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Pharmacognostic and physicochemical analysis of the leaves of *Fadogia andersonii* Robyns (Rubiaceae)

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Abstract

Fadogia andersonii has long been used in Africa in treatment of diseases including inflammation, arthritis, malaria and as aphrodisiac. The use and demand of this medicinal plants has been growing over long period of time. This study was carried out to determine some important pharmacognostic parameters of *F. andersonii* leaf. Evaluation of the fresh, powdered leaves was carried out to determine the macro-morphological, qualitative and quantitative microscopic, chemomicroscopic features, physicochemical properties and elemental analysis of the leaves. The leaves are pinnate opposite arranged, oblique lamina, glabrous appearance, elliptical in shape, entire margin with reticulate venation. The microscopy revealed straight polygonal wall of epidermal cells, paracytic stomata, unicellular trichomes, xylem, phloem, prismatic & druse crystals, and starch grains. Chemomicroscopy revealed presence of cellulose, cutin, lignin, tannins, starch, calcium oxalate crystals and gum & mucilage. The physicochemical parameters for the leaves estimated include moisture contents (13.11%), total ash value (5.67%), water soluble ash (1.33%), acid insoluble ash (2.00%), water extractive values (13.33%) and alcohol extractive values (10.67%). Elemental analysis revealed the presence of iron, copper, manganese, zinc, nickel, and lead. The pharmacognostic standard observed in this study will be of help in correct identification and quality control of *F. andersonii.*

Keywords: *Fadogia andersonii;* Standardization; Physicochemical analysis; Elemental analysis

INTRODUCTION

Standardization is a code of conduct that ensures the correct substance in correct amount for desired therapeutic effect (safety, quality and efficacy) [1,2]. It describes all measures taken during manufacturing process and quality control leading to reproducible quality of particular product [3]. Standardization confirms drug identity (authentication), determines the quality and purity [4]. In herbal medicines, plant material is used either in fragment/powdered, single or multicomponent mixture making identification difficult. Misidentified collections could lead to introduction of unsuitable or unwanted plant species in medicinal preparations. In order to avoid this problem proper identification of plant species of these powders or fragment is essential [5].

Fadogia andersonii (Rubiaceae) is an erect undershrub 1-2 ft. high, stems more or less 3-angled from a stout woody rootstock, branchlets glabrous; leaves paired or in threes, oblanceolate. Flowers are greenish-yellow while fruit yellow [6]. The plant is called by different Hausa speakers as Bita Katsira, Dan

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Goyo or Gagai depend on the uses. It is native to Benin, Burkina, Ghana, Nigeria and Togo. The plant is a popular African medicinal plant, which has long been used in Africa in treatment of diseases including amoebic dysentery, dried powdered leaf in hot infusion for the cure of inflammation, arthritis, malaria, fractures, typhoid, colicky pain, emetic. Leaves and fruit for malaria, fever. Powdered leaf mix with oil to cure wounds and boils. Whole plant is boiled to cure unknown?? sickness (Personal communication). The aqueous root extract increases sperm count, motility and epididymal weight [7].

Regardless of the medicinal applications of *F. andersonii*, there is dearth of information on the pharmacognostic and physicochemical analysis on of the leaves. Therefore, this study was carried out to determine some important pharmacognostic parameters of *F. andersonii* leaf which will assist in standardization for quality, purity and proper identification.

EXPERIMENTAL METHODS

Collection, identification and preparation of *Fadogia andersonii.* The aerial part of *F. andersonii* plant was collected in Makwai village, Zaria Local Government Area of Kaduna state, Nigeria. It was identified and authenticated in Herbarium unit, Department of Botany, faculty of Life Science, Ahmadu Bello University, Zaria with voucher specimen number ABU588. The leaves of the plant collected were washed and then air dried under shade at room temperature, ground to powder and stored in an air tight container for further use.

Macroscopic/ organoleptic examination. The general features of the fresh leaf of *F. andersonii* were studied. The size (length and width) of the lamina was measured with a ruler. The shape, composition, venation, type of the margin, apex and base of the lamina was observed and noted. Organoleptic character (odour, taste. colour and texture) of both fresh and powered leaf sample was also determined using standard methods [8].

Microscopic examination. The microscopic evaluation of the anatomical section and powdered sample of the leaves was carried out using standard methods [8,9]. The prepared sections were cleared using 70% chloral hydrate solution and boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol (%). This was then observed under the microscope and appropriate images were taken and documented. The micrometric evaluation of some of the diagnostic feature was also done.

