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# Biosystematic studies of selected *Croton* species (Euphorbiaceae) from southern peninsular India

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## Abstract

The comparative evaluation of the four *Croton* species demonstrated a strong correlation between their morphological, anatomical and phytochemical characteristics, highlighting their taxonomic significance and potential pharmacological value. The selected four *Croton* species were *Croton aromaticus* L., *Croton caudatus* Geiseler, *Croton persimilis* Müll.Arg. and *Croton zeylanicus* Müll.Arg. Morphological identification and detailed examination of different plant parts were carried out using a LABOMED CSM2 stereo microscope, while anatomical analyses were performed employing standard histological techniques with a LABOMED CXL PLUS compound microscope. Phytochemical profiling of the selected taxa was conducted through preliminary qualitative phytochemical screening and high-performance thin layer chromatography (HPTLC). Among the species studied, *C. caudatus* exhibited distinct morphological characteristics compared to the other three species. Phytochemical analysis revealed a higher concentration of secondary metabolites in the leaves than in the stem and root tissues. High-performance thin layer chromatography profiling indicated a comparatively higher abundance of phytocompounds in *C. aromaticus*, whereas lower quantities were detected in *C. caudatus* and *C. zeylanicus*. Likewise, species sharing similar anatomical characteristics often display comparable phytochemical profiles, suggesting that structural adaptations influence the synthesis, storage and distribution of bioactive compounds. The species selected with distinct anatomical features exhibited unique phytochemical patterns, reinforcing their taxonomic separation. Overall, integrating morphological, anatomical and phytochemical evidence provides a comprehensive approach for authenticating *Croton* species, resolving taxonomic ambiguities and identifying species with greater medicinal potential. This multidisciplinary evaluation establishes a great framework for quality control of herbal materials, conservation strategies and future studies incorporating molecular phylogenetics and pharmacological validation.

**Keywords:** anatomy; chemotaxonomy; *Croton*; Euphorbiaceae; high-performance thin layer chromatography; plant morphology

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## Introduction

Plants are the primary source of traditional medicinal systems, like Ayurveda, Unani and Siddha. Tropical flora and regional biodiversity play a pivotal role in the formulation of crude drug. Plant-derived compounds serve as essential constituents in the preparation of traditional medicines and are considered important sources of potential chemotherapeutic agents. Plant extracts contain various bioactive compounds that are used in the treatment of several diseases (1). Plants produce a wide range of secondary metabolites, which accumulate in different plant tissues and include various bioactive compounds (2). The present study on morphological and anatomical characteristics of selected *Crotons* species reveals certain unique features and variations among the species. The comparative phytochemical profiling of *Croton* also has a great value in the traditional medicinal practices for the preparation of various crude drugs. According to several researchers, the genus *Croton* exhibits a wide range of morphological variations, both within populations and among closely related species. Similarly anatomical analyses may reveal a range of structural characteristics that vary among different taxa. Medicinal plants usually have great diversity of chemical compounds that play a significant role in the exploration of various kinds of drugs to cure different ailments (3).

The genus *Croton* has attracted considerable attention due to its rich phytochemical composition and significant medicinal properties. The present study focuses on selected species of *Croton* through morphological and anatomical investigations, along with the development of standardised methods for phytochemical screening, contributing to the identification and characterisation of crude drugs. Among the studied species, *Croton aromaticus* L. is widely used in traditional

Ayurvedic medicine for the treatment of malaria and bronchitis. Similarly, *Croton persimilis* Müll.Arg has been traditionally employing in Thai and Indian medicine to treat chronic liver enlargement, stomachache and snake bites. In addition, *Croton zeylanicus* Müll.Arg. and *Croton caudatus* Geiseler have been reporting to possess antioxidant, anticancer and purgative properties.

The present investigation also aims to conduct a bibliometric study on the genus *Croton* to evaluate research trends, medicinal significance and phytochemical diversity. The bioactive compounds and therapeutic potential of the selected *Croton* species indicate their important role in applied biology, pharmacognosy and traditional medicine. Biosystematics studies of *Croton* species aim to establish clear taxonomic boundaries, analyse morphological and anatomical variations (such as trichomes and laticifers) and resolve evolutionary relationships using cytological and chemical data. Key objectives include formulating taxonomic keys, performing phylogenetic assessments and correlating character variations with ecological and geographical factors (4).

## Materials and Methods

### Field exploration

South India includes states of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh and Union territories of Puducherry and Mahe. The present study area Peninsular India can be subdivided into two floristic regions, Malabar and the Deccan. The Western Ghat region is floristically rich and includes coastal plains and series of hill ranges. The Western Ghat passed through the states of Southern and Northern regions of India. The taxa selected for the current analysis includes *C. aromaticus, C. caudatus*, *C. persimilis* and *C. zeylanicus* (Fig. 1).

### Morphological study

The taxonomical studies carried out by analysing micro morphological features of different plant parts. Morphological analysis was conducted using LABOMED CSM2 stereo microscope. Leica-EZ4 HD camera is used to take the photographs of each species floras and literature used to clarify the collected specimens (Fig. 2–5). Approximately 10–15 samples were collected during field visits for morphological studies and herbarium preparation. The collected specimens were carefully examined and identified with the help of standard floras and taxonomic keys to confirm the species. The herbarium specimens were prepared and preserved following standard botanical methods for further reference and documentation.

### Anatomical study

Anatomical studies executed by analysing the similarities and variations in the structure of the collected species. The collected specimen stored in 95 % formalin-aceto- alcohol (FAA) solution. The samples taken for the purpose of anatomical studies by hand sectioning. The thin sections measured approximately 200–500 µm in thickness were prepared and stained with 1 % safranin O solution for 3–5 min. Gently rinse the sections with distilled water until the water runs clear to remove excess surface dye. The sections were then sequentially dehydrated through a graded series of ethanol (30, 50, 70 and 90 %) for approximately 1 min at each concentration to remove water from the tissue cells and prepare them for counterstaining and permanent mounting. The dehydrated sections were transferred into a 0.2 % toluene solution for 10–15 sec. Immediately afterward, the sections were dipped into absolute ethanol (100 %) for a few seconds to remove excess toluene before it could mask the red safranin stain. The cleaned sections were placed on glass slides and a single drop of glycerine mounting medium was added. A coverslip was then gently lowered over each section to avoid trapping air bubbles. The stained sections were subsequently mounted for microscopic observation and anatomical analysis. The anatomical features were examined using a compound microscope (LABOMED-CXPLUS) (Fig. 6–8).

