Proximate analysis, mineral properties, and phytochemical study of *Canthium parvifolium* Roxb. stem

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Abstract

This study aims to provide proximate analysis, mineral composition, and phytochemical analysis of *Canthium parvifolium* Roxb. stems. I also added the morphology and traditional uses of *Canthium parvifolium* Roxb plants. The phytochemical screening revealed that acetone extract contains amino acids, flavonoids, phenols, steroids, and carbohydrates but no terpenoids, alkaloids, or glycosides. In methanol extract, terpenoids and alkaloids are absent; amino acids, flavonoids, phenols, steroids, carbohydrates, and glycosides are present. In water extract, alkaloids and flavonoids are absent; amino acids, flavonoids, phenols, steroids, carbohydrate, and glycosides are present.

Proximate analysis yielded the following results: a moisture content of 55.65%, a total ash value of 6.13%, an acid-insoluble ash value of 5.81%, and a water-soluble ash value of 4.23%. In mineral analysis, different mineral contents are present. This result shows potassium concentration was highest at 188552.63 mg/kg, while copper mineral concentration was lowest at 122.85 mg/kg. The result shows that macro- and micro-elements are present in sufficient amounts. So plants were useful as food supplements. Due to their medicinal properties, they are also useful in some disease cures.

Key words: Canthium parvifolium Roxb., Rubiaceae, proximity analysis, mineral composition, phytochemical test.

Abbreviations : + = Present, _=Negative, mg= Miligrams, Kg=Kilogrammes.

Plants have played a significant role in human existence for a very long time. They

provide food, medicine, shelter, fibre, and fodder for domestic animals. Humans also used

plants for food, shelter, and medicinal purposes. So many plants have promising nutritive values that could nourish the ever-increasing human population but remain underutilised due to a lack of awareness and technologies for their utilisation. Many of them are even more resilient, adaptive, and tolerant to adverse climatic conditions than conventional foods.¹

Plants are a good source of remedies and are essential to the survival of tribal and ethnic societies. All across the world, medicinal plants are used to treat both human and animal illnesses. The ethnomedical and nutritional benefits of using native plant products have piqued scientists' interest and led them to search for bioactive substances. Essential nutritional ingredients, including carbohydrates, protein, and fat, are present in medicinal plants. These elements are crucial for the needs of the human body and are employed in a variety of physiological, metabolic, and morphological processes. The ingredients in medicines, dietary supplements, and other healthcare items are obtained from natural plants. The discovery of novel, useful pharmaceutical components depends heavily on plants, particularly their phytochemical elements, such as antioxidants, hypoglycemic, and hypolipidemic phytochemical components, which are examples of phytochemical ingredients. Many medicines are derived either directly or indirectly from plant resources, which are frequently a valuable supply of medicines.12

Today, a lot of regularly used medications have herbal origins. In fact, roughly 25% of the lengthy prescriptions filled in the US contain at least one active component derived from plant matter. Some are created using plant extracts, while others are synthesised to resemble a substance found in plants. Though the ideas themselves took a very long time to develop into a body of knowledge, there has been a clear concern for health care and the treatment of disease throughout human history. Recognising the fundamental concepts underlying the biochemical mechanisms leading to drug action is a logical approach to studying medications and their effects. Now that more knowledge has been gained about how medications behave at the macromolecular level¹⁶.

Therefore, plant medicines are still a significant component of medicines utilised today, particularly in contemporary medicine and traditional medicinal systems like our traditional Ayurveda, Siddha, and Unani, as an immune modulator. The possibility of using higher plants as a source of novel medications, however, has not yet been fully investigated. Only a small portion of the estimated 250,000-500,000 plant species have had their phytochemical content analysed, and an even smaller portion have had their pharmacological qualities fully examined. In the majority of cases, pharmacological screening or exploratory research has been performed, and about 8,000 species are thought to have been investigated for potential therapeutic use^{16} .

