proteins, nucleic acids, chlorophylls etc. are required for their basic metabolic processes and found throughout the plant kingdom. These organic compounds are called primary metabolites or biomolecules. These are produced in large quantities and can easily be extracted from the plants. Many plants, fungi and microbes of certain genera and families synthesize a number of organic compounds which are not involved in primary metabolism (photosynthesis, respiration, and protein and lipid metabolism) and seem to have no direct function in growth and development of plants. Such compounds are called secondary metabolites. Family Euphorbiaceae is popularly known as the "Spurge family". The name "spurge" is derived from Medieval French "epurga" referring to the purgative properties of the seeds of the genus of the Euphorbia.

Croton L. (Euphorbiaceae) is one of the largest genera of flowering plants, with about 1300 species of herbs, shrubs, trees and occasionally lianas that are ecologically prominent and important elements of secondary vegetation in the tropical and subtropical regions worldwide. The genus is pan tropical, with some extending to temperate areas .*Croton* L. (Euphorbiaceae) is a characteristic genus of dry to moist vegetation in the tropics and subtropics worldwide, and its species can usually be recognized in the field by their pungent odor, stellate or lepidote pubescence, clear to colored latex, and leaves that turn orange before dehiscing.

Croton aromaticus L. (Euphorbiaceae) is a tree. Flowering and fruitingperiod is April-August. It is mainly used as the treatment for; bronchitis, diarrhea, dysentery, fever, malaria. It is also used as an insecticide, alternatives for synthetic fungicides. *Croton malabaricus* Bedd. is commonly called as Chunnambumaram, Pamparam, Anakuru. This tree growsup to

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28m tall and it is endemic to Southern Western Ghats. Flowering and fruiting is April-November. *Croton tiglium* is a small tree commonly called as Neervalam and Kumbhini, annual. Flowering and fruiting is January-November. It also used for the treatment of skin fungal infections. Stem, leaves and seeds have reported for antidermatophytic, anti- tumor, anti- HIV, anti- inflammatory antidermatophytic, antioxidant activities. It also used in the treatment of gastrointestinal disturbances.

Collection of materials

The plants selected for the present study are *Croton aromaticus* L., *Croton malabaricus* Bedd. And Croton *tiglium* L. They were collected from Nelliampathy of Palakkad district, Periya pass of Kannur district and Pulppally of Wayanad district of Kerala respectively.

Morphological Study

Morphological characterizations of different plant parts were carried out and the details of habit, stem, stipule, leaves, pubescent, venation pattern, petiole, inflorescence, bract, flower, calyx, sepal, androecium, gynoecium, ovary, placentation, seed, etc. were recorded for each species. Morphological characters were analysed by using LABOMED CSM2stereo microscope and photographs were taken by using Leica EZ4Hd camera.

The collected specimens were authenticated with help of available Floras and Literature such as Flora of India vol: 23 (1), Flora of The Presidency of Madras (7), A hand book of Coimbatore (30), Flora of Coimbatore (3), Flora of Agasthyamala (18), Flora of Calicut (16), Flowering plants of Trissur forests (26), Flora of Palghat (34), Flora of Courtallum (20), Flora of Nilambur (Sivarajan and Mathew, 1996), Flora of Silent valley (Manilal, 1988), Flora of Thiruvananthapuram (17), Revised handbook to the flora of Ceylon (4), Flora of Thenmalai (31), Flora of Pathanamthitta (Western Ghats, Kerala, India)(13). The specimens where deposited in Dev herbarium, St. Joseph's college Devagiri, Calicut.

