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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.*

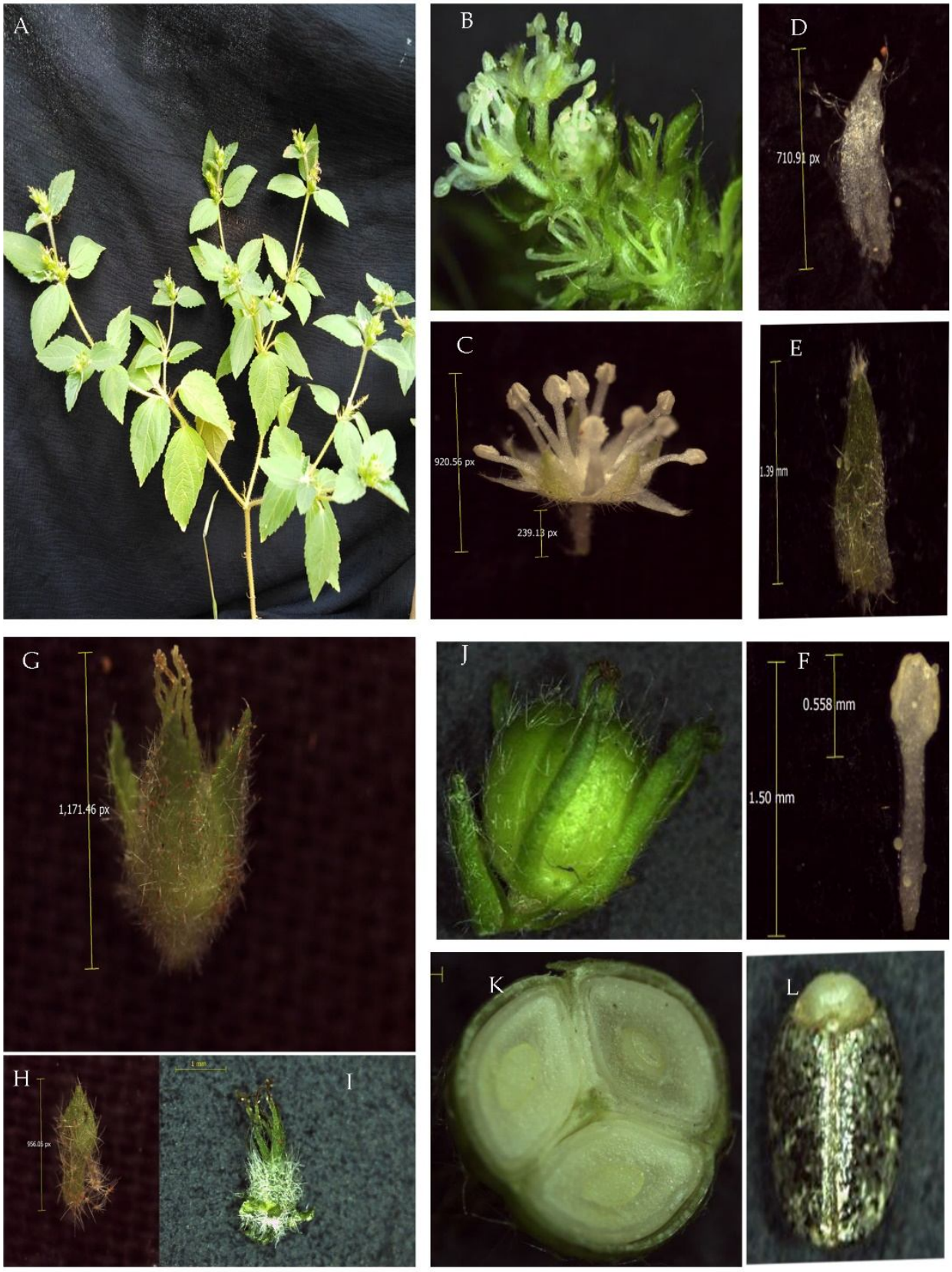

Plate.1: ***Croton hirtus*** - **A**: Habit, **B**:Inflorescence, **C**:Male flower, **D**: inner layer tepal,**E**:Outer layer tepal, **F**: Stamen,**G**:Female flower,**H**:tepal, **I**: Gynoecium, **J** : Mature fruit,**K** : C.S. of Fruit,**L**:Seed.