Plant-derived medicines have the largest global contribution to human health. These plants' primary therapeutic ability is due to a few chemical components. These chemical compounds have a specific physiological effect on the human body that is referred to as a phytochemical. These substances serve as a barrier against sickness and are non-nutritive. Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant of these phytochemicals.^{3,10} Evaluating the nutritional significance of various medicinal plant species could be helpful to understand the value of these plant species since they are also consumed as food in addition to their medical effects^{5,9}. With more than 45,000 different plant species, India is one of the world's top twelve biodiversity hotspots. Only 750–800 of them have been used by traditional healers out of the 1,500–2,000 plants that have good therapeutic characteristics. Roughly 600 plant species are used in the Siddha educational institutions of medicine: 700 in Ayurveda, 700 in Unani, and roughly 30 in modern medicine.

There are many uses for minerals. Calcium, for instance, aids in the formation of bones, teeth, and blood clots. It can be found in tofu, green leafy vegetables, and dairy products. Red blood cells, which carry oxygen from the lungs to other body cells or organs, must be built using iron minerals.

Minerals are solid inorganic compounds that crystallise and are categorised based on their trace minerals, such as molybdenum, selenium, zinc, iron, and iodine, which are only required in a few milligrammes or less.

The proximity and mineral composition of plants reveal important details about their therapeutic and nutritive value. It is possible to determine a variety of factors, including the amount of moisture, ash, volatile matter, ash, and fixed carbon. Ash is an inorganic residue that serves as an indicator for the overall mineral content of food and plant matter. When compared to other dietary components, minerals have low volatility and are not destroyed by heat. The quantity of total ash in different plants and plant components might vary greatly. Because mineral contents may be the root of a pharmacological effect, the assessment of ash contents is crucial^{4,7}.

Biodiversity refers to the variety of living organisms, including those in aquatic, marine, and terrestrial ecosystems. It has to do with the diversity of living creatures that live on the earth, such as the variation found within and between species as well as within and between the ecosystems they make up. It maintains the balance between numerous species and serves ecological, economic, and societal purposes. India is home to several endangered species of plants and animals, in addition to six key biodiversity hotspots.

Canthium parvifolium Roxb. characters :

Canthium parvifolium Roxb. is a dicot plant included in the Rubiaceae family and order Gentianales. The plant's appearance is that of a shrub with paired straight supraaxillary thorns. Leaves are simple and opposite in arrangement with interpetiolar stipules. The young branches and the lower surface of the leaves are covered with pubescence. The inflorescence is an axillary cymose with a few to several flowers. The calyx is green, and the limb is very short and undulating, 5-lobed, with a subglobose ovary portion. The corolla is tubular with five lobes, green to white or vellow in colour. There are five stamens inserted into the corolla throat. The ovary is inferior, 2-loculate, with only 1 ovule each.

Medicinal importance :

Tribals like eating these leafy vegetables, which are available everywhere naturally in forest areas. This is a famous proverb in Andhra Pradesh. *Canthium parvifolium*, as an herbal medicine, is used for the treatment of diabetes, among other major tribal groups in South India. Though the ethnomedicinal importance of this plant is known, the phytochemical basis for this kind of medicinal property is not known.¹² *Canthium parvifolium* plants have different parts that are useful in many ways. The objective of this study is to find out the proximate mineral analysis and phytochemical study of *Canthium parvifolium* stem.

Study area :

Prantij has latitude 23.4367 °N and longitude 72.8528 °E, and it is a town and a municipality in Sabarkantha district in the Indian state of Gujarat. The nickname of this tehsil is Praptipura. Different villages are located in different areas. Many shortages are found in villages. Different types of plants are located around the Prantij area. People are mostly farmers. Near the Prantij Taluka, different plants like Justiicia adhatoda, Ocimum sanctum, Azadirachta indica, Euphorbia hirta, Emblica officianils, Neuracantrhus sphaerostachyus, etc. were found. Many medicinal plants have been observed in this area and are useful in many ways.