Quantitative leaf microscopy. Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number was carried out on epidermal peelings according to Evans method and examined under microscope with aid of Camera Lucida [8].

Chemomicroscopic examination. The histochemical detection of cell walls and contents of the powdered leaves such as cellulose cell wall, lignin, starch, cutin, tannins and calcium oxalate, calcium carbonate etc. was carried out using standard method [8,9].

Physicochemical parameters. Powdered sample was subjected to physicochemical analysis; water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content was determined according to WHO [10].

Determination of elemental analysis. Five grams (5 g) of powder of *F. andersonii* leaves was taken in pre-cleaned crucibles and heated in a muffle furnace at 400 ^{o}C until there was no evolution of smoke. The crucibles were kept in a desiccator and cooled at room temperature. The ash was moistened with concentrated H_2SO_4 (1.0 mL) and heated on a heating mantle till the fumes of H_2SO_4 ceased. Sulphated ash

was dissolved in 10 mL Conc. HCl diluted to 100 mL with double distilled water or de-ionized water and filtered through Whatman filter paper. Solution was stored in a tightly capped plastic bottle and Atomic Absorption Spectrophotometry (AAS) was used to determine the different elements present [11].

RESULTS

Macroscopic/ organoleptic examination. *Fadogia andersonii* plant is an erect undershrub of 1 - 2 ft found at its natural habitat in makwai village and arrangement of leaves as shown on Plate I. The leaves are pinnate opposite arranged, oblique lamina, glabrous appearance, and elliptical in shape with entire margin. Apex of leaf is obtuse and cuneate base with reticulate venation. The size of the leaf is 10.07 x 3.83 cm, greenish in colour, rough texture and fracture with characteristics taste and weak musty odour (Table 1).

Microscopic examination. Microscopical examination of *F. andersonii* leaf revealed the presences of important diagnostic characters on both adaxial (upper) and abaxial (lower) epidermal layers, also on the transverse section of the mid rib and lamina. The epidermal cells are polygonal with anticlinal walls on both epidermal layers. The presence of paracytic or rubiaceous (irregular- celled) type of stomata with two subsidiary cells having long axes parallel to the pore of stomata on both surface but are more densely on the abaxial (lower) epidermal layers. Both epidermal layers (adaxial and abaxial) have the presences of numerous unicellular hair non-glandular trichomes, calcium oxalate crystals of both prismatic and druse type distributed along the veins and at the sponge mesophyll (Plate II A – E and Plate III A&B).

Micrometric evaluation of some of the diagnostic features. The result of micrometric determination of some diagnostic features such as; epidermal cells, stomata, trichomes, calcium oxalate crystal (prism and druse) measured at (Mag × 10) magnification viewed under microscope as shown in Table 2.

Quantitative microscopic examination. On the average, the adaxial (upper) surface has stomatal number (12.20), and stomatal index (2.96); the abaxial (lower) surface has stomatal number (29.80) and stomatal index (9.92), palisade ratio (3.27), vein termination numbers (3.60) and vein islets (5.80) were determined and recorded (Table 3).

Chemomicroscopic examination. Chemomicroscopic examination of the powdered leaf of *F. andersonii* revealed the presence of cellulose cell wall, lignin, calcium oxalate, tannins, starch, gums and mucilage and absence of calcium carbonate.

Physicochemical constant examination. The result of moisture content, total ash value, acid insoluble ash, water soluble ash, water and alcohol soluble extractive value, swelling index and foaming index of the powdered leaf of *F. andersoni* was determined and presented in Table 4 with its values.