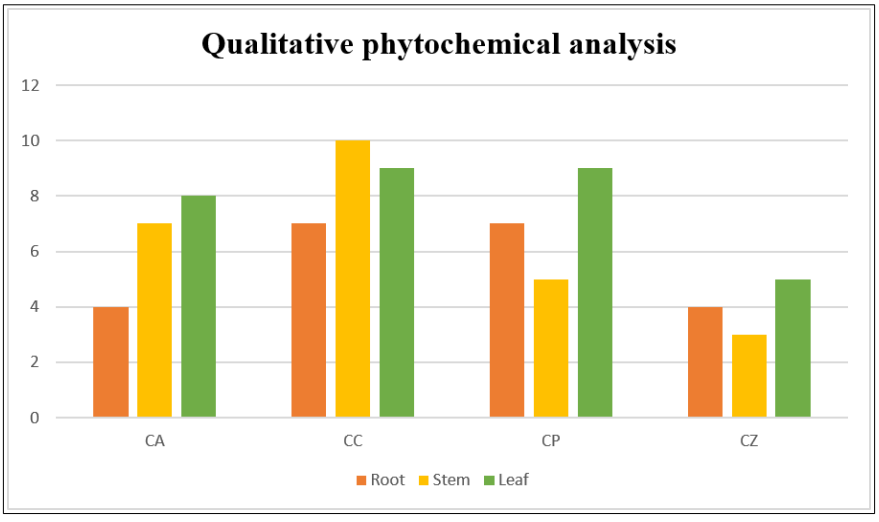



**Fig. 1.** Graphical representation of qualitative phytochemical analysis.
CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

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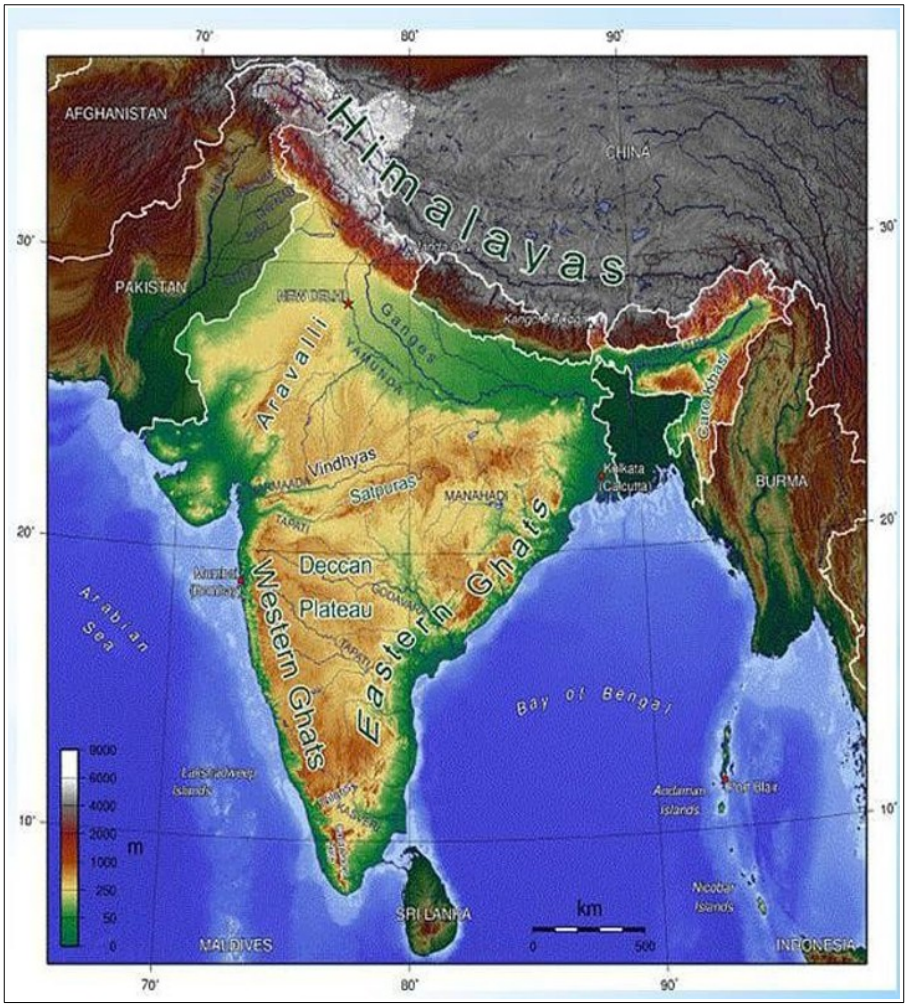



**Fig. 2.** Map of India showing study area.




**Fig. 3.** *Croton aromaticus.* A: habit; B: inflorescence; C: male flower; D: inner layer of tepal; E: outer layer of tepal; F: stamen; G: female flower; H: tepal; I: gynoecium; J: mature fruit; K: seeds.