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

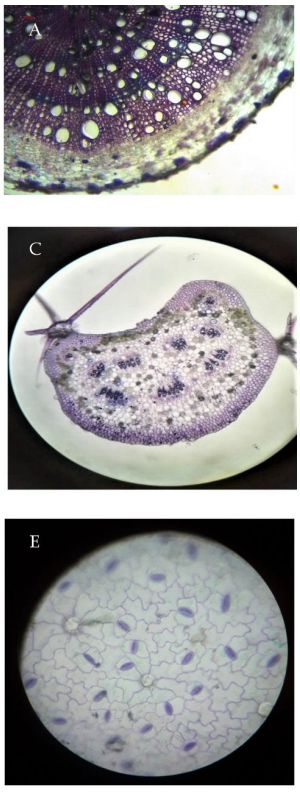

Plate.3: Anatomy of *Croton hirtus* - A:C.S.of Root, B: C.S.OF Stem, C:C.S.of Petiole,D: C.S. of Leaf,E: Paracytic type of Stomata,F:Trichome.

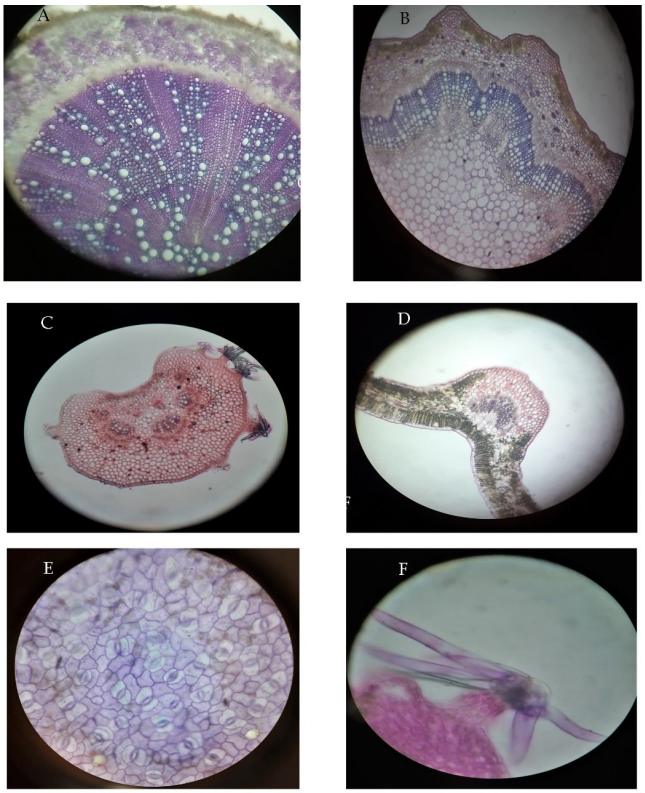

Plate.4:Anatomy of *Croton bonplandianus* - A:C.S.of Root, B: C.S.OF Stem, C:C.S.of Petiole,D: C.S. of Leaf,E: Paracytic type of Stomata,F:Trichome.

