

# Phytochemical, Pharmacognostic and Elemental Analysis of *Cayratia gracilis* (Guill. & Perr.) Suesseng

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## ABSTRACT

The plant *Cayratia gracilis* (Guill. & Perr.) Sues., family Vitaceae, is renowned for its numerous medicinal application in folks medicine especially for its analgesic properties. Phytochemical, physicochemical, microscopic and elemental analyses were carried out on the plant to establish basic monograph information for authentication of the plant samples. The moisture content was 12.7%, water extractive value was 19%, and alcohol extractive value was 3.9% in the leaves and the moisture content of 8.8%, water extractive value of 14.8% and alcohol extractive value of 1.3% in the stem. Phytochemical screening revealed the presence of 8 secondary metabolites for the leaves and 6 for the stem. The thin-layer chromatographic (TLC) profile of the stem revealed 4, 3, and 2 spots for the hexane, ethyl acetate and methanol extracts respectively, while the leaf revealed 5, 2 and 4 clear spots for hexane, ethyl acetate and methanol extracts respectively. Atomic Absorption Spectroscopy (AAS) of the plant shows the presence Zn, Cu, Mn, Mg, Fe, Na, and absence of metals like Pb and Cr. The study reveals microscopic characters that are useful as diagnostic parameters for *Cayratia gracilis*. Information obtained from this study is important in establishing diagnostic indices for identification, standardization, and also in monograph development of the plant which has ethno-medicinal uses.

## INTRODUCTION

*Cayratia gracilis* (Guill. & Perr.) Suesseng belongs to the family Vitaceae, It is synonymous to *Cissus gracilis* Guill. & Perr. and is locally called 'Teydebedik' in Senegal, 'Basari' in Guinea, 'Kissipendekelo' in Sierra Leone, 'Dagbaniwowo' in Ghana, and 'Anaya' or 'Yaakuwarfataakke' in Hausa language of Nigeria (Burkill, 2000). It can be found in shady and damp sites of the savannah and forest region, it can be found in most African countries like Senegal, Guinea, Sierra Leone, Ghana, Nigeria, some regions of West Africa and Tropical Africa (Burkill, 2000). The Genus *Cayratia* comprises of about 50 different species and they are vastly distributed. *Cayratia gracilis* (Figure 1) is a herbaceous climber or perennial herb with opposed leaves and branched tendrils, it has slender stems of up to 7.5m with

alternating leaves of 3-5 foliate and petiole of about 4 – 6cm long. The leaflets are ovate or elliptical with oblong triangular stipules; it has a cuneate or cordate base and an acuminate apex (Descoings, 1972; Verdcourt, 1993).

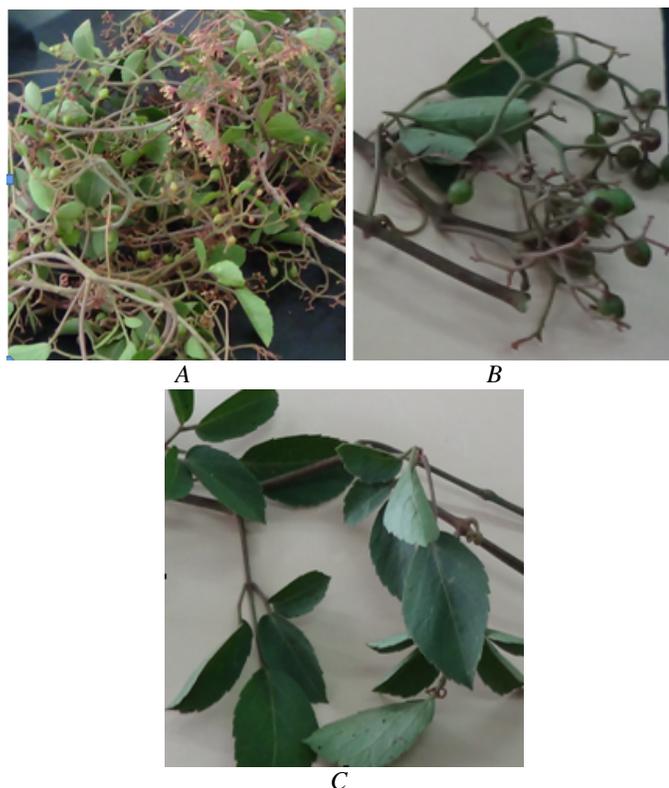
The leaves of *C. gracilis* are occasionally eaten as vegetable in Sudan but its use as vegetable is limited elsewhere. It is considered in Senegal as a famine food that needs to be cooked together with *Amorphophallus* roots (Burkill, 2000).

