

Research Article

Phytochemical and mineral contents of *Croton sparsiflorus* in Bahawalpur

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Abstract

Although a huge quantity of plants has been studied for their medicinal importance, there is still a large number of plant species that have never been subjected to systematic phytochemical studies, proximate or mineral contents analysis. In this study, a phytochemical analysis, proximate, and mineral contents investigations have been carried out on indigenous medicinal plant of Pakistan namely *Croton sparsiflorus* Morong (syn. *Croton bonplandianum* Baill.) The proximate and phytochemical compositions of *Croton sparsiflorus* were investigated following standard procedures. The mineral composition was determined by atomic absorption spectrophotometer and a flame photometer. The result indicated the healthy mineral level of *Croton sparsiflorus*. It showed a considerable amount of Zinc (49.2 ppm), Iron (333.6 ppm), Manganese (7.6 ppm), and Copper (23.14 ppm). Phytochemical studies of *Croton sparsiflorus* confirmed the presence of alkaloids, flavonoids, saponins, phenols, proline, and carbohydrates in varying concentrations. The proximate analysis showed that the dry contents of *Croton sparsiflorus* were 98.95%. The moisture content in the dry sample was found to be 0.73%. The crude protein (crude protein included both true protein and non-protein nitrogen) content was 10.63%. This study is another confirmation of the earlier stated facts that this plant is a good source for bio-prospecting. The study further revealed that the plant, *Croton sparsiflorus* used has substantiated to be very essential in medical research and development, because of the phytochemicals that are contemporaneous and can be utilized in the medical field. Twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus* seem to have good nutritious, and suitable mineral elements worth crucial to preserve good well-being.

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1. Introduction

There are 300 genera and 5000 species, particularly shrubs, trees, and non-sugar plants, in the Euphorbiaceae family. It is widespread and can be found in the subtropics of the world [1]. The perfume and cup-shaped flowers of this family are distinctive. Male and female flowers are found in a single flower from several of the species of this family and each contributes to a single stamen. On the other hand, there are some species with separate sexes seeds, and some species have male, female and bisexual flowers in combination. There are 24 genera in Pakistan in the *Euphorbiaceae* family [1]

The genus consists of a variety of biologically active compounds, predominantly diterpenoid esters, clerodane, labdane, kaurane, trachylobane, pimarane etc [2]. *Croton* species, like most *Euphorbiaceae*, can produce latex that in some species may be red in colour, suggesting medicinal properties [3]

Sifting of literature revealed the previously known alkaloids, diterpenes and sterols of this species [4], [5]. *Croton sparsiflorus* is a woody shrub of a height of 1-5 m and containing rolling branches. The plant is grown on the roadside, on drainage canals, on plantations and on sandy clay grounds [1]. It has been verified that it is grown in Brazil, Argentina, America, India, Bangladesh, and Pakistan [4], [6]. The facility is located in Pakistan near to Khyber Pakhtun Khwa, Islamabad territory, Punjab areas mostly Bahawalpur and some areas of Sindh.



Fig. 1. Foliage of *Croton sparsiflorus*

2. Materials and Methods

2.1. Collection of Plant Materials

Croton sparsiflorus plant samples (leaves, stems, inflorescences, twigs and roots) were collected from the farm at The Islamia University Bahawalpur, in March 2020. The plant material collected were green and fresh.

2.2. Qualitative Phytochemical Analysis

The sample was verified aimed at the occurrence of bioactive composites by means of following standard methods described by [7]



Fig. 2. Phytochemical Test Protocols in Laboratory

2.3. Test for proteins

For the confirmation of proteins test Ninhydrin test was performed. 2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in a boiling water bath for 1-2 minutes, observed for the formation of purple colour the presence of proteins [8].

2.4. Test for carbohydrates

For the confirmation of carbohydrates test Molisch's test was performed. 2 ml of extract, 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid were added, Formation of Purple colour at the inter phase of the two layers indicated the presence of carbohydrates [8].

2.5. Test for Phenols and Tannins

2.1 ml of crude extract was blended with 1.9 ml of FeCl_3 mixture at 2%. Presence of phenols and tannins was suggested by a black coloration or sometimes blue and green [9].

2.6. Test for flavonoids

For the confirmation of flavonoids, alkaline test was performed. 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by addition of concentrated sulphuric acid, appearance of yellow colour indicated the presence of flavonoids [10].

2.7. Test for Saponins

In a test tube, 2ml of crude extract was mixed with 5.0ml distilled water and vigorously shaken. The existence of saponins was assumed based on the formation of solid foam [11].