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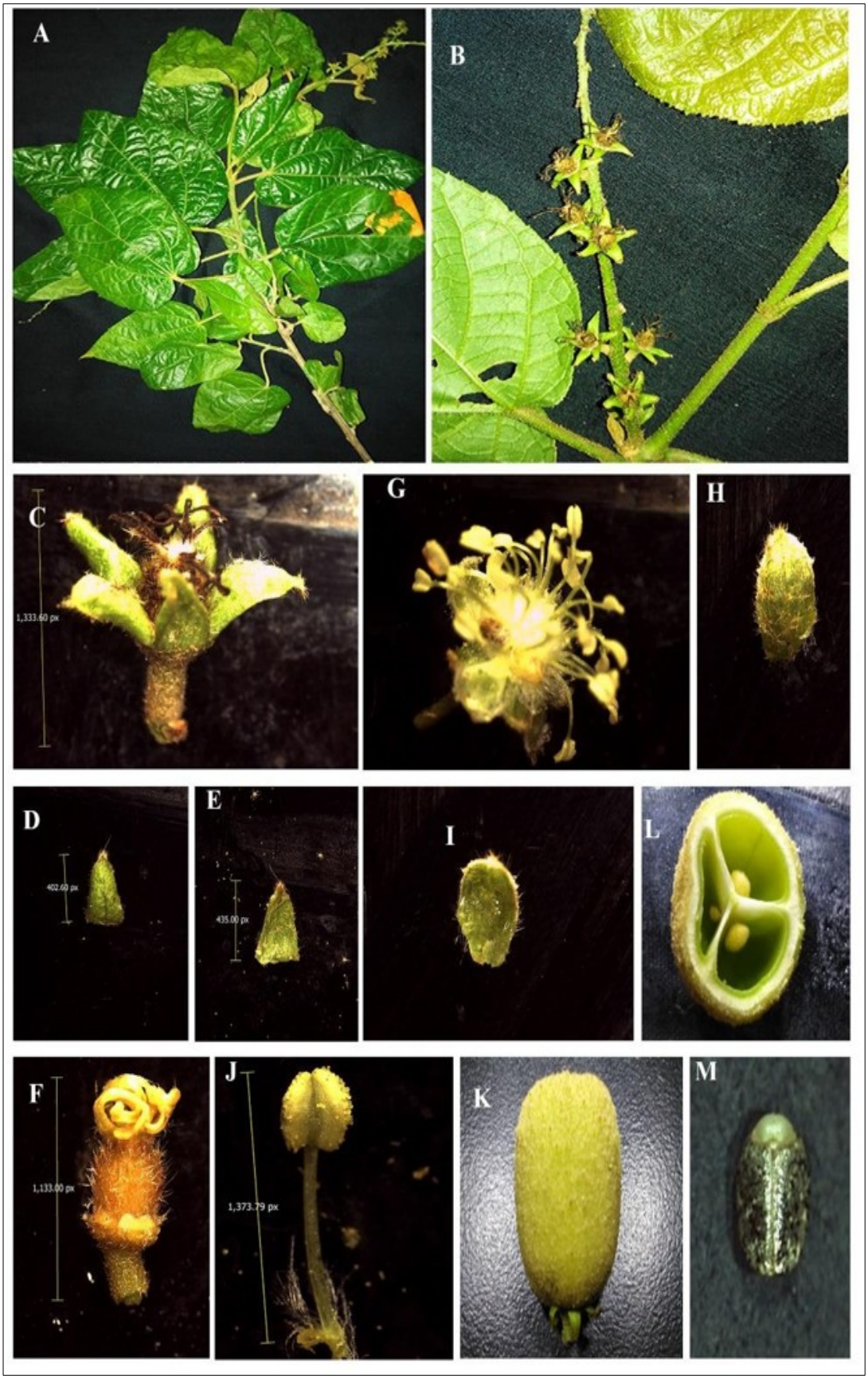



**Fig. 4.** *Croton caudatus.* A: habit; B: inflorescence; C: female flower; D: outer layer of tepal; E: inner layer of tepal; F: gynoecium; G: male flower; H: outer layer of tepal; I: inner layer of tepal; J: stamen; K: fruit; L: cross section of fruit; M: seed.

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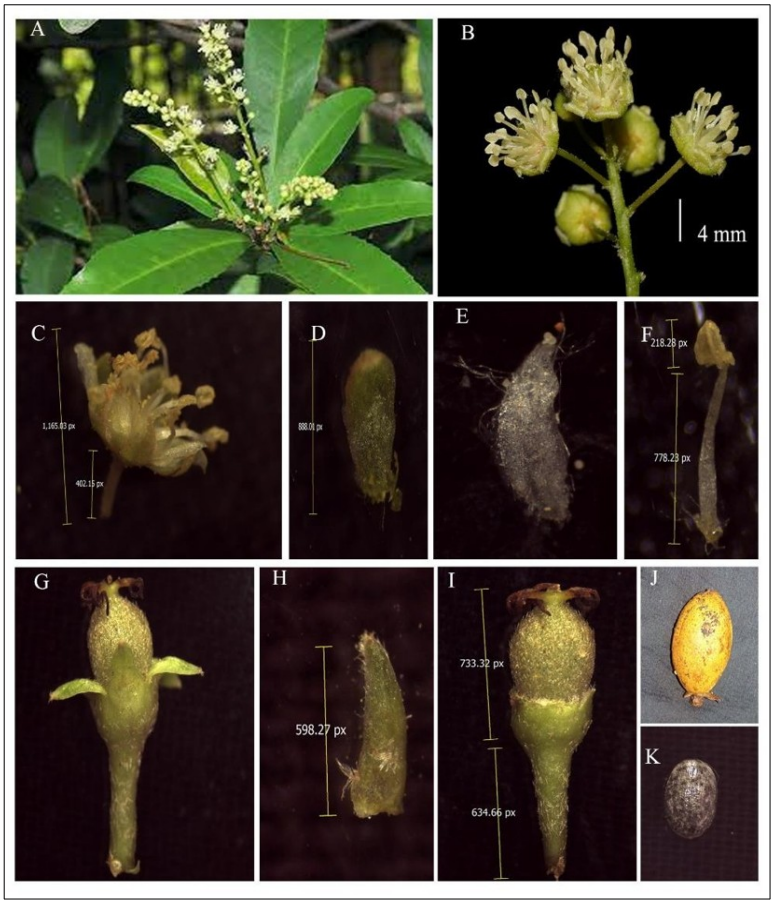



**Fig. 5.** *Croton persimilis.* A: habit; B: inflorescence; C: male flower; D: inner layer of tepal; E: outer layer of tepal; F: stamen; G: female flower; H: tepal; I: gynoecium; J: mature fruit; K: seeds.




**Fig. 6.** *Croton zeylanicus.* A: habit; B: inflorescence; C: male flower; D: inner layer of tepal; E: outer layer of tepal; F: stamen; G: female flower; H: tepal; I: gynoecium; J: mature fruit; K: seeds.