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

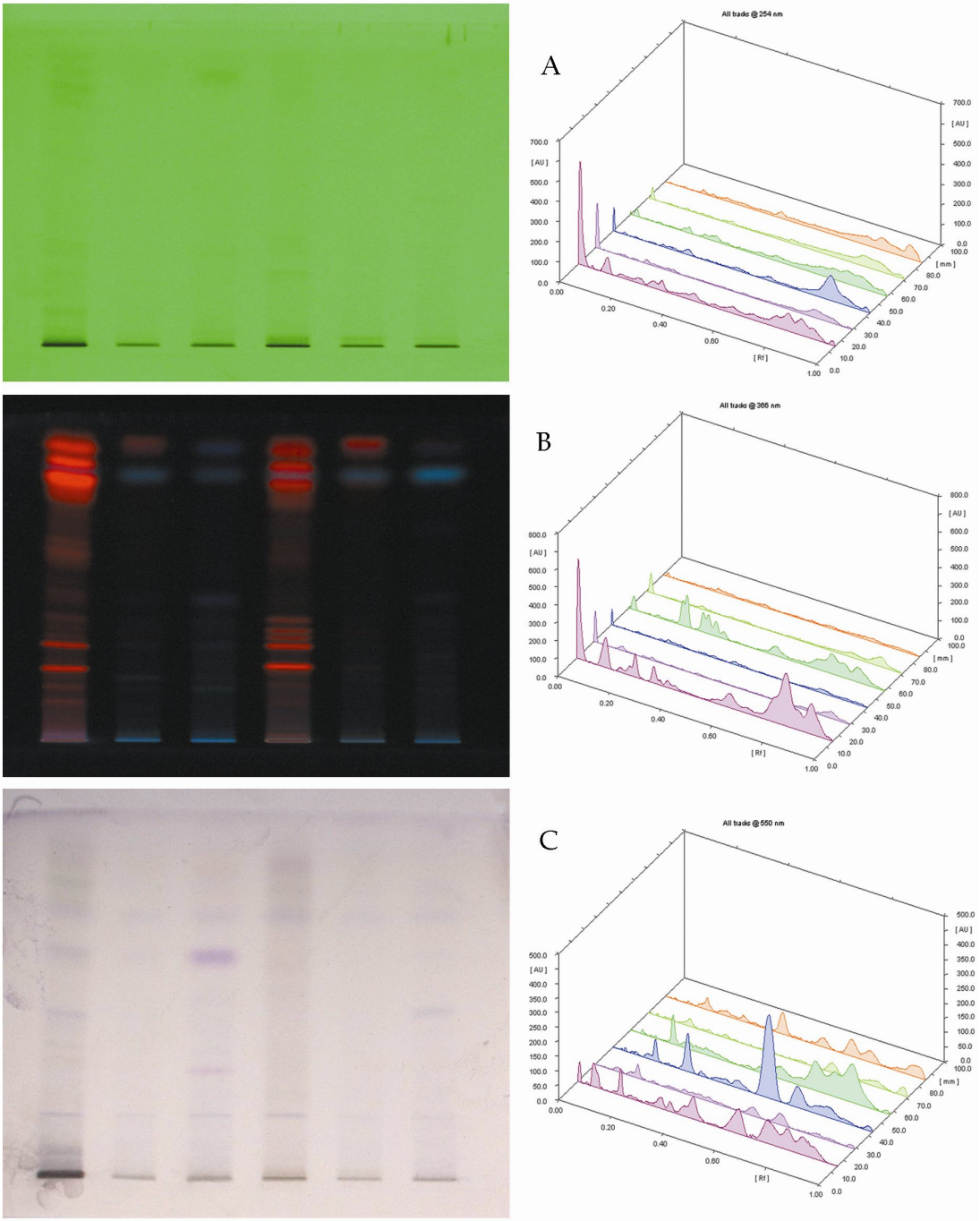

Plate 5: HPTLC profile of root, stem & leaf of ***C.hirtus*** & ***C.bonplandianus***. **A**: HPTLC chromatogram& densitometric scanning image under 254nm. **B:** HPTLC chromatogram & densitometric scanning image under 366nm, **C:** HPTLC chromatogram & densitometric image under 550nm. Track 1: Leaf 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.*