2.8. Test for Steroid

1ml of crude extract combined with 2ml chloroform, and concentrated H_2SO_4 was applied in a sideways motion. The existence of steroids was demonstrated by a red color in the lower chloroform layer. Another experiment was carried out by combining crude extract with 2ml chloroform. The mixture was put with 2ml of concentrated H_2SO_4 and acetic acid. The presence of steroids was demonstrated by the appearance of a greenish coloration.

2.9. Test for Terpenoids

0.5 ml crude extract was dissolved in 2 mL chloroform and evaporated until it was fully dry. 2ml concentrated H_2SO_4 was applied to this, and it was heated for around 2 minutes. The existence of terpenoids was suggested by a greyish color [10].

2.10. Test for Alkaloids

2ml crude extract was combined with 2 ml of 1% HCl and gently heated at low flame. The reagents of Mayer and Wagner were then added to the mixture. The alkaloids existence was determined by the turbidity of the resulting precipitate [9].

3. Quantitative Analysis

3.1. Determination of Proximate Analysis

Moisture, crude protein, and crude fat make up the proximate composition. The AOAC (2005) methods were used to assess the ash content and dry content of a powdery sample of *Croton sparsiflorous*.



Fig. 3. Quantitative Test Protocols in the Laboratory

3.2. Determination of Mineral Composition

To determine mineral content used the flame photometer for determination of Perchloric acid and nitric acid (1:2) mixture: 480 ml per-chloric acid (68%) in 950 ml HNO₃ (66-68%), thoroughly mixed, cooled, and packed in an amber glass container. For the desired micronutrients, standard solutions were chosen.

Calculations

Micronutrients (ppm) = Reading x 50

4. Results & Discussion

4.1. Phytochemical Qualitative Studies

Phytochemical qualitative studies of the plant revealed that leaves, stem, and other parts of *Croton sparsiflorous* possess alkaloids, flavonoids, saponins, phenols, proline, and carbohydrates.

Results indicates (as shown in Table 1) that all phytochemicals were present in stem of it. While twigs contain only flavonoids and carbohydrates.

4.2. Alkaloids

The qualitative phytochemical results showed that Alkaloid were present in the leaves, stem, and inflorescence and absent in twigs and roots of *Croton sparsiflorous* (Table 1).

4.3. Flavonoids

Results indicates (as shown in Table 1) that Flavonoids were present in all parts of *Croton sparsiflorous* such as twigs, leaves, roots, stem, and inflorescence.

Table 1.

Different parts of *Croton sparsiflorus* showing presence and absence of different phytochemicals.

<i>Croton sparsiflorus</i> Parts	Alkaloids	Flavonoids	Saponins	Phenol	Steroids	Carbs.
Twigs	–	+	–	–	–	+
Leaves	+	+	–	+	–	–
Roots	–	+	+	–	+	+
Stem	+	+	+	+	+	+
Inflorescence	+	+	–	–	+	–

4.4. Saponins

The analysis of saponins in different parts of *Croton sparsiflorus* revealed that the concentration of saponins in the roots and stem (Table 1). However, saponins were absent in twig, inflorescence, and leaves of it.

4.5. Phenols

The present experiment revealed that *Croton sparsiflorus* possess phenol contents in leaves and stems. However, the phenol contents were absent in twig, inflorescence, and roots as shown in (Table 1).

4.6. Steroids

The analysis of saponins in different parts of *Croton sparsiflorus* revealed that the roots, stems, and inflorescence have concentration of steroids. However, in twig, and leaves steroids were absent (Table 1).

4.7. Carbohydrates

The results revealed that analysis of carbohydrates in different parts of *Croton sparsiflorus* indicates that the roots, stems, and twigs have concentration of carbohydrates. However, carbohydrates were absent in inflorescence and leaves (Table 1).

5. Mineral Contents

To determine the mineral composition of different parts (leaves, twigs, and roots) of *Croton sparsiflorus* using a flame photometer and a spectrophotometer. In the first step for sample preparation, collected representative samples of *Croton sparsiflorus* leaves, twigs, and roots, cleaned and washed the samples thoroughly with deionized water to remove any contaminants, and then dried the samples at a controlled temperature. In the second step, the samples were digested. Weigh the dried plant samples using an analytical balance, digest the samples using suitable digestion reagents to extract mineral content, and then follow the recommended digestion procedure for each plant part [12]. For flame photometer analysis Prepare mineral standards of known concentrations for copper, iron, manganese, and zinc, then calibrate the flame photometer using the standards. Analyze the digested samples with the flame photometer and record the readings for copper, iron, manganese, and zinc concentrations in parts per million (ppm). Moreover, for spectrophotometer analysis Prepare mineral standards of known concentrations for copper, iron, manganese, and zinc, and use the spectrophotometer to measure the

absorbance of the digested samples. Compare the absorbance values with the standard curve to determine mineral concentrations, and then record the readings for copper, iron, manganese, and zinc concentrations in ppm.