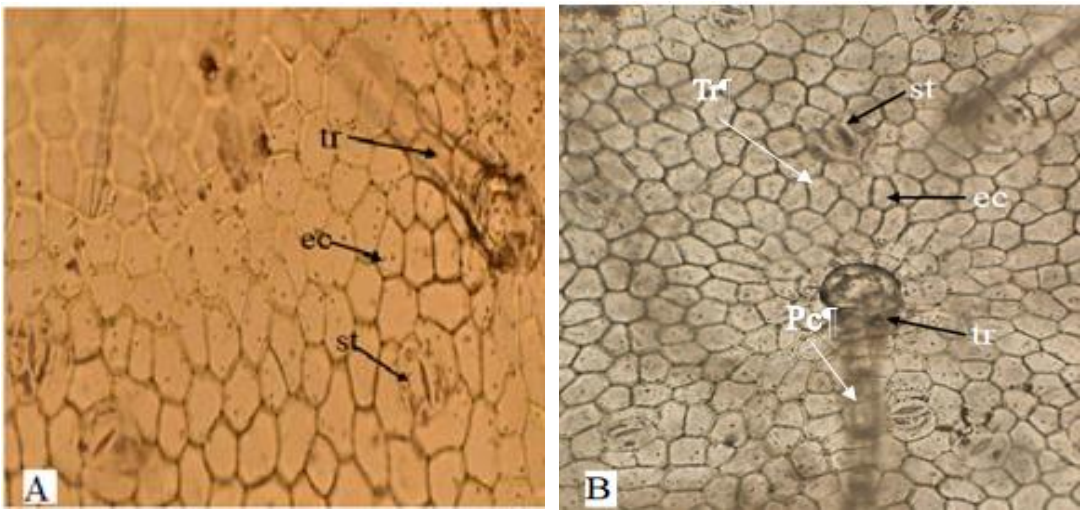
Elemental composition. The elemental analysis carried out on the powdered sample of *F. andersonii* leaf revealed the presence and concentration of copper, Iron, Zinc, Manganese, Lead and Nickel (Table 5).

Plate I: *Fadogia andersoni* **A:** in its natural habitat at Makwai village, Zaria Local Government Area of Kaduna state, Nigeria; **B:** showing arrangement of leaf and size

Table 1*:* Macroscopic/ Organoleptic Characters of Fadogia andersonii leaf

CHARACTERS	OBSERVATIONS
Surface appearance	Glabrous
Shape	Elliptical
Margin	Entire
Lamina	Oblique
Apex	Obtuse
Base	Cuneate
Venation	Reticulate
Arrangement	Opposite
Colour	Green
Size (cm)	10.07 x 3.83 ± 0.64 x 0.28*cm
Odour	Weak musty
Taste	Characteristic
Texture	Rough
Fracture	Rough