Anatomical Study

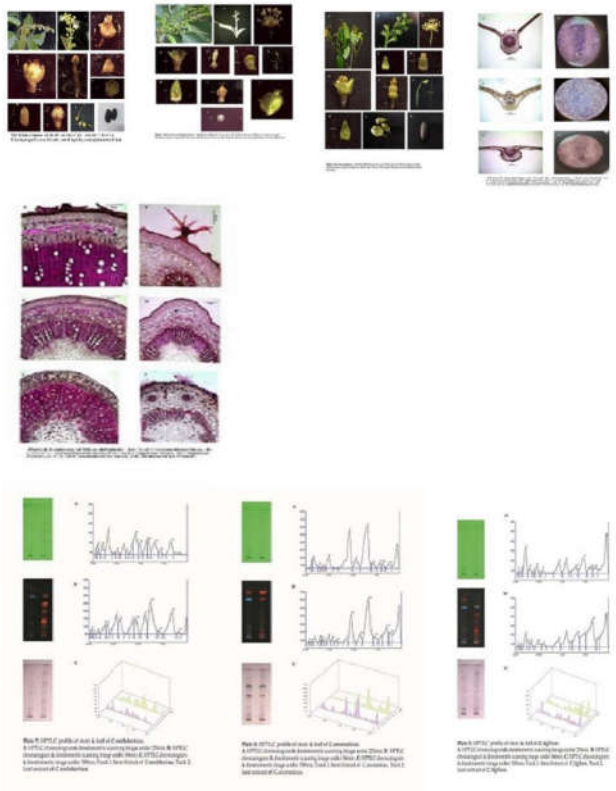
Anatomical studies were done for identifying and comparing the selected species of *Croton* such as *C. aromaticus* L., *C. malabaricus* Bedd. And *C. tiglium* L. The cross sections of leaf, petiole and stem of these species were taken by hand sectioning. The thin sections were stained in Saffranin (24). The stained materials were mounted using glycerine and observed under compound microscope-LABOMED CXLPLUS.

Preparation of extract for phytochemical analysis

The methanolic extract of leaf and stem of selected *Croton* species were prepared for the investigation. The collected material was subjected to cleaning, drying and powdering. The extraction was done using Reflex condenser. 10g of each powdered material of leaves and stem were taken in the RB flask containing methanol (200ml). The setup at boiling temperature was kept for about three hours. The extracts thus obtained were filtered and concentrated to 30ml in a water bath. Then this extract again diluted and used for preliminary phytochemical tests. While the concentrated extract were subjected to HPTLC analysis.

Phytochemical Studies

The stored extract was diluted and used for the various phytochemical studies. A preliminary phytochemical analysis done to determine the presence of phytochemical components such as alkaloids, coumarins, flavonoids, glycosides, phenols, quinones, Saponins, steroids, tannins and terpenoids.



Tests for Alkaloids

1. ***Mayer's test:*** To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids (32).
2. ***Hager's test:*** To a few ml of plant sample extract, two drops of Hager's reagent are added along the sides of test tube. Appearance of yellow precipitate indicates the presence of alkaloids (32).
3. ***Wagner's test:*** A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive (32).

Tests for Coumarins

To 2 ml of the extract add 3 ml of 10% NaOH solution. Formation of yellow colour indicates the presence of coumarins (23).

Tests for Flavonoids

1. ***Alkaline reagent test:*** 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids (10).

2. ***Lead acetate test:*** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids (32).

Tests for Glycosides

Keller Killiani's test: To the test solution, 2ml of glacial acetic acid containing a few drops of $FeCl_3$ solution was added. 1ml of conc. H_2SO_4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxy sugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer (28).

Tests for Phenols

1. ***Ferric chloride test:*** 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol (25).
2. ***Lead acetate test:*** 10mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of phenol (25).

Tests for Quinones

To 2 ml of the extract add 3 ml of HCl. Formation of yellow colour indicates the presence of quinones in the sample (8).

Tests for Saponins

Foam test: The stock solution (1 ml) was taken in a test tube and diluted with 20 ml of distilled water. It was shaken by hand for 15 minutes. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

Tests for Steroids

Salkowski tests: To 2 ml of extract, add 2ml chloroform and 2 ml concentrated H2SO4 and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of steroids.

Tests for Tannins

Braymer's test: 5ml solution of the extract was taken in a test tube. Then 1ml of 5% Ferric Chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins (Rishikesh et al., 2013).