The result of mineral analysis of *Croton sparsiflorus* (Table 2) indicated that the minerals detected were, copper, iron, manganese, and zinc. The leaves showed a high level of iron content (89 ppm) followed by zinc (50 ppm) while copper and manganese were detected in also good amounts in leaves (9.2 ppm and 24 ppm) respectively. The result showed the healthy mineral level of the leaves. On the other hand twigs showed a high level of iron content (243 ppm) followed by zinc (65 ppm) and copper in low amounts (3.5 ppm) and manganese was absent in twigs. The result showed good level of mineral in the twigs apart from copper and manganese. While roots showed a high level of iron content (119 ppm) followed by copper (79.1 ppm), zinc (30 ppm) and manganese was in low level (7 ppm). The result showed good level of mineral in the roots.

Table 2.

Croton sparsiflorus showing minerals composition after mineral contents analysis

Minerals	Twigs	Root	Stem	Leaves	Inflorescence
Zn (ppm)	65	30	41	50	60
Cu (ppm)	3.5	79.1	4.6	9.2	19.3
Fe (ppm)	243	119	819	89	398
Mn (ppm)	ND	7	7	24	ND

Furthermore, stem showed a high level of iron content (819 ppm) followed by zinc (41 ppm) and manganese and copper in low amounts (7 ppm and 4.6 ppm) respectively. The result showed good level of mineral in the stem apart from copper and manganese. In addition, inflorescence showed a high level of iron content (398 ppm) followed by zinc (60 ppm) and copper (19.3 ppm). While manganese was absent in inflorescence. The result showed good level of mineral in the stem apart from manganese.

5.1. Zinc (Zn)

Highest concentration (65 ppm) of zinc was observed in the Twigs of *Croton sparsiflorus* while its lowest concentration (30 ppm) was detected in the roots as shown in (Fig.4). The values recorded after mineral analysis in leaves, inflorescence and stem (50 ppm, 60 ppm, and 41 ppm) respectively.

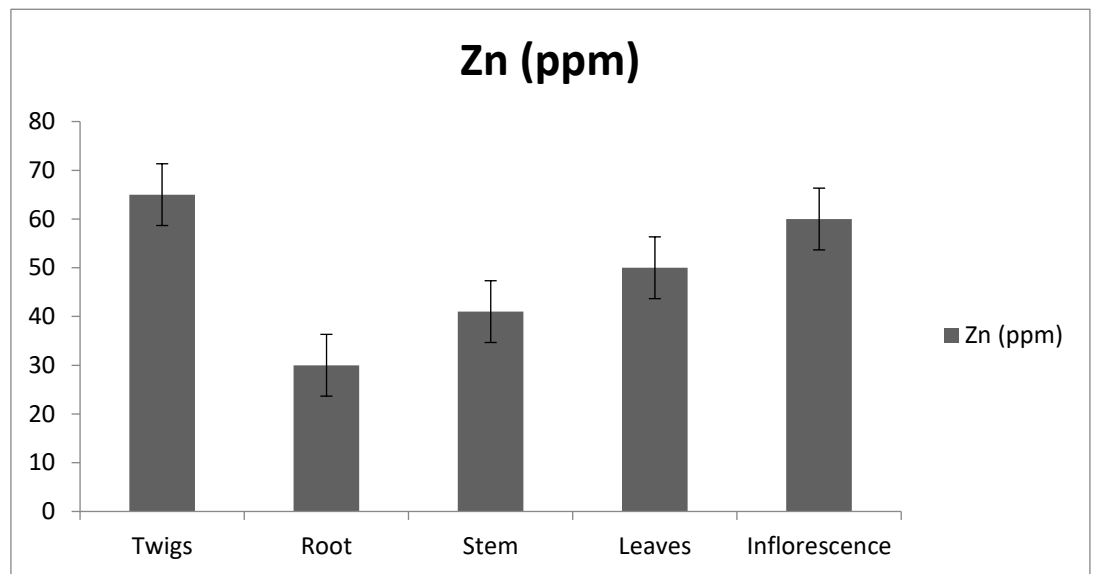


Fig. 4. Zinc contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

5.2. Copper (Cu)

Highest concentration (79.1 ppm) of copper was observed in the roots of *Croton sparsiflorus* followed by inflorescence (19.3 ppm). While its lowest concentration (3.5 ppm) was detected in the twigs as shown in (Fig.5). The values recorded after mineral analysis of leaves and stem (9.2 ppm, and 4.6 ppm) respectively.