*****Average values and Standard Error of Mean of ten determinations.



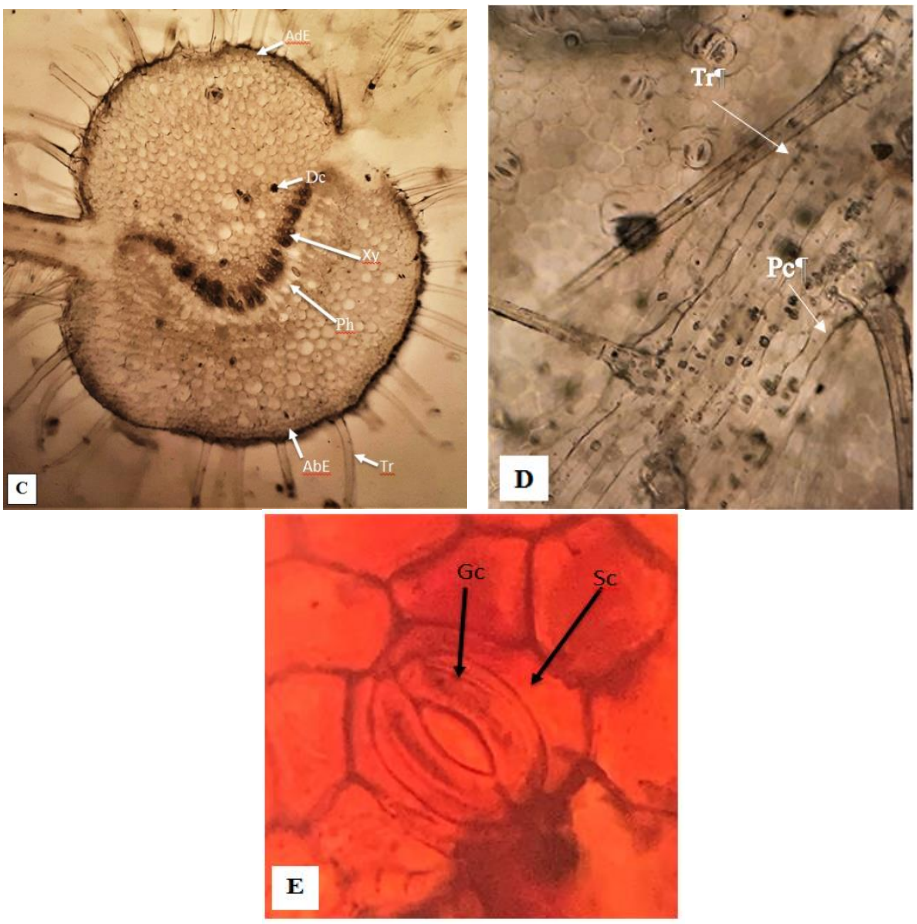


Plate II: Photomicrograph of fresh leaf of *F. andersonii* **A:** Adaxial (upper) leaf surface; **B:** Abaxial (lower) leaf surface; **C:** Transverse Section of midrib; **D:** Epidermal layer showing type of trichome and calcium oxalates crystal; **E:** Epidermal layer showing paracytic stomata stained with safranin (Mag×160). AdE: Adaxial epidermis; AbE: Abaxial epidermis; Dc: Druse crystals; Xy: Xylem; Ph: phloem; Tr: Trichome; St: Stomata; Ec: Epidermal cell; Gc: Guard cell and Sc: Subsidiary cell.

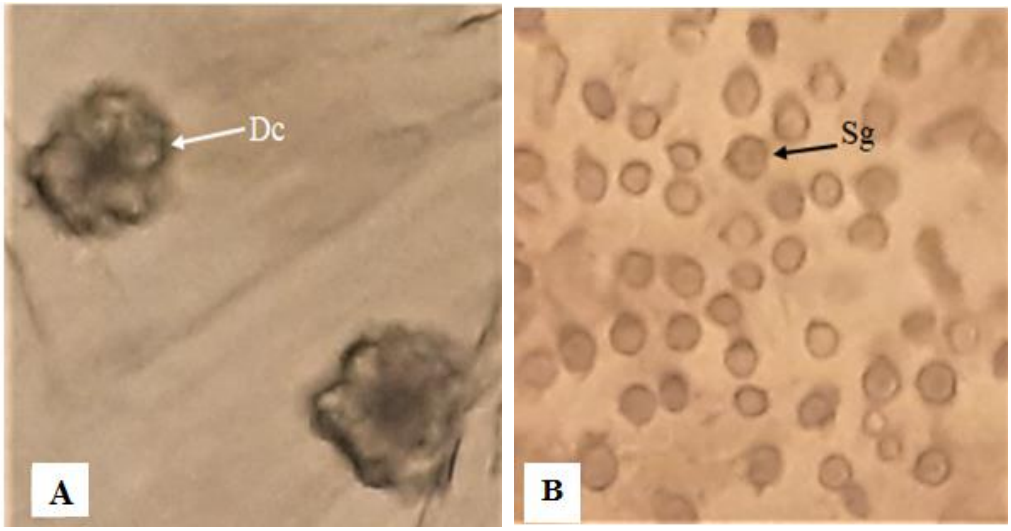


Plate III: Photomicrograph of powdered leaf of *F. andersonii* showing **A:** Druse crystals; **B:** Starch grains (Mag×160). Dc: Druse crystal and Sg: Starch grain**.**

Table 2: Micrometric Evaluation of Some of the Diagnostic Features

Diagnostic Features	Size in μm ± SEM*
Epidermal cells	14.14 ± 0.69 x 9.52 ± 0.75
Stomata	9.14 ± 1.07 x 5.17 ± 0.27
Trichomes	68.27 ± 1.74 x 8.70 ± 0.33
Calcium oxalate crystal	
Prism	3.54 ± 0.33 x 2.72 ± 0.43
Druse	5.44 ± 0.00 x 5.71 ± 0.27

*****Average values of five measurements, SEM: Standard Error of Mean

Table 3: Quantitative Microscopic Values of the Leaf of *Fadogia andersonii*

Evaluative Parameter	Values (range)
Upper surface	
Stomatal number	10.00 - 12.20 - 15.0
Stomatal index	2.44 - 2.96 - 3.61
Palisade ratio	3.00 - 3.27 - 3.75
Lower surface	
Stomatal number	24.00 - 29.80 - 38.00
Stomatal index	5.66 - 6.92 - 8.68
Veinlet termination	3.00 - 3.60 - 4.00
Vein islet number	5.00 - 5.80 - 7.00

Table 4: Physicochemical constant of the powdered leaf of *Fadogia andersonii*

Parameter	Value (%w/w) ± SEM *
Moisture contents	13.11 ± 0.19
Total Ash Value	5.67 ± 0.17
Water soluble ash	1.33 ± 0.17
Acid insoluble ash	2.00 ± 0.00
Water soluble extractive value	13.33 ± 0.89
Alcohol soluble extractive value	10.67 ± 0.33