In Tanzania, the pulped leaves are rubbed topically on slight incisions to treat lumbago. The crushed leaves mixed with *Momordica foetida* Schum. are used to stop the irritation caused by a spitting cobra when rubbed on the affected area (Burkill, 2000). The decoction of the leaves can be used for the relief of painful menstruation in women, its infusion can be used in the relief of child birth labour, it is said to have analgesic properties and can be used as a pain relief, it can also be used to prevent scurvy (bleeding gum) (Personal communication). The fresh roots are chewed or boiled and the decoction used as a cough remedy (Ferry, 1974).

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The leaves of *C. gracilis* as well as those of several other Asiatic species of the Genus were found to contain several flavonoids including cyanidin, delphinidin, kaempferol, myricetin and quercetin (Slamet, 2001). The aerial parts of the *Cayratia gracilis* is said to contain triterpene epifriedelanol, a compound with demonstrated anti-tumour activity, whereas the stem, leaves and roots contain cyanic acid (Slamet, 2001).



**Fig. 1:** Pictures of *Cayratia gracilis* (A: whole plant; B: fruits; C: leaves)

The medicinal plant, *C. gracilis* like other plants is very sensitive and easily and variously affected by environmental and edaphic factors (Saber, 1975). Any change in any of these factors depreciates its medicinal potentials or lose its activities (OAU/STRC, 1979). Not only its age but stage of development, time of collection are of great importance as to its value as an agent of therapy (Saber, 1975). Information on the mineral content, phytochemistry and pharmacological properties of *Cayratia gracilis* is limited.

In view of its use as a vegetable and many medicinal uses, as well as results from related Asiatic species, this work is aimed at finding the phytochemical constituents, microscopy, pharmacognostic and elemental composition of the leaf and stem of *C. gracilis* found in Abuja, Nigeria.

## MATERIALS AND METHOD

### Collection of plant

The *Cayratia gracilis* plant was collected from Idu Industrial area, Abuja, Nigeria in July, 2013. The plant was identified in the herbarium of the National Institute for

Pharmaceutical Research and Development, Abuja, Nigeria by a Taxonomist.

### Plant sample processing

The fresh leaves and stem was air dried indoors for about 8-9 days. The leaves and stem was powdered using mortar and pestle, but later blended with electronic blending machine. The powdered samples were stored in air tight containers for the phytochemical analysis.

### Microscopic examination

Leaves of *Cayratia gracilis* was cut at the median portion. Some of it was soaked in concentrated nitric acid and some in sodium hypochlorite and left for about 48hours. The appearance of air bubbles showed that the epidermis was ready to be separated.

The sample was then transferred to petri dishes containing water and with the use of fine forceps and dissecting needle; the upper and lower epidermis was separated. Each sample was stained with saffranin and sudan IV and then mounted on slides in glycerol. The edges of the cover slip were sealed with nail varnish to prevent dehydration.

### Determination of Physicochemical constants

Determination of the moisture content and extractive values of the powdered leaves and stem were carried out according to standard methods (Sofowora, 2008; Evans, 2002; African pharmacopeia, 1986).