Tests for Terpenoid

Copper Acetate test: 2 ml of the extracts is dissolved in water and treated with a 3-4 drops of copper acetate solution. Formation emerald green color indicates the presence of diterpenes.

To 2 ml of the extract add 1 ml of chloroform and 2 ml of H_2SO_4.Formation of reddish brown colour indicates the presence of terpenoids.

High performance thin layer chromatography (HPTLC) studies

HPTLC is a sophisticated and automated form of TLC. HPTLC is the fastest of all chromatographic methods. In HPTLC aluminium plate precoated with silica gel 60 F 254 was used as stationary phase. Chromatograms were developed in a saturated twin trough chamber. Mobile phases employed in this study were prepared by mixing toluene, ethyl acetate and methanol in the ratio 8:2:0.1 respectively. 10 µl of the samples were applied on precoated plate using Camag automatic TLC sampler 4. The plates were dried and the spots were visualized sequentially under UV light at 254 and 366 nm. Densitometric scanning of the plates was done by using Camag TLC scanner at 254 nm and 366 nm.

RESULTS

Morphological description of selected plant species

Morphological features of vegetative as well as reproductive parts were carried out. Results showed the different species exhibited both similarities and dissimilarities in morphology.

Systamatic Position of Croton Aromaticus L.

Class: Dicotyledons
Sub Class: Monochlamydeae
Series: Unisexuales
Family: Euphorbiaceae
Genus: *Croton* L.
Species: ***Croton aromaticus*** L.
Local Name: Theppadi

Synonyms: *C. aromaticus* Gartn., *C. aromaticus* Miq., *C. aromaticus* var. *lacciferus* (L.) Trime Trees, branchlets stellate, brown tomentose. Leaves to 12 x 6 cm,simple, ovate, acuminate, cordate at base, 3-ribbed from base, glands 2 pairs; upper leaves with acute base; petiole 2-3 cm long. Inflorescence 10 cm long, densely stellate hairy, white; Male flower 3-5mm long, above, unisexual, actinomorphic, bracteate, Perianth biseriate, tepel 10, outer layer 1-2mm,5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.6 -1mm, 5, fused, white, stellate hairs , Stamens 0.8-1mm, 10, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flower 7 mm across; sepals ovate, acute; petals obovate, ciliate; styles 3, bifid, glabrous. Capsule8-10mm long, ovoid, trilocular, green, hirsute; seeds 4-6mm, 3, black and cream patches, glaberous (Plate-1).

Fl. & Fr.: April- October.
Habit: Tree
Habitat: Terrestrial.
Distribution: Palakkad, Kottayam, Idukki, Kollam, Pathanamthitta, Malappuram, Kozhikode, Thrissur

Systamatic Position of Croton Malabaricus Bedd

Class: Dicotyledons
Sub Class: Monochlamydeae
Series: Unisexuales
Family: Euphorbiaceae
Genus: *Croton* L.
Species: Croton malabaricus Bedd.
Local Name: Kolavanchi, Thenadal, Pambaram, Chunnambumaram

Table 1 Anatomical comparison of leaves of *C. aromaticus* L., *C. malabaricus* Bedd. and *C. tiglium* L. (Plate-5)