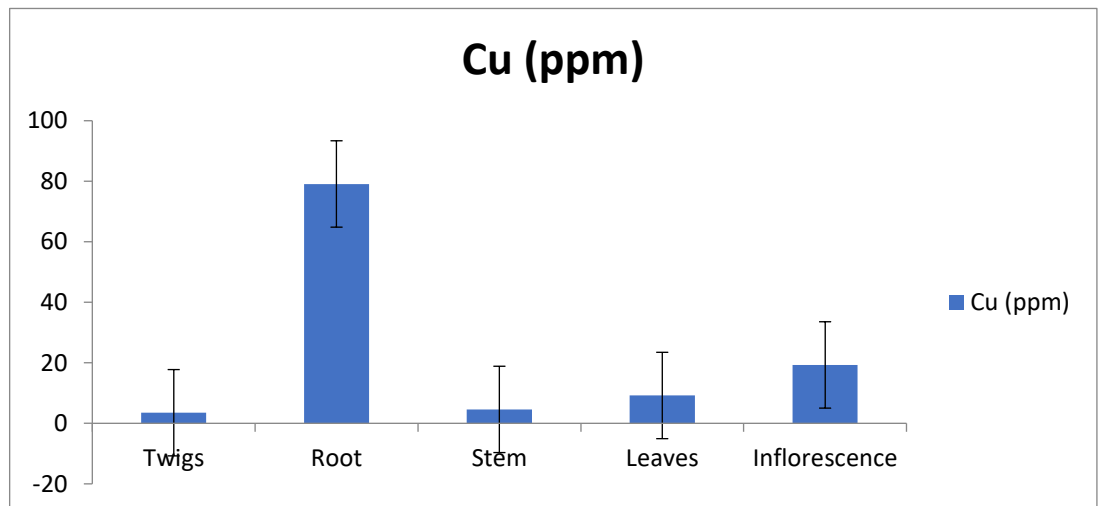


Fig. 5. Copper contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

5.3. Iron (Fe)

Highest concentration (819 ppm) of iron was observed in the stem of *Croton sparsiflorus* followed by inflorescence (398 ppm). While its lowest concentration (89 ppm) was detected in the leaves as shown in (Fig. 6). The values recorded after mineral analysis of twigs and roots (243 ppm, and 119 ppm) respectively.

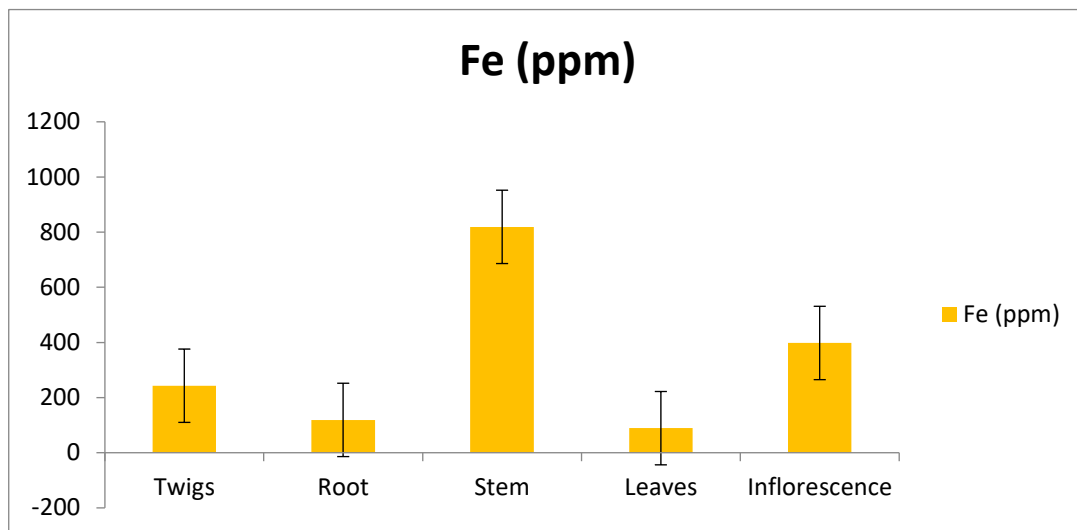


Fig. 6. Iron contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

5.4. Manganese (Mn)

Highest concentration (24 ppm) of manganese was observed in the leaves of *Croton sparsiflorus*. The values recorded after mineral analysis of *Croton sparsiflorus* of both roots and stem were (7 ppm). While its concentration was not detected in the twigs and inflorescence as shown in (Fig.7)