Plant material collection and identification:

The whole plant of *Canthium parvifolium* Roxb. was collected from the roadside areas of Vadrad, Kamalpur Roadside Area, and Pranitj, India. The plant specimen was identified by Prof. Dr. Kaushik C. Patel, Department of Botany, at Smt. S.M. Panchal Science College, Talod.

Phytochemical Study : Qualitative analysis of a powder sample :

Powder from plant samples :

The collected powder extract samples were washed with water and then put in shaded, dry place for drying. Then make a powder sample with the help of a grinder. And used in different phytochemical tests.

Preparation of plant extracts :

Two g of powdered plant material was dissolved in 10 ml of three different solvents, like Acetone, Methanol, and water, for 24 hours. This solution was sonicated for 10 minutes and then filtered.

Extraction of plant materials :

Extraction was carried out in 500 ml of ethanol at 600 °C for 8 hours using the Soxhlet extractor. After extraction, the extracts were dried at room temperature until they solidified.

Preliminary phytochemical analysis :

Different organic solvent extracts of *Canthium parvifolium*. Whole plant powder were used to screen the following phytochemicals: carbohydrates, alkaloids, phenolic compounds, flavonoids, amino acids, glycosides, and terpenoids. Phytochemical's qualitative tests were carried out in extracts following the standard procedures as described by Sofa-Ware¹⁵, Trease and Evans¹⁶, and Harborne². The various extracts of *Canthium parvifolium* obtained with different organic solvents were subjected to the same phytochemical tests to screen the following phytochemicals:

Qualitative Screening :

Qualitative analysis was done to identify the presence of different phytochemicals like alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins, And amino acids using standard procedures.

Test for flavonoids :

Take 2 ml of extract, then add a few drops of 20% sodium hydroxide. An intense yellow colour is observed. To this, a few drops of 70% dilute hydrochloric acid were added, and the yellow colour disappeared.

The formation of a yellow colour in this observation indicates the presence of flavonoids in the sample extract. Then the yellow colour disappeared.

Test for Phenols :

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; the formation of a blue colour indicates the presence of phenols in the sample extract.

Test for Cardiac Glycosides :

One ml of each extract, 0.5 ml of glacial acetic acid, and 3 drops of a 1% aqueous ferric chloride solution were added.

The formation of a brown ring observed at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for Terpenoids :

Take 1 ml of the extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulfuric acid. The formation of a reddish-brown precipitate indicates the presence of terpenoids in the sample extract.

Test for steroids :

Add 2 ml of chloroform and concentrated H_2SO_4 to the 5 ml of aqueous plant crude extract. In the lower chloroform layer, a red colour appeared that indicated the presence of steroids in the sample extract.

Test for amino acids : Folin's *test :*

Take 1 ml of sample and add 1 ml of Folin's phenol reagent, followed by the addition of 1N sodium carbonate. The blue colour that appeared indicates the presence of tyrosine and tryptophan.

Test for alkaloids :

In the second portion, a pair of drops of Meyer's chemical agent were added. A creamy white precipitate indicated the presence of alkaloids within the sample extract.

Tests for carbohydrates (Barfoed's test):

Take One millilitre (1 mL) of an aqueous solution of the extract and 1 mL of Barfoed's reagent were added into the test tube and heated in a water bath for 2 minutes. The red precipitate showed the presence of monosaccharides.

Determination of total Ash :

Incinerate about 2 to 3 g accurately weighed of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450 until free from carbon, cool, and weigh. If carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash-less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450. Calculate the percentage of ash with reference to the air-dried drug.

Determination of Acid-Insoluble Ash :

Boil the ash obtained in the above method for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible or on an ash-less filter paper; wash with hot water; and ignite to a constant weight. Calculate the percentage of acid-insoluble ash with reference to the airdried drug.