	C. aromaticus	*C. malabaricus*	*C. tiglium*
Epidermis	Single layered, parenchymatous, thick walled, compactly packed, Cuticular. In the mid rib, lower epidermis convex and upper epidermis is linear.	Single layered, parenchymatous, thick walled, compactly packed, Cuticular. In the mid rib region upper epidermis is slightly convex and lower epidermis is rectangular.	Single layered, parenchymatous, thick walled, compactly packed, Cuticular. In the mid rib, lower epidermis broadly convex and upper epidermis slightly convex.
Stomata	Paracytic, surrounded by 2 subsidary cell. Stomatal pore elliptical in outline.	Paracytic, surrounded by 2 wavy subsidary cells. Stomatal pore elliptical in outline.	Paracytic, surrounded by 2 subsidary cell. Stomatal pore elliptical in outline.
Trichome	Stalked, glandular, unicellular, short, branched at base and arises from lower and upper epidermis.	Stalked, hairy, unicellular, long, branched at base and arises from lower epdermis.	Absent
Mesophyll	Differentiated into palisade and spongy parenchyma, single layered palisade parenchyma, covered with tannin cells; spongy parenchyma oriented towards lower epidermis, 2-3 layered, tannin cells present.	Differentiated into palisade and spongy parenchyma, Palisade parenchyma single layered, spongy parenchyma 2-3 layered, spicular cells present.	Differentiated into palisade and spongy parenchyma, Palisade parenchyma single layered, covered with tannin cells; spongy parenchyma 2-3 layered tannin cells present.
Midrib	Outer layers of cells are made up of 3-4 small sclerenchymatous cells and inner layers of cells are made up of 7-8 layers of parenchyma cells.	Outer region made up of 3-4 layered small sclerenchymatous cells and inner region consist of 14-15 layers of parenchyma cells, tannin cells present.	Consist of a sclerenchymatous cap, palisade parenchyma seen just beneath the sclerenchymatous cap, above the lower epidermis 3-4 layered sclerenchymatous cells and inner region made up of parenchyma cells.
Vascular bundles	Arranged as two sets of bundles; it is U shaped towards the lower epidermis and seen as another small patch towards the upper epidermis, conjoint, collateral, endarch and closed.	Conjoint, collateral, endarch, closed, towards the lower epidermis it is U shaped and towards the upper epidermis two bundles are seen.	Conjoint, collateral, endarch, closed, towards the lower epidermis it is U shaped and towards the upper epidermis bundles are seen as a linear line.
Xylem	Made up of xylem tracheids, xylem vessels and xylem parenchyma. In abaxial bundle xylem arranged in U shape, Protoxylem oriented towards centre and metaxylem oriented towards periphery	Made up of xylem tracheids, xylem vessels and xylem parenchyma. Protoxylem towards the centre and metaxylem towards the periphery	Made up of xylem tracheids, xylem vessels and xylem parenchyma. Seen outside the xylem, protoxylem towards the centre and metaxylem towards the periphery.
Phloem	Seen outside the xylem. Made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed.	Seen outside the xylem. Made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed. Present in very less amount	Seen outside the xylem, made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed

Table 2. Anatomical comparison of petioles of *C. aromaticus* L., *C. malabaricus* Bedd.*and C.tiglium* L. (Plate-4)

	C. aromaticus	*C. malabaricus*	*C. tiglium*
Epidermis	Single layered, wavy in outline, barrel shaped, thick walled, compactly packed and cuticular.	Single layered, barrel shaped, thick walled, compactly packed and cuticular.	Single layered, wavy in outline, barrel shaped, thick walled, compactly packed and cuticular.
Trichome	Stalked, glandular, unicellular, short, branched at base.	Stalked, hairy, unicellular, long, branched at base.	Absent
Hypodermis	Made up of small sclerenchymatous cells. 7-8 layered, tannin cells present.	Made up of small sclerenchymatous cells, 6-7 layered, spicular cells present.	Made up of small sclerenchymatous cells, 6-7 layered.
Cortex	Made up of parenchymatous cell, 10-11 layers of cells, tannin cells and calcium oxalate crystals are present.	Made up of parenchymatous cells, reduced, 2-3 layered, two bundles present at the cortex, few tannin cells, calcium oxalate crystals and spicular cells are present.	Reduced, made up of parenchymatous cells, two bundles present at one side, few calcium oxalate crystals and tannin cells present, small thick walled cells are present as discontinuous ring.
Vascular bundle	Bundles are 7 in number in which 5 in a circle with wavy outline and 2 are at the two upper sides, conjoint, collateral, endarch and closed.	Xylem and phloem seen as continuous ring, two bundles present outside this ring, conjoint, collateral, endarch and closed	Xylem and phloem present as ring, conjoint, collateral, endarch and closed.
Xylem	Seen as patches, made up of xylem parenchyma, xylem vessels and xylem tracheids. Protoxylem oriented towards centre and metaxylem oriented towards periphery. In the peripheral 2 bundles; protoxylem towards the upper side and metaxylem towards the lower portion	Seen as a continuous ring, protoxylem seen toward the centre and metaxylem towards the periphery, made up of xylem parenchyma, xylem vessels and xylem tracheids.	Made up of xylem parenchyma, xylem vessels and xylem tracheids. Arranged as ring, protoxylem towards the centre and metaxylem towards the periphery.
Phloem	Seen outside the xylem, seen as wavy ring in the central bundle and circular around outer bundle, tannin cells present.	Seen outside the xylem as a continuous ring. Made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed.	Seen outside the xylem, made up of phloem parenchyma which is irregularly shaped, unequal in size and compactly packed, present in a very scare amount.
Pith	Parenchymatous with large and spherical cells, tannin cells and calcium oxalate crystals are present.	Massive, parenchymatous, spicular cells and few calcium oxalate crystals are present.	Massive, parenchymatous, few tannin cells present