### Phytochemical analysis

The powdered leaves and stem were screened for the presence of secondary metabolite such as carbohydrate, tannins, saponins, anthraquinones, flavonoids, alkaloids, terpenes, resins, balsam, and sterols. The phyto-chemical screening were carried out following standard methods (Sofowora, 2008; Evans, 2002)

### Thin-layer chromatography

Thin layer chromatography (TLC) of the extracts was carried out to determine the number of components present in each extract. TLC plates pre-coated with K<sub>5</sub> silica gel were used. Spotting was done using capillary tubes. The hexane and ethyl acetate extracts were developed in a solvent system of hexane/ethyl acetate, ratio 9:1, while the methanol extract was developed in a solvent system of ethyl-acetate and methanol in ratio 3:2. The plates were sprayed with dilute sulphuric acid, heated in an oven for 3 min at 110 °C and observed for spots after (Evans, 1996) and the retardation factor (R<sub>f</sub>) for each spot was determined.

### Elemental Analysis

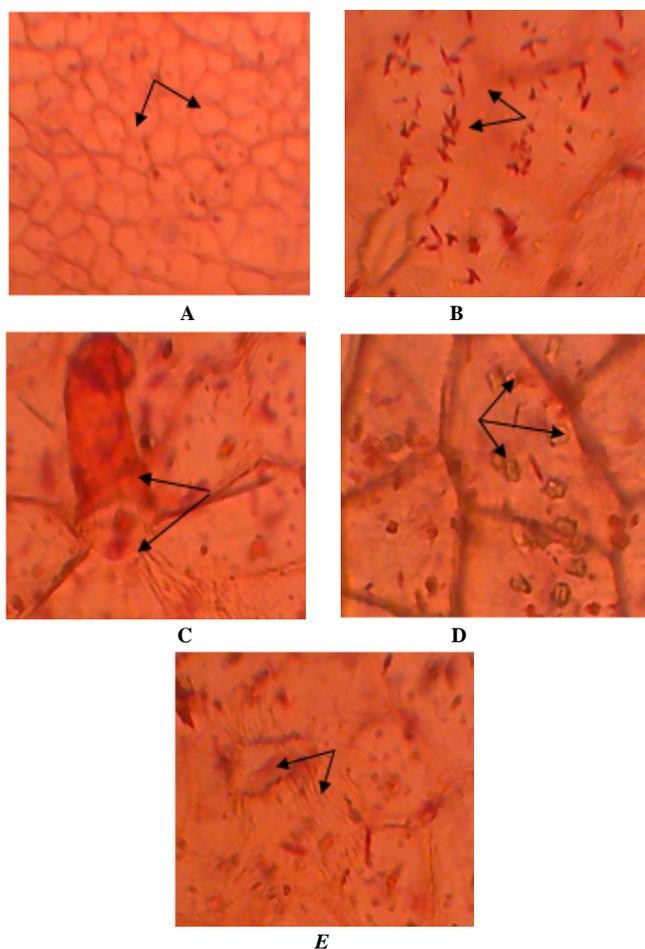
Elements such as Zinc (Zn), Copper (Cu), Manganese (Mn), Magnesium (Mg), Iron (Fe), Sodium (Na), Lead (Pb) and Chromium (Cr) were assessed qualitatively and quantitatively

using the method of Association of Official Analytical Chemist (AOAC, 1980) with the aid of Atomic Absorption Spectrometer (AAS) (GBC AVANTA Model) using hollow cathode lamp of the elements and a fuel rich flame (air-acetylene). Standards and digested samples were aspirated and the mean signal responses were recorded at each of the elements respective wavelengths.