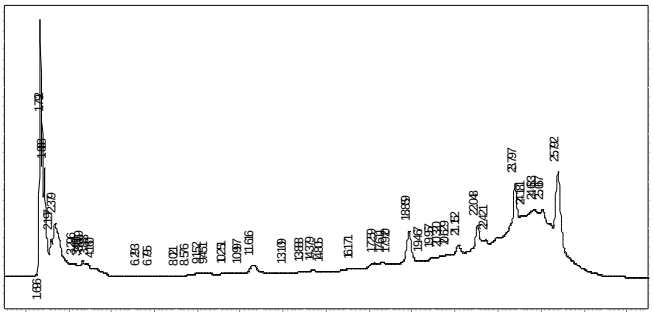

Fig. 1: HPLC chromatogram of the leaf extract of *C. hirtus*

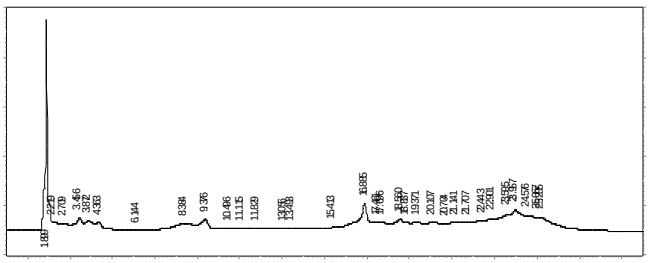

Fig. 2: HPLC chromatogram of the leaf extract of *C. bonplandianus*

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

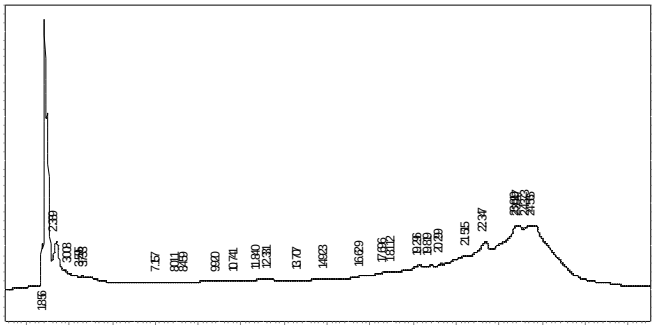

Fig. 3: HPLC chromatogram of the stem extract of *C. hirtus.*

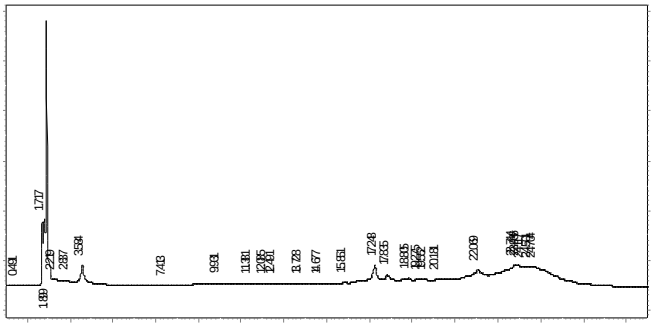

Fig. 4: HPLC chromatogram of the stem extract of *C. bonplandianus*

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.*

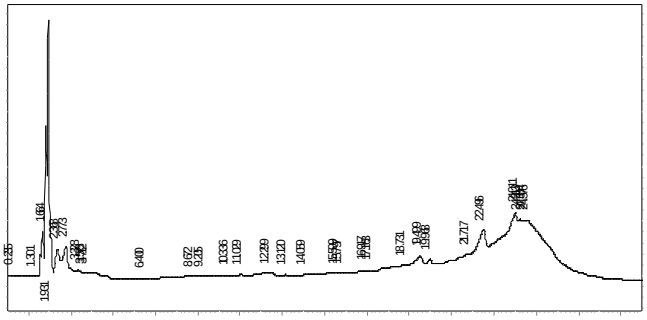

Fig. 5: HPLC chromatogram of the root extract of *C. hirtus.*

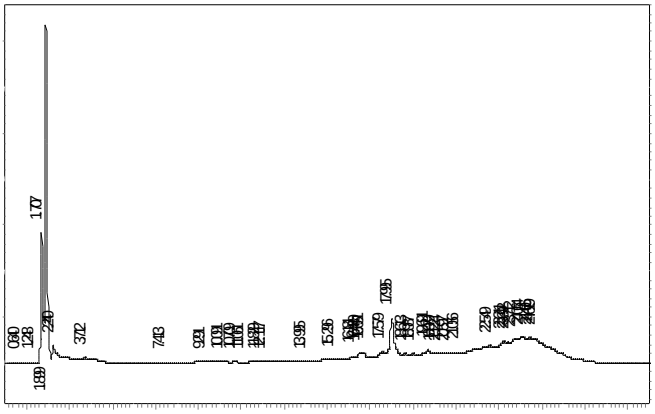

Fig. 6: HPLC chromatogram of the root extract of *C. bonplandianus*

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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