Synonyms: *Oxydectes malabarica* (Bedd.) Kuntz.

Trees, to 20 m high, bark greyish-white, smooth; branchlets stellate-hairy. Leaves simple, alternate, 7-24 x 3-12 cm, rhombic-ovate, broadly ovate or elliptic, apex acuminate, base cuneate, obtuse or round, margin entire, glabrous or with silvery stellate hairs and reddish glands beneath, chartaceous; stipules 12-18 mm long, lateral, filiform, scarious; petiole 10-125 mm long, slender, stellate scales present; prominently, 3-4-ribbed from base; lateral nerves 4-6 pairs, pinnate, prominent, intercostaescalariform, obscure.

Table 3 Anatomical comparison of stems of *C. aromaticus* L., *C. malabaricus* Bedd.and *C.tiglium* L. (Plate-4)

	C. aromaticus	*C. malabaricus*	*C. tiglium*
Epidermis	Single layered, wavy outline, covered by cuticle	Single layered, wavy outline, covered by cuticle.	Single layered, covered by cuticle, tannin cells are seen as a continuous layer just beneath the epidermis.
Trichome	Numerous trichomes which is stalked, glandular, unicellular and branched at base.	Stalked, hairy, Unicellular, long, branched at base.	Absent
Cortex	Reduced, differentiated as outer, middle and inner cortex. Outer cortex is sclerenchymatous, tannin cells and spicular cells present. Middle cortex is chlorenchymatous, completely filled with tannin cells, calcium oxalate crystals are present. Inner cortex is parenchymatous with few tannin cells and calcium oxalate crystals. Spicular cells are seen as small patches, discontinuous, 1-3 layered with intercellular space.	Reduced, differentiated as outer and inner cortex. Outer cortex is sclerenchymatous, outer layers of cells are small and inner layers of cells are large, tannin cells present. Inner cortex is parenchymatous with few tannin cells, spicular cells are seen as discontinuous ring.	Massive, differentiated as outer and inner cortex. Outer cortex is sclerenchymatous. Inner cortex is chlorenchymatous with tannin cells. Patches of spicular cells are seen as discontinuous ring.
Vascular bundle	Conjoint, collateral, endarch and open, massive.	Conjoint, collateral, endarch and closed.	Conjoint, collateral and open and endarch.
Xylem	Massive, made up xylem tracheids, xylem vessels and xylem parenchyma. Arranged in a wavy ring. Protoxylem arranged towards the pith and shows spiral thickening. Metaxylem arranged towards the periphery and shows bordered pitted thickening, tannin cells present.	Made up of xylem tracheids, xylem vessels and xylem parenchyma. Seen outside the xylem, protoxylem towards the centre and metaxylem towards the periphery, wavy outline and tannin cells present.	Massive, wavy in outline, made up of xylem tracheids, xylem vessels and xylem parenchyma. Seen outside the xylem, protoxylem towards the centre and metaxylem towards the periphery.
Phloem	Seen outside the xylem, made up of small sized irregularly placed cells which are compactly arranged.	Seen outside the xylem. Made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed. Seen as discontinuous ring.	Present in very few amount, seen outside the xylem, made up of phloem parenchyma which are irregularly shaped, unequal in size and compactly packed.
Pith	Massive, wavy in outline, sometimes seen as intruded into the xylem, made up of parenchymatous cells, peripheral region has small sized cells and large sized cells are present towards the inner side, tannin cells and calcium oxalate crystals are present.	Massive, made up of parenchyma cells, outer layers of cells are small and inner layers of cells are large, few tannin cells present.	Massive, wavy outline, intruded into the xylem, outer layers of cells are small and inner layers of cells are large.