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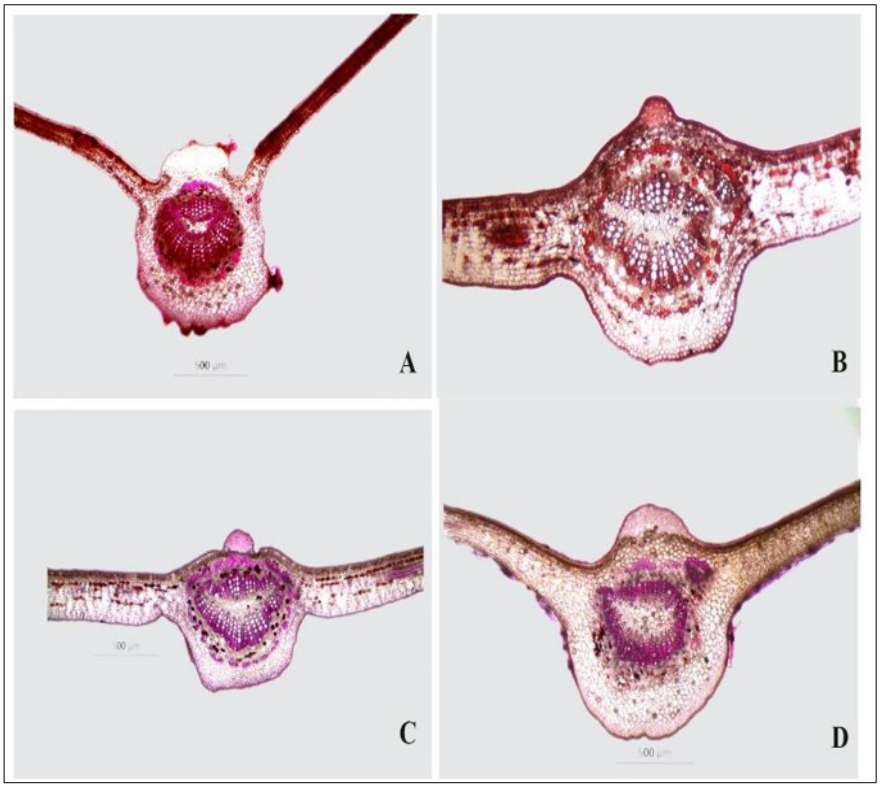



**Fig. 7.** Anatomical study of leaves of *Croton* species. A: transverse section of leaf of *Croton aromaticus*; B: transverse section of leaf of *Croton caudatus*; C: transverse section of leaf of *Croton persimilis*; D: transverse section of leaf of *Croton zeylanicus.*




**Fig. 8.** Anatomical study of stem of *Croton* species. A: transverse section of stem of *Croton aromaticus*; B: transverse section of stem of *Croton* caudatus; C: transverse section of stem of *Croton persimilis*; D: transverse section of stem of *Croton zeylanicus*.

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## Extraction methods

Collected samples species have being extracted for the phytochemical analysis. Samples underwent several processes and were ground into a powder 180–250 µm. Each powdered material (10 g) taken in the reflex condenser used to prepare the methanolic extractions of each sample (200 mL). The extracts obtained were filtered, concentrated to a final volume of 30 mL and placed in a water bath. The concentrated extracts were then subjected to qualitative phytochemical analysis.

## Phytochemical analysis

The prepared samples were subjected to various qualitative and quantitative analyses. Qualitative analysis was performed to identify the presence of different phytochemical constituents, while quantitative analysis was carried out using techniques such as high-performance thin-layer chromatography (HPTLC).

## Qualitative phytochemical analysis

### Alkaloids test

**1. Analysis of alkaloids using Mayer's reagent:** A 2 mL portion of the extract was taken in a test tube and two drops of Mayer's reagent were added. The formation of a white, creamy precipitate indicated the presence of alkaloids in the sample (5).

**2. Analysis of alkaloids using Hager's reagent:** Two drops of Hager's reagent were added to 2–4 mL of the sample solution. The formation of a yellow precipitate indicated the presence of alkaloids in the sample (5).

**3. Analysis of alkaloids using Wagner's reagent:** A 2–4 mL portion of the sample solution was taken in a test tube and 2–5 mL of Wagner's reagent was added. The formation of a dark brown precipitate confirmed the presence of alkaloids in the sample (5).

### Analysis of carbohydrates using Molisch's test

A 2 mL portion of the extract was mixed with an α-naphthol ($C_{10}H_8O$) solution and shaken well. Then, 2–3 drops of concentrated sulfuric acid ($H_2SO_4$) were slowly added along the sides of the test tube. The formation of a violet ring at the interface indicated the presence of carbohydrates in the sample (6).

### Analysis of reducing sugar

**Fehling's reagent:** A 2–3 mL portion of the sample solution was taken in a test tube and Fehling's reagent, prepared by combining Fehling's solutions A and B, was added. The test tube containing the mixture was then heated to boiling. After a few minutes, the formation of a brick-red precipitate of cuprous oxide ($Cu_2O$) indicated the presence of reducing sugars in the sample (7).

**Benedict's reagent:** A 0.5 mL portion of the aqueous sample solution was taken in a test tube and 5 mL of Benedict's reagent was added. The mixture was then heated in boiling water for 5 min and allowed to cool for a few minutes. The formation of a red precipitate of $Cu_2O$ confirmed the presence of reducing sugars in the sample (8).

### Flavonoids test

**1. Test for alkaline reagent:** A 2 mL portion of 2 % sodium hydroxide (NaOH) solution was added to the plant extract, resulting in the development of a yellow colour. The yellow colour disappeared upon the addition of two drops of dilute acid to the sample. This colour change indicated the presence of flavonoids (9).

**2. Test for lead acetate:** Two to three drops of lead acetate [$Pb(C_2H_3O_2)_2$] solution was added to the sample solution. The formation of a yellowish precipitate confirmed the presence of flavonoids in the sample (9).

**3. Foam test:** A small amount of the sample solution was taken in a test tube and shaken vigorously for 15 min. The formation of a persistent foam layer on the surface of the solution indicated the presence of saponins (10).

### Tannin analysis

**Braymer's test:** A 5 mL portion of the extract solution was taken in a test tube and 1 mL of 5 % ferric chloride ($FeCl_3$) solution was added. The formation of a greenish-black precipitate indicated the presence of tannins in the sample (10).

### Steroids tests

**Salkowski tests:** A 2 mL portion of the extract was mixed with 2 mL of chloroform and 2 mL of concentrated $H_2SO_4$ and shaken well. The chloroform layer developed a red colour, while the acid layer exhibited greenish-yellow fluorescence, indicating the presence of steroids in the sample (10).

### Protein analysis

**Millon's test:** A 5 mL of Millon's reagent is added to 3 mL of extract solution. The appearance of white precipitate which slowly turns to pink or red when heated gently indicates the presence of proteins (11).