Determination of water-soluble Ash :

Boil the ash for 5 minutes with 25 ml of water, collect insoluble matter in a Gooch crucible or on an ash-less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight rep Resents the water-soluble ash Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Mineral analysis

Sample preparation :

 $0.5 \text{ gm Ash} + 3 \text{ ml HNO}_3 + 2 \text{ ml HCL}$ Add 50 ml of distilled water and put it on a hot plate for acid digestion until the water is up to 25 ml. Then cool, filter with Watchman filter paper No. 42, and make up the volume to 100 ml in a volumetric flask with distilled water. The supernant was decanted, and the liquid was analyzed for the levels of Magnesium, Manganese, Sodium, potassium, iron, copper, Copper and Calcium present using standard procedures using an atomic absorption spectrophotometer (AAS).⁹.

Morphology of Canthium parvifolium Roxb plant :



Canthium parvifolium Roxb. Plant in Natural Habitat

The herb *Canthium parviflorum* Roxb. Belongs to the family Rubiaceae. It is a thorny shrub. The biological type for the genus consists of specimens originally described by Jean-Baptiste Lamarck as *Canthium parviflorum* Roxb.¹⁵.

Canthium parvifolium Roxb is included in the Rubiaceae family as a shrub or tree, having deciduous leaves with spines on the stem. Leaves are simple and opposite in arrangement with interpetiolar stipule, flower cymose, and axillary. The Rubiaceae are trees, shrubs, or, infrequently, herbs, comprising about 450 genera and 6500 species, including some lianous forms. The leaves are simple and usually entire and are opposite or sometimes whorled; stipules are present and interpetiolate. The flowers are nearly always bisexual and actinomorphic, often heterostylous, and usually in cymose inflorescences. The calyx is somewhat reduced and has 4-5 lobes, or sometimes the lobes are absolute, or rarely one of them is greatly expanded and brightly colored. The sympetalous corolla is mostly 4-5 lobed, occasionally with 3 or up to 10 lobes. The androecium consists of as many stamens as corolla lobes and is adjacent to the corolla tube or epigynous zone. Alternate with the lobes. The gynoecium consists of a single compound pistil of two or rarely more carpels, a single style, and a nearly always inferior ovary with the same number of locules.¹⁷

Geographical distribution :

Canthium parvifolium Roxb species are predominantly found in Southeast Asia, especially in Thailand and the Philippines. A small number of species are found in India, Sri Lanka, and Bangladesh. Only a limited number of species are found on the African continent, especially in southern and eastern Africa.

Traditional medicine :

In Ayurveda, the medicinal herb Canthium parviflorum (Rubiaceae) has been extensively used to treat kapha, diarrhoea, strep throat, fever, leucorrhoea, intestinal worms, and general weakness. In several communities in the Shimoga area of Karnataka, India, this plant has a long history of use for treating snakebite and is reported to have wound-healing properties. The goal of the current study was to evaluate the antioxidant capacity of C. parviflorum solvent extracts. The Orissan tribes have long used the roots of this plant to cure neck swelling and the fruits to treat headaches. This plant has been used pharmacologically as a diuretic, an astringent, an anthelmintic, an antidysentric, and an antispasmodic. We learned through the ethnomedical study that many residents of the region of Vellore have been using the plant and its varied parts for a variety of diseases in those places for centuries. Therefore, the entire plant was used for our current examination to learn more about the presence of different phytochemical constituents and their associated activities.¹¹

Sr No.	Content	AcetoneExtract	MethanolExtract	Water extract
1	Alkaloids	_	_	_
2.	Flavanoids	+	+	_
3.	Amino acid	+	+	+
4	Phenol	+	+	+
5	Steroids	+	+	+
6	Glycoside	_	+	+
7.	Terpanoids	_	_	+
8.	Carbohydrates	+	+	+