Flowers 5-10 mm, white, in erect terminal racemes; bracts small; Male flower1-3 mm, above, unisexual, actinomorphic, bracteate and pedicellate; Pedicel 0.3-1 mm long, cauline, white, stellate hairs, 2-seriate; 5-partite, glandular at base; tepel 10, outer layer 1-3mm,5, fused, obovate, green, stellate hairs, inner layer spathulate, 0.4 -1mm, 5, fused, white, stellate hairs, Stamens 0.7-1mm, 10 -15, basifixed, white, polyandrous, anther globose, dehiscent-longitudinal, white; Female flowers 3-5mm, below, unisexual, actinomorphic, hypogynous, bracteate and pedicellate; pedicel 0.7 mm long, cauline, stellate hairs, green, bract 1-3mm, green, hairy, Perianthuniseriate, tepel 0.6-1 mm, 5 ovary stellate hairy, 3-celled, ovules one in each cell; styles long, slender, pistillode absent. Fruit capsule 2.5 x 2 cm, obovoid, depressed above, brown tomentose; seeds 13 x 8 mm, oblong, mottled with brown (Plate-2).

Fl. & Fr.: April-November
Habit: Tree
Habitat: Terrestrial.

Distribution: Palakkad, Idukki, Kollam, Pathanamthitta, Malappuram, Thiruvananthapuram, Thrissur, Wayanad, Kannur.

Systamatic Position of Croton Tiglium L.

Class: Dicotyledons
Sub Class: Monochlamydeae
Series: Unisexuales
Family: Euphorbiaceae
Genus: *Croton* L.

Species: ***Croton tiglium* L.**
Local Name: Neervalam

Synonyms: *C. acutus* Thunb.,*C. arboreus* Schecut, nom. Rej., *C. birmanicus* Mull. Arg., *C. camaza* Perr., *C. himalaicus* D.G.Long, *C. jamalgota* Buch.-Ham., *C. muricatus* Blanco, nom. Illeg., *C. officinalis* (Klotzsch) Alston, nom. Superfl., *C. tiglium* (L.) Raf.,*Oxydectesbirmanica*(Mull. Arg) Kuntze, *O. tiglium* (L.) Kuntze, *O. blancoana* Kuntze, *Tiglium officinale* Klotzsch

Small trees, annual, up to 15m height; Stem terete, glaberous, green; Leaves 10.6-13.8 × 3.6-4.3cm simple, alternate, ovate, stipulate, petiolate –3.5-4 cm, hairy with stellate hairs, trinerved at base; lateral nerves 1 - 6 pairs, membranous, yellowish green, acuminate, serrulate, round, pinnately veined glaberous, green; Inflorescence 5 - 18 cm long, pedicels 2 - 5 mm long, terminal raceme. Flowers pedicellate. Male flowers: 8-8.5mm, biseriate, outer layer 1.5 - 4 x 1, 2.5-3mm, 5 fused hairy with stellate hair green; inner layer 2 - 4 x 0.7 - 2 mm; Stamen 3-4 mm long , 14-20 basifixed ,yellow; Female flowers: pedicels 2 - 8 mm long; tepals oblong, triangular or lanceolate, 2 - 4.5 x 0.7 - 3 mm ovary obovoid , 2.5 - 4 x 2 - 3.5 mm, tomentose; styles 3.5 - 7 mm long, free, bifid. Capsules obovoid to oblong, 3 lobed, 1.6 - 2.5 x 1.3 - 2 cm, subglabrous; seeds 3-4mm, black (Plate-3).