### Glycosides tests

**Keller Killiani's test:** To the test solution, 2 mL of glacial acetic acid containing 2–3 drops of $FeCl_3$ solution was added. Then, 1 mL of concentrated $H_2SO_4$ was carefully added along the side of the test tube. The formation of a brown ring at the interface indicated the presence of deoxy sugars associated with cardenolides. A violet ring appeared beneath the brown ring, while a greenish ring gradually developed throughout the acetic acid layer (12).

### Phenolic tests

**1. Test for ferric chloride:** Ferric chloride solution was added to the sample solution. The formation of a dark-coloured precipitate indicated the presence of phenolic compounds in the sample (13).

**2. Test for lead acetate:** A 2–3 mL portion of $Pb(C_2H_3O_2)_2$ solution was added to the sample. The formation of a yellow precipitate indicated the presence of phenolic compounds in the plant extract (13).

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### Amino acid analysis

**Ninhydrin test:** A 2 mL portion of the sample solution was mixed with 2–5 mL of ninhydrin solution. The mixture was then placed in a water bath and boiled for 2 min. The formation of a purple colour in the test tube indicated the presence of amino acids in the sample (14).

### Terpenoids test

**Test for diterpenes using copper acetate:** The sample was dissolved in water and 2–3 drops of copper acetate [$Cu(CH_3COO)_2$] solution were added. The development of a bright green colour indicated the possible presence of diterpenes in the sample (14).

### Quantitative phytochemical analysis

### High-performance thin layer chromatography

High-performance thin-layer chromatography, an advanced form of thin-layer chromatography (TLC), was used for the separation and analysis of phytochemical constituents. The stationary phase consisted of an aluminium plate coated with silica gel 60 $F_{254}$, while the mobile phase comprised a mixture of toluene, ethyl acetate and methanol in a ratio of 7 : 3 : 1 (v / v / v) respectively. A 5 µL aliquot of the sample was applied to the plate and chromatographic development was carried out in a saturated twin-trough chamber. The distance between the tracks was maintained at 15.00 mm. The developed TLC plates were analysed using a CAMAG TLC scanner through densitometric scanning at wavelengths of 254 nm and 366 nm.

## Results

### Morphological features of selected taxa

The morphological characteristics of each part of the selected taxa were analysed. The results revealed that some characteristics were closely related among the taxa, whereas others exhibited variations (Table 1).

### Anatomical characterisation of selected plant species

The anatomical studies revealed that some characteristics were common to all the selected *Croton* species, whereas others exhibited variations, particularly in the distribution and types of trichomes and the deposition of secondary metabolites, such as tannins and calcium oxalate crystals. The anatomical characteristics of the different plant parts of the selected taxa were described in Tables 2–5 and illustrated in Fig. 6–8.

### Qualitative phytochemical profiling of selected species of *Croton*

The phytochemical profiling of the selected *Croton* taxa revealed variations in the distribution of different phytoconstituents among the root, stem and leaf extracts. Alkaloids were detected in most of the selected taxa but were absent in the stem of *C. persimilis* and the leaves of *C. zeylanicus*. Similarly, coumarins were present in the root extracts of *C. aromaticus* and *C. persimilis*, whereas they were absent in the leaf extracts of *C. zeylanicus*.

**Table 1.** Morphological comparison of *Croton*

| Morphological features | CA | CC | CP | CZ |
|---|---|---|---|---|
| Habit | Tree with stellate branchlets | Scandent woody climber | Small trees | Small tree with terete branchlets adorned with stellate-scaly texture |
| Leaves | Simple, ovate, acuminate and cordate at the base, 3 prominent ribs from the base, two pairs of glands are present, a petiole 2–3 cm | Ovate, cordate, elliptic, oblong to orbicular in shape, the petioles measure 0.5–7 cm in length and the stipules are lacerate with glandular tips, ranging from 4–15 mm long | Simple and alternate, elliptic, obovate, oblanceolate, swelling at both the tip and base | An ovate-lanceolate, acuminate tips accompanied by bracteates, one pair of sessile glands present supported by petioles measuring 1–3 cm |
| Inflorescence | Racemose, densely stellate hairy and white | Racemose 8–35 cm long | Racemose | Racemose 6 cm long |
| Flower | **Male flowers:** 3–5 mm, actinomorphic and bracteate, biseriate, with 10 tepals, stamens 10, 0.8–1 mm, basifixed, white, polyandrous, possess globose anthers, milky | **Male flowers:** Pedicels are 3–9, sepals 5–6 and 1–3.5 × 1–3.5 mm, oblong, elliptic, or triangular; petals 5, elliptic to spathulate, 2–4 × 1–3 mm; stamens 18–40, 3.5–6 mm, with oblong or obovoid anthers 0.8–1.3 mm | **Male flowers:** Ovate obtuse sepals, elliptic-lanceolate woolly petals and 12 inflexed stamens with hairy lower halves | **Male flowers:** 5 tepals with glabrous filaments, 3–5 mm, biseriate arrangement, the stamen 1–2 mm long, 12–14 bifid anthers, pubescent and cream |

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|---|---|---|---|---|
| | **Female flowers:** 7 mm across, ovate and acute sepals, ciliate obovate petals and 3 bifid, glabrous style | **Female flowers:** Pedicels 1–5 × 1–1.8 mm; sepals 5, oblong, elliptic to ovate, 2–6 × 1–4 mm, often boarded with sub sessile glands; petals 0–5, filiform or subulate, 0.5–2.5 mm long and a globose or oblong ovary | **Female flowers:** Ovate acute ciliate sepals, obovate woolly-petaled flowers, a 3 celled ovary with one ovule in all the cells, 3 styles | **Female flowers:** 5 sepals, 4 mm ovate, obtuse; petals 5 obovate, tomentose, green; ovary green stellate hairy; styles 3, glabrous |
| Fruit | Capsule measures 4–8 mm in length, ovoid, trilocular, green and hirsute | Capsules are globose, oblong or obovoid, 1.5–3 × 1.5–2.5 cm, bluntly 3 or 6 angled, often muricate, pubescent | Lepidote scaled, sub globose, depressed capsule, 3 lobed | Capsule 1.3 × 1 cm, ovoid |
| Seed | Seeds 3, 3–4 mm in size, exhibit black and cream patches, also glabrous | Seeds 3, 3–4 mm in size, exhibit black and cream patches, also glabrous | Seeds 3 with black patches | 8 × 7 mm, oblong, mottled with brown |

CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

**Table 2.** Anatomical comparison of selected species of *Croton* (leaf)

| Anatomical features | CA | CC | CP | CZ |
|---|---|---|---|---|
| Epidermis | Glandular, unicellular trichomes present on both surfaces | Without any trichomes on both lower and upper epidermis | Trichomes absent, paracytic stomata with two subsidiary cells | Glandular, unicellular trichomes present on lower surface |
| Vascular bundle | Collateral | Conjoint, collateral, endarch, closed, wavy in outline | Conjoint, collateral and closed, wavy outline | Conjoint, collateral, endarch, closed |
| Mesophyll | Circular outline and arranged with intercellular space, few of chloroplast, tannin cells present | Chloroplast present abundantly, tannin cells and specular cells are present | Chloroplasts present | Abundant chloroplast, tannin cells present |
| Observations | Presence of tannin cells | Presence of chloroplast, tannin cells and spicular cells | Trichomes absent, chloroplast observed few in number | Tannin cells present |

CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

**Table 3.** Anatomical comparison of selected species of *Croton* (petiole)

| Anatomical features | CA | CC | CP | CZ |
|---|---|---|---|---|
| Epidermis | Unicellular | Trichomes orientating from epidermal cells are branched at base, glandular | Numerous trichomes, stalked, glandular, long, unicellular | Trichomes present |
| Cortex | Crystals are present | Made up of parenchymatous, thin-walled cells arranged in 9–11 layers, with intercellular spaces and calcium crystals; small patches of spicular cells, tannin cells and laticifers are also present | A massive parenchymatous zone is present, containing numerous calcium crystals | Tannin cells are present on both side |
| Vascular bundle | Bundles are 7 in number | Numerous | 7–8 bundles, conjoint, collateral and closed, with a wavy outline | Continuous ring, conjoint, collateral, endarch and closed |
| Observations | Numerous trichomes | Tannin cells, lactiferous | Numerous calcium crystals present | Tannin cells present |

CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus*.

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**Table 4.** Anatomical comparison of selected species of *Croton* (stem)

| Anatomical features | CA | CC | CP | CZ |
|---|---|---|---|---|
| Epidermis | Numerous trichomes, glandular, unicellular and branched at base | Trichomes absent | Trichomes absent | Trichomes absent |
| Cortex | Reduces, 4–5 layered, tannin cells present, specular cells | Spicular cells seen in discontinuous patches, tannin cells present abundantly | Tannin cells scattered, sclerenchymatous patches present | 8–9 layered paranchymatous cells, tannin cells absent |
| Xylem | Bordered pitted thickening, tannin cells present | Tannin cells absent | Tannin cells absent | Tannin cells absent |
| Pith | Present | Tannin cells absent | Tannin cells absent | Tannin cells absent |

CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

**Table 5.** Anatomical comparison of selected species of *Croton* (root)

| Anatomical features | CA | CC | CP | CZ |
|---|---|---|---|---|
| Periderm | Ruptured and form wide fissures | Ruptured and discontinuous layer of cells | Ruptured and form wide fissures | Ruptured and form wide fissures |
| Cortex | Parenchymatous, few sclerenchymatous patches present | 8–10 layers of chlorenchymatous cells are followed by irregularly shaped collenchymatous cells containing starch grains, small and large patches of sclereids are seen above the phloem | Parenchymatous, sclerenchymatous patches present, tannin cells are scattered | Parenchymatous, tannin cells are found |
| Vascular bundle | Present | Numerous vascular bundles present, arranged in a circular fashion, open | Present | Present |
| Phloem | Phloem parenchyma cells, irregularly arranged | Parenchyma with deposition of starch grains | Phloem parenchyma cells, irregularly arranged | Phloem parenchyma cells, irregularly arranged . |
| Xylem | Proto xylem 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 vascular bundle | Reduced, made up of irregularly shaped parenchyma cells which are loosely packed | Highly reduced, attached to vascular bundle | Reduced, made up of irregularly shaped parenchyma cells, which are loosely packed |

CA: *Croton aromaticus*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

Flavonoids were among the major phytoconstituents identified in the selected taxa. They were present in the root extracts of *C. caudatus*, *C. persimilis* and *C. zeylanicus*. In the stem extracts, flavonoids were detected in all selected *Croton* species except *C. zeylanicus*. Likewise, flavonoids were present in the leaf extracts of *C. aromaticus, C. caudatus* and *C. persimilis*. Glycosides were also detected in most of the selected taxa but were absent in *C. zeylanicus*.

Phenolic compounds were detected in most of the samples but were absent in the root extracts of *C. aromaticus* and *C. caudatus*, as well as in the stem extracts of *C. aromaticus*, *C. caudatus* and *C. zeylanicus*. Quinones exhibited variation in their distribution among the selected species and plant parts. They were detected in the root extracts of *C. aromaticus* but were absent in those of the other selected species. In the stem extracts, quinones were detected in *C. caudatus* and were reported in the other species as well. In the leaf extracts, quinones were present in *C. aromaticus* and *C. persimilis* but were absent in *C. caudatus* and *C. zeylanicus*.

Saponins were absent in most of the examined samples but were detected in *C. caudatus* and the leaf extracts of *C. zeylanicus*. Steroids were present in most of the selected taxa but were absent in the root extracts of *C. caudatus*, the stem extracts of *C. persimilis* and the leaf extracts of *C. zeylanicus*. Tannins were detected in several samples, including the root extracts of *C. caudatus* and the stem extracts of *C. aromaticus*, *C. caudatus* and *C. persimilis*. However, tannins were absent in the leaf extracts of *C. zeylanicus*. Terpenoids were detected in most of the examined samples but were absent in the root extracts of *C. aromaticus* and *C. zeylanicus*, as well as in the stem extracts of *C. zeylanicus* (Table 6).

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**Table 6.** Qualitative phytochemical analysis

| Sl. No. | Phytochemical constituents | Name of test | Root | | | | Stem | | | | Leaf | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CA | CC | CP | CZ | CA | CC | CP | CZ | CA | CC | 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 | Kellerkillani's test | + | + | + | - | + | + | + | - | + | + | + | - |
| 5 | Phenol | Ferricchloride 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*; CC: *Croton caudatus*; CP: *Croton persimilis*; CZ: *Croton zeylanicus.*

Overall, the preliminary phytochemical screening of the selected *Croton* species demonstrated that the distribution of phytoconstituents varied among the different plant parts, including the roots, stems and leaves. These findings highlighted the interspecific and organ-specific variations in the phytochemical composition of the selected taxa.