Table-1. Phytochemical value of *Canthium parvifolium* Roxb stem plants

+=Present, =Absent

The phytochemical evaluation result shows that acetone extract Where alkaloids, glycosides, and terpenoids are lacking, secondary metabolites such as flavonoids, amino acids, carbohydrates, and steroids are abundant. Alkaloids and terpenoids are absent from the methanol extract, while flavonoids, amino acids, steroids, glycosides, carbohydrates, and phenols are all present. According to Nurul Nadia et al. (2015), flavonoids are phenolic compounds that are typically found in plants and have antioxidant qualities. They are present in aqueous extracts along with amino acids, carbohydrates, phenols, steroids, terpenoids, and glycosides. For plants to produce fruits and seeds and attract pollinators, phenolic chemicals are essential. In allelopathy, phenolics also play a significant role. Alkaloids and flavonoids are missing¹⁴. Secondary metabolites called terpenoids provide plants with their antibacterial and antifungal properties.

Gibberellins (diterpenes), one type of terpene, play a fundamental role in the growth and development of the plant body.²² The largest and same number of phytochemicals are present in both aqueous and methanol extracts. There are extremely few phytochemicals in acetone extract. In all three solvents' extracts, alkaloids were missing.

These secondary metabolites have boosted its physiological activity as well as its therapeutic efficacy. A wide spectrum of pharmacological properties of the flavonoid that was discovered include antioxidant, antiinflammation, antiplatelet, anti-allergic, cytotoxicity, and reduced risk for heart disease. The plant's identified phytoactive component is well known for its pharmacological properties, which include antibacterial and antifungal effects.

Table-2. Proximate analysis of

Canthium parvijolium Roxb. Plant				
Sr. no	Parameter	Value W/W %		
1	Moisture content	55.65		
2	Total ash value	6.13		
3	Insoluble ash value	5.81		
4	Soluble ash value	4.23		

A Canthium parvifolium Roxb stem sample had a moisture content of 55.65%. Ash content totals 6.13%. In evaluating the presence or absence of foreign organic matter, such as metallic salts or silica, the total ash is particularly crucial. We calculated the insoluble ash value at 5.81% and the soluble ash value at 4.23% from the total ash content. In comparison to both soluble and insoluble ash values, the total ash value is higher. The value of insoluble ash is greater than that of soluble ash.

Sr. No	Parameter	Mean \pm Sd	Unit
1	Available Potassium	188552.63±0.03055	Mg/Kg
2	Calcium	916.80±0.3	Mg/kg
3	Magnesium	301.10±0.05773	Mg/kg
4	Sodium	61973.68±0.02516	Mg/kg
5	Copper	122.85±0.0208	Mg/kg
6	Iron	3969.77±0.02645	Mg/kg
7	Manganese	430.46±0.025166115	Mg/kg

Table-3. Mineral analysis of different component in Canthium parvifolium Roxb stem

The outcome of the above table demonstrates that the number of minerals found in the plant sample varies. Minerals are organised in decreasing sequence as follows: K>Na>Fe>Ca>Mn>Mg>Co. This sample has the highest content of potassium (188552.62 \pm 0.03055 Mg/kg). It might lower blood pressure, prevent water retention and stroke, assist in kidney stone and osteoporosis prevention, and reduce blood pressure. Sodium, with a concentration of 61973.68 mg/kg, is the second-highest amount discovered. A small quantity of sodium is required by the human body to convey nerve impulses, contract and relax muscles, and maintain the proper ratio of water to minerals. Our bodies are said to require 500 mg per day. Iron, at 3969.77± 0.02645 mg/kg, comes in third place. Haemoglobin, a class of protein that delivers oxygen in red blood cells, contains iron as a key component.

The above table result shows that the mineral amount observed is different in the plant sample. Minerals are arranged according to decreasing order: K>Na>Fe>Ca>Mn>Mg>Co. The highest amount of potassium found in this sample is 188552.62 \pm 0.03055 mg/kg. It may help reduce blood pressure and water retention, protect against stroke, and prevent osteoporosis and kidney stones. The second highest amount found is sodium (61973.68 \pm 0.02516 mg/kg concentration).