Fl. & Fr.: January – November.
Habit: Small Tree
Habitat: Terrestrial.

Distribution: Indo-Malesia. Occasional; in evergreen forests, Wayanad, Nilambur.

Anatomical description of selected plant species

The comparative anatomical description of the leaves, petioles and stems of *C. aromaticus* L., *C. malabaricus* Bedd. And *C. tiglium* L. were discussed in Table 1, 2 and 3 (Plate-4 and 5)

Preliminary Phytochemical Analysis

Alkaloids were present among the leaves of three *Crotonsps*.and the stem of *C. aromaticus.* It was absent in the stems of *C. malabaricus* and *C. tiglium.* Leaf and stem of *C. aromaticus* and the stem of *C. tiglium* shows the presence of coumarins where as it was absent in the leaves of *C. malabaricus, C. tiglium* and stem of *C. malabaricus.* Flavonoids were absent in the leaves of *C. malabaricus* and *C. tiglium*. and was present in the leaf of *C. aromaticus* and stem of these three *species*. Leaf of *C. malabaricus,* stem and leaf of *C. tiglium* shows positive result towards the phenol test. Quinones were present only in the leaves of *C. aromaticus*and stem of *C. tiglium*. Saponins were present in the leaves of *C. malabaricus* and *C. tiglium* and absent in the leaf of *C. aromaticus* and stem of these three species. The leaves of *C. malabaricus* lack the presence of the common constituent tannins. Glycosides, steroids and terpenoids were present in the leaves and stems of all species (Table-4).

HPTLC analysis

Comparative HPTLC analysis of the stem extracts of C. aromaticus., C.malabaricus Bedd. and C. tigliumL.

UV 254nm: Under 254nm compounds at Rf 0.20 were present in *C. aromaticus, C. malabaricus* and *C. tiglium.* Bands at Rf 0.09 and 0.49 was present in *C. aromaticus* and *C. tiglium.* Bands at Rf 0.32 and 1.60 are present in *C. malabaricus C. tiglium.* Bands at 0.28, 0.64, 0.93, 1.31, 1.43, 1.56, 1.65, 1.75 were specific for *C. aromaticus.* Bands at 1.37, 1.72 were specific for *C. malabaricus.* Bands at 0.12, 0.35, 1.48, 1.92 were specific for *C. tiglium* (Plate-6, 7 and 8).

UV 366nm: Under 36nm compounds at Rf 1.45 was present in *C. aromaticus* and *C. tiglium.* Bands at Rf 0.16, 0.32, 1.70 were present in *C. malabaricus* and *C. tiglium.* Bands at Rf 0.09, 0.23, 0.29, 0.80, 1.38, 1.66, 1.75, 1.91 were specific for *C. aromaticus.* Bands at Rf 0.20, 0.99, 1.57, 1.80, 1.95 were specific for *C. malabaricus.* Bands at 0.48, 0.63, 0.95, 1.62 were specific for *C. tiglium* (Plate-6, 7 and 8).

Comparative HPTLC analysis of the leaf extracts of C. aromaticus L., C.malabaricus Bedd. and C. tiglium L.

UV 254nm: Under 254nm compounds at Rf 1.57 was present in *C. aromaticus* and *C. malabaricus.* Bands at Rf 0.10, 1.96 was present in *C. aromaticus* and *C. tiglium.* Bands at Rf 0.35 was specific for *C. malabaricus* and *C. tiglium.* Bands at Rf 0.28, 0.80, 1.44, 1.48, 1.75 were specific for *C. aromaticus.* Bands at Rf 0.58, 0.65, 0.89, 1.16, 1.96 were specific for *C. malabaricus.* Bands at Rf 0.01,0.14, 0.23, 0.50, 1.83 were specific for *C. tiglium* (Plate-6, 7 and 8).