## RESULTS AND DISCUSSION

### Microscopic examination

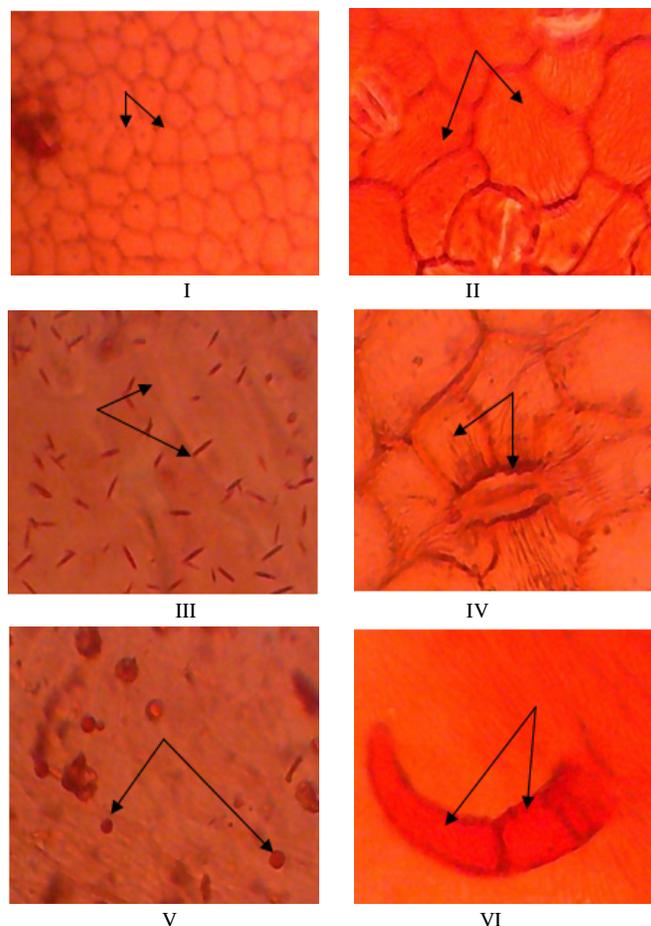
Figure 2 shows the photomicrograph of the upper epidermal layers of the leaves of *Cayratia gracilis* with the polygonal shaped cells of the upper surface, spindle shaped crystals of calcium oxalate, glandular trichomes with striated base, prism crystals of oxalate, and the anomocytic stomata.



**Fig. 2:** Photomicrographs of upper epidermal layer of *Cayratia gracilis* leaf.

**Key:** A: Polygonal shaped cells B: Spindle shaped crystals C: Glandular trichomes with striated base D: Prismatic crystals E: Anomocytic stomata with striated surrounding base

The lower epidermal layer of *Cayratia gracilis* showed polygonal shaped cells, highly striated cells obstructing the epidermal cells, scattered spindle shaped crystals, anomocytic stomata with striations, multicellular glandular trichomes which are more concentrated towards the venations (Figure 3).



**Fig. 3:** Photomicrograph of lower epidermal layer of *Cayratia gracilis* leaf.

**Key:** i: polygonal shaped cells, ii: highly striated cells obstructing the epidermal cells, iii: Scattered spindle shaped crystals, iv: anomocytic stomata with striations, v: Oil globules, vi: multicellular glandular trichomes which are more concentrated towards the venations.

Epidermal characteristics observed, such as, striations on the epidermal cells, abundance of multi-cellular trichomes, prismatic and spindle shaped crystals, anomocytic stomata cell shapes, etc., can also be used as diagnostic features for the authentication of the plant samples.

### Physicochemical parameters

Table 1 shows the results of the physicochemical parameters of the leaf and stem of *C. gracilis*. The qualitative evaluation of the leaf and stem sample showed the alcohol extractive values to be 3.9% and 1.3% respectively and the water extractive values to be 19% and 14.8% respectively.

This suggests that water is the best extractive solvent for the plant materials. The values of the moisture content of the leaf and stem (12.7% and 8.8%) were between the official ranges of 8-14.5 for vegetable drugs (African pharmacopeia, 1989). This implied that the plant does not retain much water to support microbial attack after drying and when stored under good condition. This may have good implication on the shelf-life of the well-packaged crude vegetable drug.