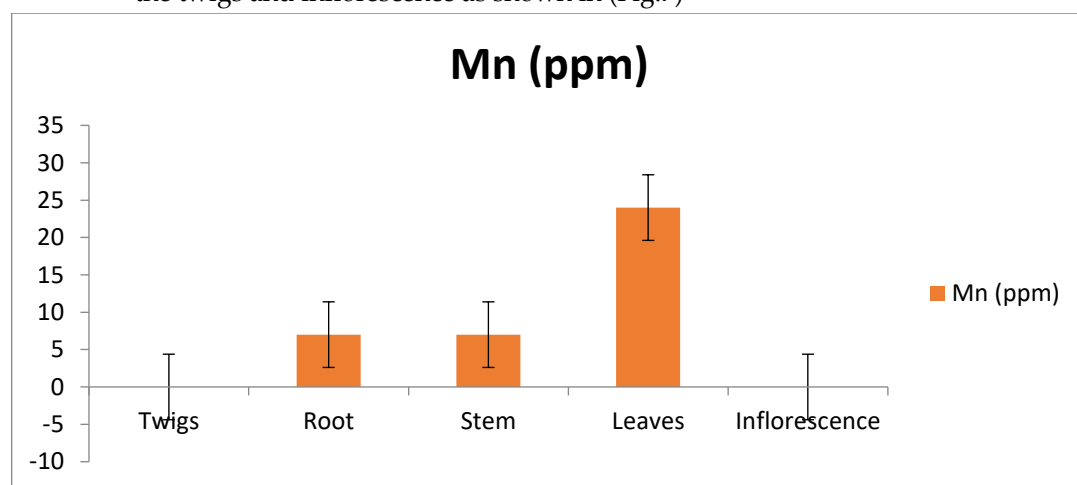


Fig.7. Manganese contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

6. Proximate Analysis

The Proximate analysis results of *Croton sparsiflorus* as shown in (Table 3). This showed that leaves have highest amount of dry content (98.97%), crude fat (31.15%), crude protein (25.21%), Ash (14.10%), and moisture content (1.03%). Stem has the highest amount of dry content (99.58%), crude fat (9.30%), crude protein (5.51%), Ash (5.50%), and moisture content (0.42%). While twigs have highest amount of dry content (99.35%), crude fat (9.40%), crude protein (7.15%), Ash (5.90%), and moisture content (0.65%). On the other hand, roots have highest amount of dry content (99.35%), crude fat (7.40%), crude protein (5.51%), Ash (6.20%), and moisture content (0.85%). In addition, inflorescence have

highest amount of dry content (97.73%), crude fat (8.89%), crude protein (9.60%), Ash (7.93%), and moisture content (0.73%).

Table 3. Proximate composition of twigs, roots, stem, leaves, and inflorescence of *Croton sparsiflorus*.

Parameters	Twigs	Root	Stem	Leaves	Inflorescence
Moisture Content (%age)	0.65	0.85	0.42	1.03	0.73
Dry Content (%age)	99.35	99.15	99.58	98.97	97.73
Ash (%age)	5.90	6.20	5.50	14.10	7.93
Crude Fat (%age)	9.40	7.40	9.30	31.15	8.89
Crude Protein (%age)	7.15	5.51	5.69	25.21	9.60

7. Moisture Content

Moisture content has the highest amount in leaves (1.03%), followed by roots (0.85%). While inflorescence, twigs and stem have (0.73%, 0.65%, and 0.42%) respectively as shown in (Fig. 8).