UV 366nm: Under 366nm compounds at Rf 0.02, 0.10, 0.40, 0.98 was present in *C. aromaticus* and *C. malabaricus.* Bands at Rf 0.83, 0.95, 1.48 was present in *C. aromaticus* and *C. tiglium.* Bands at Rf 0.50, 0.58, 0.88 was present in *C. malabaricus* and *C. tiglium.* Bands at Rf 0.17, 0.23, 0.30, 0.55, 0.61, 0.73, 1.67, 1.75, 1.94 were specific for *C. aromaticus.* Bands at Rf 0.15, 0.35, 0.65, 0.75, 1.17, 1.26, 1.58, 1.77 were specific for *C. malabaricus*. Bands at Rf 0.27, 0.36, 0.43, 1.05, 1.14, 1.60, 1.70, 1.88 were specific for *C. tiglium* (Plate-6, 7 and 8).

DISCUSSION

Studies on effect of *Croton aromaticus* in controlling crown rot disease of Embul banana in combination with modified atmosphere and cold storage *(*5). Inhibitory effects of the ethanolic extract of C. aromaticus against test pathogens were investigated (36). The effects of essential oils from *Croton tiglium* L. on intestinal transit in mice was reported by Wang, (36). The effects of *Croton tiglium* oil (CO) on intestinal transit in mice was also investigated. *Croton tiglium* (Euphorbiaceae) is widely used as a herb for treatment of gastrointestinal disturbances. Activity-guided isolation of a novel protein from *Croton tiglium* with Antifungal and antibacterial activities were studied by Shahid *(*27).

Table 4 Comparative preliminary phytochemical tests for *Croton sps.*

SI No.	Phytochemical constituents	Name of test	Leaf			Stem		
			C. aromaticus	*C. malabaricus*	*C. tiglium*	*C. aromaticus*	*C. malabaricus*	*C. tiglium*
		Mayer's test	+	+	+	+	-	-
01	Alkaloids	Hager's test	+	+	+	+	-	-
		Wagner's test	+	+	+	+	-	-
02	Coumarins		+	-	-	+	-	+
03	Flavonoids	Alkaline reagent test	+	-	-	+	+	+
		Lead acetate test	+	-	-	+	+	+
04	Glycosides	Keller killani's test	+	+	+	+	+	+
05	Phenol	Ferric chloride test	-	+	+	-	-	+
		Lead acetate test	-	+	+	-	-	+
06	Quinones		+	-	-	-	-	+
07	Saponins	Foam test	-	+	+	-	-	-
08	Steroids	Salkowski's test	+	+	+	+	+	+
09	Tannins	Braymer's test	+	-	+	+	+	+
10	Terpenoids	Copper acetate test	'	+	+	+	+	+
			+	+	+	+	+	+

Morphological comparison revealed slight variations in size and shape, number of stamens. Anatomical comparison of different parts revealed that the variation in trichomes, midrib and xylem and pith. Preliminary phytochemical tests show clear difference between these three species. Phytochemical constitutes such as glycosides, steroids and terpenoids are

uniformly present in the leaves and stem of these three species. Whereas alkaloids, coumarins, phenol, quinones, saponins and tannins are present specifically. The comparative HPTLC studies showed greater similarities between these three species. However, differences were observed in terms of number and density of bands between *C. aromaticus*, *C. malabaricus* and *C. tiglium*. Maximum numbers of bands were observed in leaves than stem. Stem extracts of *C. aromaticus* shows highest number of bands, whereas *C. malabaricus* performed least number of bands. Some of the bands are sharing in *C. malabaricus* and *C. tiglium*, similarly *C. aromaticus* and *C. tiglium*. Leaf extract of *C. aromaticus* performed greatest number of bands, whereas *C. malabaricus* shows least number of bands. Most of the compounds present in *C. aromaticus*. *C. aromaticus* will become a topic of discussion among researchers and scientists to reveal the active principle behind it for pharmacological applications in the near future.

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