To transmit nerve signals, contract and relax muscles, and keep the right ratio of water to minerals, the human body needs a tiny quantity of sodium. It is estimated that our bodies need 500 mg per day. The third-highest amount is iron at 3969.77 ± 0.02645 mg/kg. Haemoglobin, a form of protein found in red blood cells that transports oxygen from the lung to every region of the body, is largely composed of iron. The amount of calcium is 916.80±0.3 mg/kg. In order to give bones and teeth structure and hardness, the body stores the mineral calcium in these tissues. The body uses vitamin D to absorb calcium. The content of Mn is 430.46±0.0251661 mg/kg. Together with vitamin K, Mn has a role in blood clotting and hemostasis. Magnesium and copper are the following minerals in order of concentration, with copper coming in at 122.85±0.0208 mg/ kg and magnesium at 301.10 ± 0.05773 mg/kg. Magnesium aids the body in controlling blood pressure, blood sugar levels, and muscle and neuron activity. Copper stimulates genes and supports the immune and nervous systems.

According to Wagey et al., there are carbohydrates, saponins, steroids, phenolics, tannins, proteins, glycosides, and flavonoids in a leaf sample of *Canthium parvifolium* Roxb when it is dissolved in various solvents. Alkaloids were found in the ethanol and acetone extracts of the stem sample; carbohydrates and glycosides were found in all solutions tested by Benedict; steroids and saponin were found; and cardiac glycosides and flavonoids were also present. Alkaloids were absent in the water extract, acetone, and methanol used in the current investigation. In contrast to the Wagey study, this outcome is different in this investigation. Carbohydrates, steroids, amino acids, phenol, flavonoids, glycosides, and steroids are all present in all three solvents. Terpanoids are only present in one solvent. In addition to providing food for both plants and animals, minerals also play other significant roles in the environment. According to research by Muhammad Shaibu Auwal et al.8, Inorganic chemical elements play a crucial structural role in cellular processes and have been found to be necessary for nutrition. For plant parts, phytochemical research is crucial. In comparison to *Momordica charantia* (13.08 + 0.05%), *Gossypium hirsutum* (15.04 + 0.01%), and *Gossypium hirsutum* (18.72 + 0.05%), the moisture content is higher at 55.65%. The total ash value is also lower, at 6.13%. (M.J. Ayeni et al., 2015) Comparing the total ash value to *Alteranthera sessilis*, it is 6.13% higher. 8.5 1.67, *Alternanthera sessilis*' acid insoluble value of 5.18% is higher than 3.2 0.78, and the soluble ash value of 4.23 is higher than 1.8 \pm 1.25.

In Canthium Parvifolium Roxb. plants, proximate, phytochemical, and mineral analyses have been reported. Some phytochemical components, including flavonoids, amino acids, steroids, glycosides, terpenoids, and carbohydrates, have been found to be effective in proving the medicinal use of this plant. Carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites are all natural antioxidants found in plant components. Peroxide decomposers, enzyme inhibitors, and synergists are examples of such antioxidants. The ash content is measured by the amount of minerals present overall in the plant. Because these minerals function as inorganic co-factors in metabolic processes, there wouldn't be anyPossibly a compromised metabolism. (Franklin Uangboje Ohikhena et al., 2017). Which serve as an antioxidant's second line of defense. The data from the preliminary phytochemical screening will be helpful in determining the drug's inventiveness. Ash values are a dependable tool for spotting adulteration. Overall, these phytochemicals and antioxidants provide Canthium parvifolium with great medical value. This study suggests that Canthium parvifolium Roxb's nutrients and phytochemicals may act as a food source and supplement, providing a range of nutritional advantages. Additionally, it offers proof of the shrub's medicinal properties, which makes it a potential alternative treatment for several illnesses. The study of plants has made significant contributions to medicine.

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