## Quantitative phytochemical studies

The quantitative analysis of these phytochemicals revealed characteristic $R_f$ values, with certain constituents being unique to specific extracts, while others were common to all extracts. The preliminary phytochemical analysis discussed under qualitative phytochemical profiling.

## Comparative high-performance thin layer chromatography analysis of *Croton* species at 254 nm

At 254 nm, HPTLC analysis revealed distinct bands at characteristic $R_f$ values in different plant parts of the four *Croton* species. In *C. aromaticus*, bands at $R_f$ 1.48 and 1.66 were observed in the leaves, while bands at $R_f$ 0.64, 1.31 and 1.65 were characteristic of the stems. The roots exhibited bands at $R_f$ 0.06 and 0.15. In *C. caudatus*, a band at $R_f$ 1.24 was unique to the leaves, whereas bands at $R_f$ 0.07 and 0.24 were detected in the roots. The leaves of *C. persimilis* showed characteristic bands at $R_f$ 0.44, 0.53, 0.66 and 0.75, while a band at $R_f$ 0.87 was unique to the stems. The roots of this species exhibited bands at $R_f$ 0.41, 0.48 and 0.95. In *C. zeylanicus*, bands at $R_f$ 0.51, 0.74 and 0.81 were observed in the leaves, whereas bands at $R_f$ 0.71 and 0.96 were characteristic of the stems. Additionally, bands at $R_f$ 0.69 and 0.77 were reported for *C. zeylanicus*; however, the corresponding plant part requires clarification (Fig. 9).

## Comparative high-performance thin layer chromatography analysis of *Croton* species at 366 nm

At 366 nm, bands at $R_f$ 0.39, 0.51 and 0.54 were observed in the leaves of *C. caudatus*, while bands at $R_f$ 0.13 and 0.45 were detected in its roots. A band at $R_f$ 0.87 was unique to the leaves of *C. persimilis*. In *C. zeylanicus*, the leaves exhibited characteristic bands at $R_f$ 0.32, 0.68, 0.74 and 0.81 (Fig. 10).

## Comparative high-performance thin layer chromatography analysis of *Croton* species at 550 nm

At 550 nm, HPTLC analysis revealed distinct chromatographic bands at characteristic $R_f$ values across the different plant parts of the four *Croton* species. In *C. aromaticus*, bands at $R_f$ 0.09, 0.48 and 0.63 were observed in the leaves, while a band at $R_f$ 1.06 was unique to the stems. The roots exhibited bands at $R_f$ 0.33, 0.81 and 0.89. In *C. caudatus*, bands at $R_f$ 0.37 and 0.77 were detected in the leaves, whereas the stems showed bands at $R_f$ 0.16, 0.28, 0.46 and 0.87. The roots of *C. caudatus* exhibited bands at $R_f$ 0.04, 0.41 and 0.67. In *C. persimilis*, a band at $R_f$ 0.61 was unique to the leaves, while the stems showed bands at $R_f$ 0.24 and 0.53. A band at $R_f$ 0.31 was unique to the roots of this species. In *C. zeylanicus*, a band at $R_f$ 0.74 was unique to the leaves (Fig. 10).

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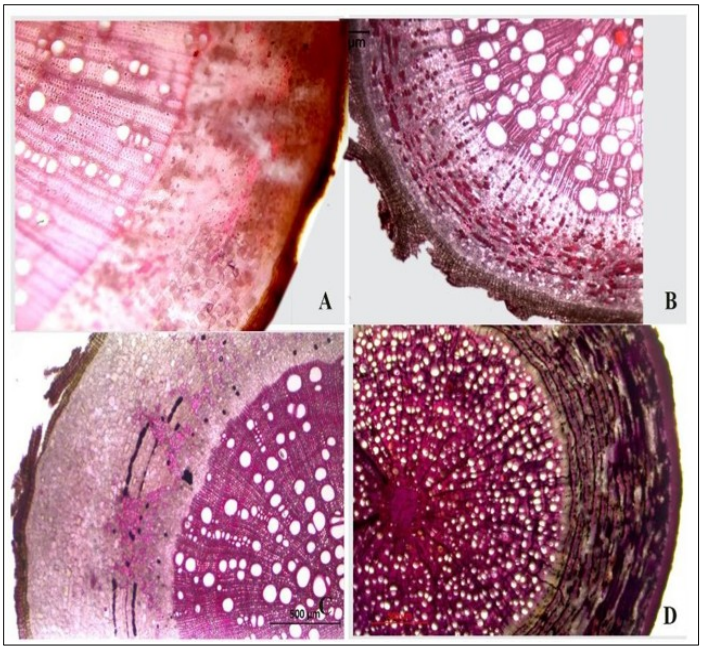



**Fig. 9.** Anatomical study of root of *Croton* species. A: transverse section of root of *Croton aromaticus*; B: transverse section of root of *Croton caudatus*; C: transverse section of root of *Croton persimilis*; D: transverse section of root of *Croton zeylanicus.*