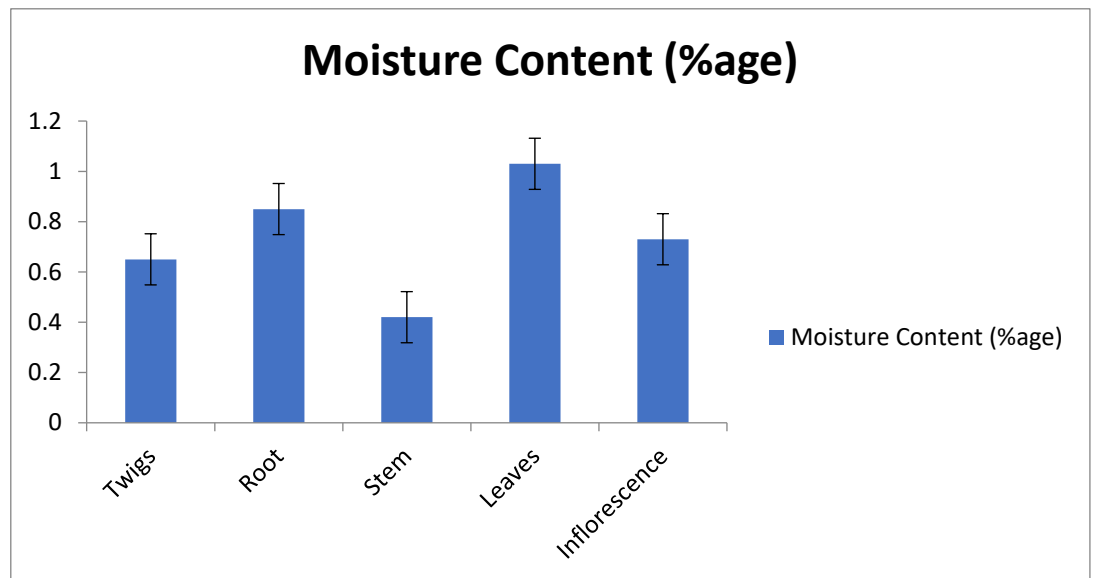


Fig.8. Moisture contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

7.1. Dry Content

Highest amount of dry content was found in stems (99.58%) followed by twigs, roots, leaves, and inflorescence have (99.35%, 99.15%, 98.97%, and 97.73%) respectively as shown in (Fig. 9).

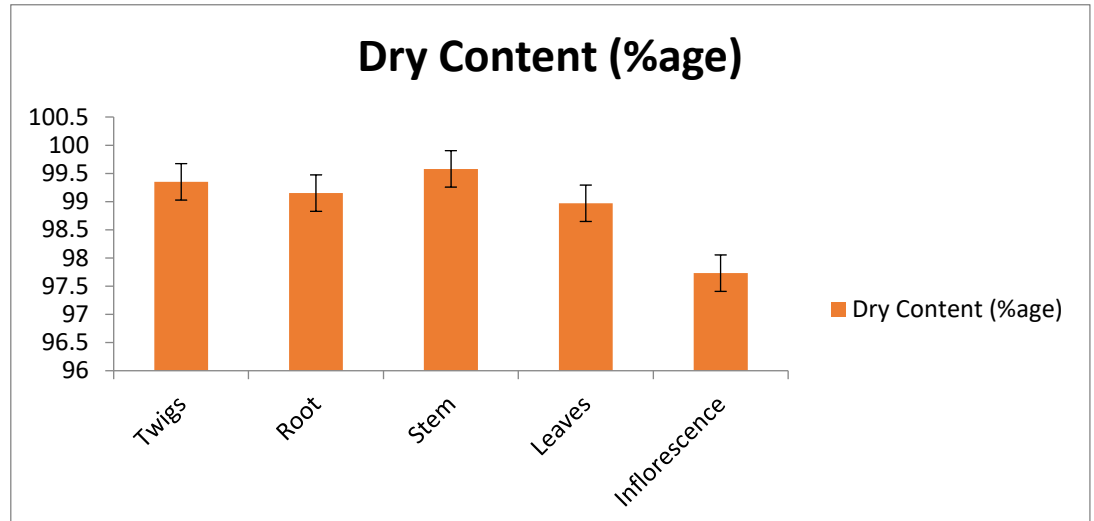


Fig. 9. Dry contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

7.2. Ash

Ash has the highest amount in leaves (14.10%), followed by inflorescence (7.93%). While roots, twigs and stem have (6.20%, 5.90%, and 5.50%) respectively as shown in (Fig. 10).