**Table 1:** Result of the physicochemical parameters of the leaves and stem of *C. gracilis*.

Parameter	<i>C. gracilis</i> leaves (%)	<i>C. gracilis</i> stem (%)
Moisture content	12.7	8.8
Alcohol extractive value	3.9	1.3
Water extractive value	19	14.8

### Phytochemical Screening

The result of phytochemical screening is shown in Table 2. The results revealed the presence of several metabolites in the leaf and stem of *Cayratia gracilis*. The metabolites present for the leaves were carbohydrate, tannins, saponins, flavonoids, Balsams, Resins, alkaloids and sterols while those present in the stem include carbohydrate, saponins, flavonoids, terpenes, balsams, resins and alkaloids. The usefulness of these metabolites in phytomedicines or treatment of ailment has been well documented. Tannins have astringent and anti-diarrhoea activity, and also used for treatment of sexually transmitted diseases. Saponins are used for the treatment of gastro-intestinal infections and cardiovascular diseases. The activity of flavonoids as free radical scavengers has been established and are being exploited in the management of inflammatory diseases such as tumour, and in oxidative stress related diseases (Robertson and Heber, 1956; Haslem, 1989; Evans, 2002). Therefore, the presence of these metabolites in these plants supports their uses in treatment of various ailments traditionally (Burkil, 2000).

**Table 2:** Result of the phytochemical analysis of the leaves and stem of *Cayratia gracilis*.

Secondary metabolite	Leaves	Stem
Carbohydrate	+	+
Tannins	+	-
Saponins	+	+
Flavonoids	+	+
Anthraquinones	-	-
Terpenes	-	+
Balsams	+	+
Resins	+	+
Alkaloids	+	+
Sterols	+	-

**Key:** + = Detected; - = Not detected

### Thin Layer Chromatography (TLC)

The results of TLC of the leaf and stem extracts are as shown in table 3. The hexane and ethyl acetate extracts gave 5 and 4 spots and 2 and 3 spots for the leaf and stem respectively, while the methanol extract for the leaf and stem gave 4 and 1 spots.

**Table 3:** Results of TLC analysis of the extracts of the leaf and stem of *Cayratia gracilis*.

Spots	Hexane extract (R <sub>f</sub> )		Ethyl acetate extract (R <sub>f</sub> )		Methanol Extract (R <sub>f</sub> )	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	0.05	0.06	0.76	0.63	0.09	0.96
2	0.09	0.08	0.98	0.72	0.24	
3	0.14	0.15		0.96	0.74	
4	0.21	1.00			0.96	
5	0.30					

The TLC fingerprinting is indicative of the presence of secondary metabolites on the plant and can be used to identify, standardize and differentiate between the plant species and other species of the same Genus.

### Elemental Analysis

The result of elemental analysis is shown in Table 4. Seven essential metals were screened but two of the metals, (Pb and Cr) were not detected while five (Mg, Zn, Fe, Cu, Na and Mn) were detected and quantified. Zn, Fe, Mn, Mg and Cu are essential metals which play important role in various cellular metabolic processes, including normal growth and development, including the development of the brain. Some of these metals enable the body to utilize vitamins (C, B, biotin etc.) and neutralize free radical, prevent diabetes, enhance growth of connective tissues and help in blood formations.

The dietary limits of Zn, Fe and Mn are 100µg/g, 10 – 60mg/day and 11µg/g respectively, according to WHO (1998) and Kaplan *et al.*, (1993). The deficiency of these essential metals may result in loss of sense of touch, smell (Hunt, 1994), gastrointestinal infections, nose bleeding (Hunt, 1994) birth defects and reduced fertility. All the metal analyzed were within WHO permissible limits and may not result in any health hazards to consumers since *Cayratia gracilis* is eaten as food, and it is also encouraging that the plant is Pb and Cr free.

**Table 4:** Elemental Analysis Result of leaves of *Cayratia gracilis*.

Elements	Pb	Cr	Zn	Cu	Mn	Fe	Na	Mg
Inferences	-	-	+	+	+	+	+	+
Quantity (µg/g)	0.00	0.00	26.5	3.30	44.25	37.95	77.15	326.50

Key: - means not detected, + means detected.

### CONCLUSION

*Cayratia gracilis* is rich in secondary metabolites and can be a potential source of phyto-medicines and leads for new drugs. The diagnostic characters generated from this study will be useful in identification and the results will also be useful in standardization towards quality assurance and in preparation of monograph on the plant. We recommend that further studies should be carried out to verify the claimed traditional uses of the plant.

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