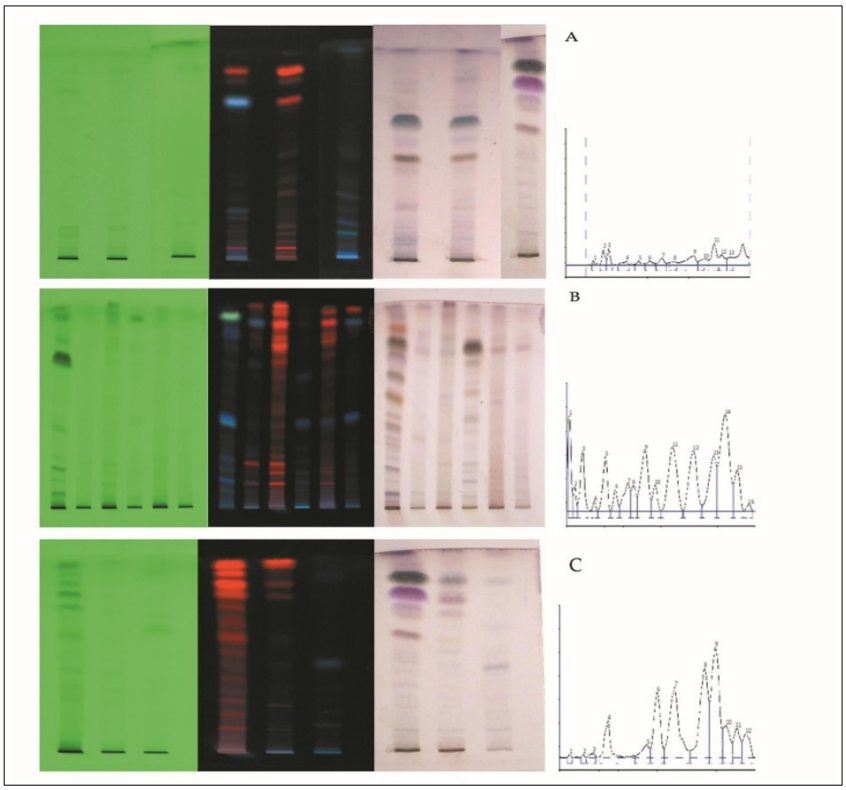



**Fig. 10.** High-performance thin layer chromatography profile of *Croton aromaticus, Croton caudatus, Croton persimilis* and *Croton zeylanicus.*

A: high-performance thin layer chromatography of *Croton aromaticus* under 254 nm, 366 nm and 550 nm (track 1: leaf extract of *Croton aromaticus*, track 2: stem extract of *Croton aromaticus*, track 3: root extract of *Croton aromaticus*); B: high-performance thin layer chromatography of *Croton caudatus* and *Croton persimilis* under 254 nm, 366 nm, 550 nm (track 1: leaf extract of *Croton caudatus*, track 2: stem extract of *Croton caudatus*, track 3: root extract of *Croton caudatus*, track 4: root extract of *Croton persimilis*, track 5; stem extracts of *Croton persimilis*, track 6; leaf extracts of *Croton persimilis*); C: high-performance thin layer chromatography of *Croton zeylanicus* under 254 nm, 366 nm, 550 nm (track 1: leaf extract of *Croton zeylanicus*, track 2: stem extract of *Croton zeylanicus*, track 3: root extract of *Croton zeylanicus*).

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## Discussion

The genus *Croton*, one of the largest genera in the family Euphorbiaceae, is known for its diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antihypertensive, antidiabetic, antinociceptive and wound-healing properties. These activities are attributed, in part, to the wide diversity of phytochemicals present in the genus. Several classes of secondary metabolites have been reported in *Croton* species, including diterpenoids such as crotofolanes, clerodanes, kauranes and labdanes, as well as other terpenoids, alkaloids and flavonoids (15, 16). The phytochemical profiles of *Croton* species have also revealed the presence of alcohols, hydrocarbons and various phenolic compounds, including flavonoids, lignin, coumarins and tannins, along with alkaloids, cyanogenic glycosides and glucosinolates. Flavones, flavonols, steroids, saponins and alkaloids have also been reported in this genus (17). Consistent with these findings, the present study revealed the presence of several phytochemical groups, including phenolic compounds, alkaloids, flavonoids, coumarins and glycosides. These findings further support the phytochemical diversity of the genus *Croton* and highlight its potential significance as a source of biologically active compounds with possible therapeutic applications (17, 18).

Microscopic examination also revealed important diagnostic features of the studied *Croton* species, including unicellular and glandular trichomes, tannin-containing cells and laticiferous cells. These anatomical characteristics may serve as useful diagnostic markers for the identification and authentication of species within the genus. Similarly, *Croton draco* Schltdl. & Cham., an arborescent member of the family Euphorbiaceae, has been reported to contain latex and condensed tannins (19), further highlighting the anatomical and phytochemical characteristics of this genus.

The chemical diversity of the genus *Croton* is comparable to the large number of species it contains, providing a valuable opportunity for the discovery of novel anticancer agents. Therefore, the pharmaceutical potential of the *Croton* genus is highly significant. Natural products derived from these plants represent an important source for the development and synthesis of new drugs because their pharmacological activities are often comparable to those of conventional medicines (20).

The present study contributes to the exploration of the medicinal potential of *Croton* species through detailed taxonomic identification and anatomical investigations, which are essential for the accurate identification, authentication and prevention of adulteration of crude drugs. Furthermore, the findings provide a foundation for future phytochemical and pharmacological investigations of the genus *Croton* (21).

The present study also has significant taxonomic implications for the identification and delimitation of the selected *Croton* species. Variations in morphological characteristics, including leaf architecture, indumentum, venation patterns, inflorescence structure and reproductive features, provide valuable diagnostic characters for distinguishing the investigated taxa. Anatomical features, such as epidermal characteristics, stomatal distribution, trichome diversity, vascular tissue organisation and crystal occurrence, further support species-level identification, particularly when morphological characters overlap among closely related taxa. In addition, the observed phytochemical profiles demonstrate variation among the investigated species and may provide supplementary taxonomic evidence when interpreted alongside morphological and anatomical data. The integration of these complementary character sets therefore offers a more comprehensive approach to species discrimination and taxonomic assessment within *Croton*. Collectively, these findings may contribute to improved species identification, support the assessment of relationships among closely related taxa and provide a baseline for future investigations incorporating molecular and phylogenetic approaches.

## Conclusion

The present study represents a preliminary step toward further taxonomic investigations by establishing a baseline for the identification and authentication of the selected *Croton* taxa. The findings may facilitate future studies by integrating molecular data and phylogenetic analyses to clarify relationships among closely related species. Further pharmacological investigations, including appropriately designed clinical trials, are necessary to establish the therapeutic efficacy and safety of bioactive compounds derived from these species. Moreover, the morphological, anatomical and phytochemical characteristics documented in this study may contribute to the authentication of genuine source plants used in herbal formulations, thereby helping to prevent adulteration and ensuring the quality and identity of plant-based raw materials.

## Authors' contributions

BR carried out the studies. RH carried out the review and rewriting. BT carried out supervision. RKN guided phytochemical analysis. All authors read and approved the final manuscript.

## Compliance with ethical standards

### Conflict of interest

Authors do not have any conflict of interests to declare.

### Ethical issues

None

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