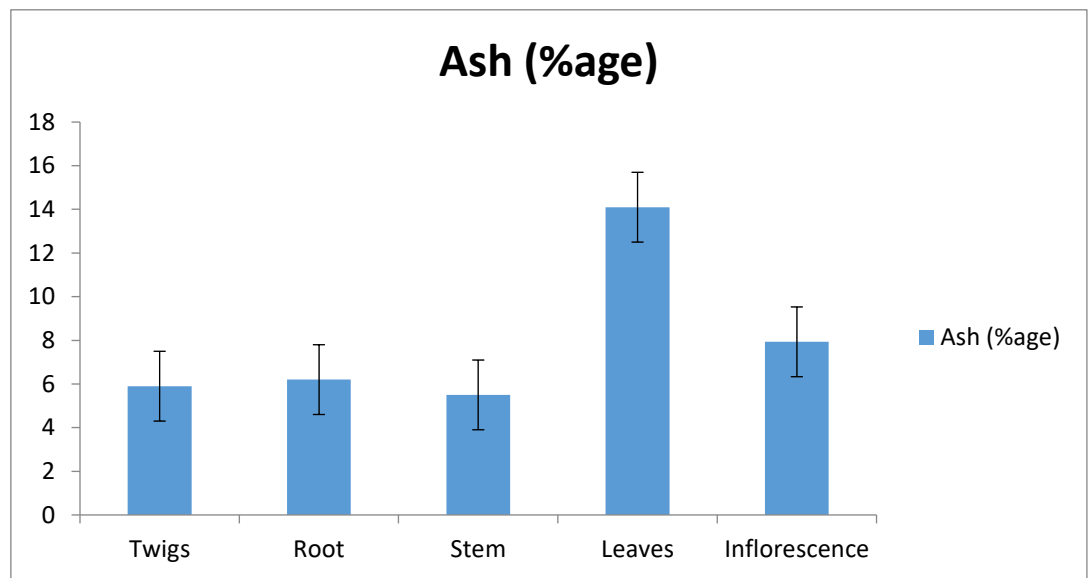


Fig. 10. Ash contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

7.3. Crude Fat

Crude Fat has the highest amount in leaves (31.15%), followed by twigs (9.40%). While stem, inflorescence and root have (9.30%, 8.89%, and 7.40%) respectively as shown in (Fig. 11).

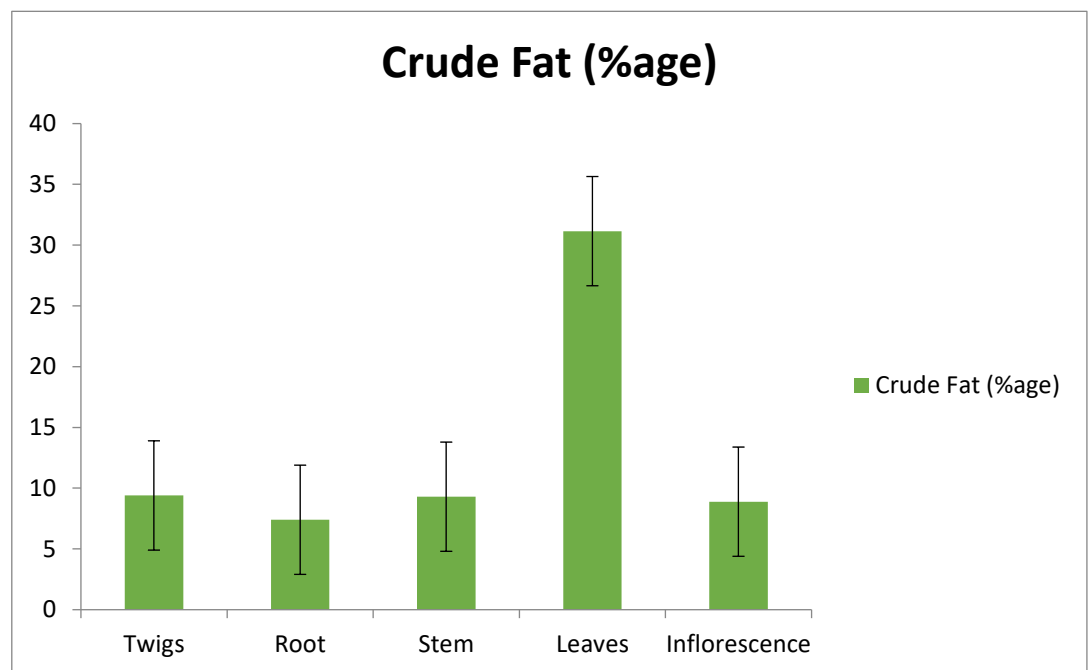


Fig. 11. Crude fat contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

7.4. Crude Protein

Crude Protein has the highest amount in leaves (25.21%), followed by inflorescence (9.60%). While, twigs, stem and roots have (7.15%, 5.69%, and 5.51%) respectively as shown in (Fig. 12).

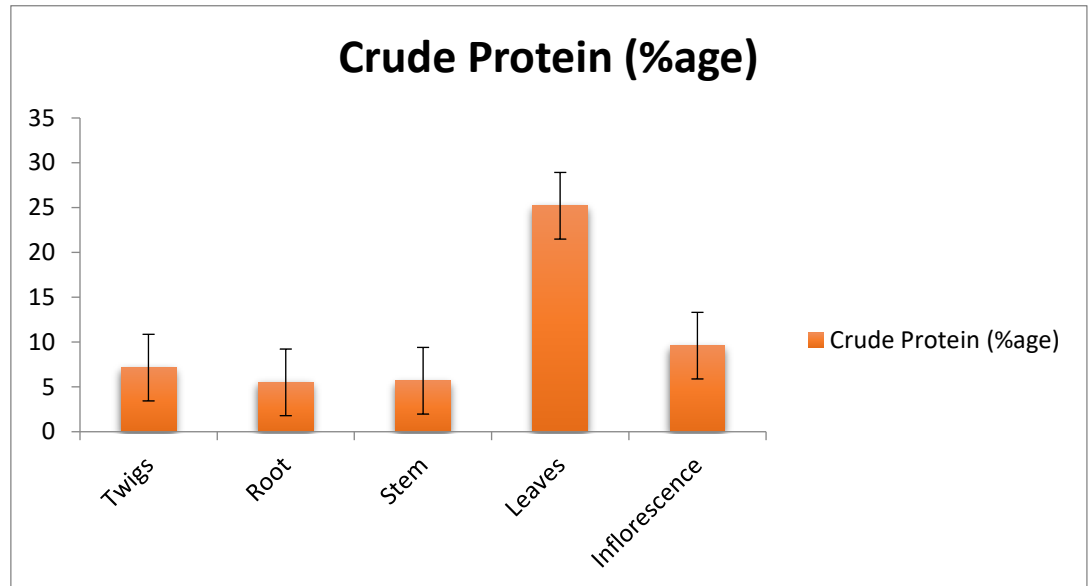


Fig. 12. Crude protein contents in the twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus*.

8. Discussion

Croton sparsiflorus was analysed for its phytochemical, proximate, and mineral composition (*Euphorbiaceae*). Alkaloids, flavonoids, saponins, phenols, proline, and carbohydrates were found in different concentration during phytochemical analysis. Its twigs, leaves, roots, stem, and inflorescence appeared to have medicinal properties as well as the essential mineral elements for good health.

Plants containing tannins are used as astringents, diuretics, and against gastrointestinal tumours, according to [13]. Flavonoids in floras have therapeutic effects, according to [13], including antioxidant and anti-inflammatory properties. The potential to supports its antioxidant function. The dry substance of *Croton sparsiflorus* was found to be 98.95 percent in a general study. In this case, the dampness capacity was determined to be 0.73 percent. The rough protein content was 10.63 percent (unrefined protein contained both genuine protein and non-protein nitrogen). The percentages of debris and rough fat material were 7.93% and 13.23%, respectively.

Incorporating restorative plants into our daily diets can aid in the management of clogging issues [14]. Dietary strands also help to reduce cholesterol and fatty acids, as well as protect against cancer and stomach problems [15]. A well-known member of this genus is *Croton tiglium*, a shrub native to Southeast Asia. *C. tiglium* oil has been used in traditional Chinese medicine to treat severe constipation or heal lesions, and is used as a purgative. It was observed that croton seeds could also be used to treat diarrhea. It is a source of the organic compound phorbol.

Table 2 demonstrates the mineral composition of *Croton sparsiflorus*. Zinc (49.2 ppm), Iron (333.6 ppm), Manganese (7.6 ppm), and Copper are all present in significant amounts (23.14 ppm). Zinc is needed for protein fusion, cell separation and replication, invulnerability, and sexual abilities, according to [16].

Magnesium is necessary in the human plasma and extracellular liquid, where it maintains osmotic balance. It can also help people avoid heart attacks and reduce circulatory strain. To regulate weight, iron promotes the oxidation of bio-particles, which inclines an individual to various illnesses. It is also essential for haemoglobin aggregation [17], is a component of unique catalysts and proteins. This validates the use of *Croton sparsiflorus* in traditional medicine as a blood tonic due to its blood enhancing properties.

The findings revealed that the *Croton sparsiflorus* contains therapeutically important constituents. The existence of these phytochemicals add therapeutic value and physiological assets to the *Croton sparsiflorus*, according to natural examinations. As a result, *Croton sparsiflorus* removes and separated mixtures may be considered a good hotspot for useful medications. For *Croton sparsiflorus*, the traditional treatment method is strongly recommended. It is expected that the combination of solid data on common objects, combinatorial sciences, and high-throughput screening methods would increase the clarity with which characteristic items and meanings are described can be utilized in drug disclosure missions and advancement measure, subsequently giving new practical prompts different infections.

9. Conclusion

The present study shows the presence of phytochemicals, minerals and supplements in *Croton sparsiflorus*, which may thusly legitimize the two it's nourishing and ethno therapeutic advantages to human well-being. The examination further uncovered that the plant, *Croton sparsiflorus* utilized have end up being vital in drug innovative work, in light of the phytochemicals that are available. The leaves additionally showed a significant degree of dampness and mineral substance. Twigs, leaves, roots, stem, and inflorescence of *Croton sparsiflorus* appear to have great nutritive and appropriate mineral components esteem important to maintain good health.

Finance

Muhammad Khalid Afzal used his personal finances to complete this project

Conflict of interest

Both authors do not have any conflict of interest.

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