*****Average values of five determinations. SEM: Standard Error of Means

Table 5: Elemental Composition of Powder leaf of *F. andersonii*

Elemental	Concentration (ppm) ± SEM	[12] limit* (ppm)
Copper	14.4 ± 0.0004	3.00
Iron	286.6 ± 0.0050	20.00
Zinc	21.31 ± 0.0008	27.40
Lead	26.3 ± 0.0011	0.43
Manganese	36.3 ± 0.0001	2.00
Nickel	5.0 ± 0.0004	1.63

*****For edible plants; ppm: Parts per million

DISCUSSION

Macroscopic and microscopic evaluations of crude drugs are aimed at identification of right variety and search for adulterant in plant materials [10]. Macroscopically (Plate I), the leaves of *F. andersonii* plant are pinnate opposite arranged with a size of about 10.07 x 3.83 cm averagely, glabrous appearance, oblique lamina, elliptical in shape with entire margin. Apex of leaf is obtuse and cuneate base with reticulate venation. While the organoleptic (Table 1) characters revealed greenish in colour, rough texture and fracture with characteristics taste and weak musty odour.

Microscopically, it has unicellular non-grandular trichomes (Plate IIA&B) covering both epidermal layers (adaxial and abaxial) with average size of 68.27 x 8.70 μm. The epidermal cells have straight polygonal wall

measuring average size of 14.14 x 9.52 μm. The stomata (Plate IIE) are paracytic or rubiaceous type, found on both adaxial (upper) and abaxial (lower) epidermis, dense on the abaxial (lower) epidermis with average size of 9.14 x 5.17 μm. Calcium oxalate crystals (Plate IID & IIIA) found are prismatic and druse type, measured average size of 3.54 x 2.72 μm and 5.44 x 5.71 μm. They are distributed along the veins and stored at the ground tissue. The powered leaves revealed presence and nature of starch grains (Plate IV D). Similarly this has been reported by Bruce *et al*. [13] and Raman *et al*. [14] on the leaf of *Fadogia cienkowski* and *Fadogia agretis* found to have the presence of paracytic stomata, unicellular nonglandular trichomes, calcium oxalate crystals of both prismatic and druse type, starch grains.

In transverse-section of midrib (Plate IID) showed unicellular trichomes covering adaxial (upper) and abaxial (lower) epidermal layers. The vascular system in midrib consists of a 'U'-shape single collateral vascular bundle with xylem toward the adaxial (lower) and phloem toward the abaxial (lower) side. The vascular tissue in the leaves of many Rubiaceae may be arranged in U, O or V shaped and organization of the vascular system have a higher taxonomic value in Rubiaceae [15,16]. Ground tissue consisting mostly of parenchyma cells are major portion of the midrib. Druse crystals are found on the ground tissue. These agreed with the general anatomical characters described for Rubiaceae Family that may also be used as taxonomic characteristic [14,17]. Anatomical features of the internal structures of plant drugs provides important diagnostic features for the identification of fresh and powdered crude drugs and detection of adulterants in plant materials [18].

The chemo-microscopy of the powdered leaves of *F. andersonii* revealed the presences of some cell wall materials and inclusion such as cellulose, cutin, lignins, calcium oxalate, tannins, starch and gums & mucilage except calcium carbonate that was found to be absent. These cell wall materials and inclusions are able to carry out functions of mechanical strengthening, protections, regulate growth, insulation and reinforcing vascular plants without being topple over [19]. The change in colour, structures and chemical reactions were valuable in the identification of powdered drugs as it was largely based on the forms, presence or absence of certain cell types and cell inclusions [20]. The results of macro and microscopically in this study can be used as diagnostic tool for proper identification and standardization of *F. andersonii* leaf to prevent adulteration and substitution, especially when the plant material is used in the powdered form for medicinal plant.

Physico-chemical constant serve an important role in standardization and quality control by means of purity, stability and phytochemical composition of plant drugs [21]. The results of physicochemical parameters of powdered leaf of *F. andersonii* showed the percentage moisture content determined by loss on drying method was 13.11% (Table 4) and when compared with Bruce *el al.* [13], which reported moisture content value of 4.6 %. The general requirement of moisture content in crude drug is that, it should not be greater than 14% [22] and the value observed in this research work was within the accepted range. Determination of the moisture content helps prevent degradation of drug during storage. The lower the value, the less likelihood of degradation of drug and suggests better stability of product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of it promotes mould and bacterial growth [10].

Ash values are used to determine purity and quality of crude drug. The total Ash value is an indication of inorganic residue after the plant drug is incinerated; these represents both the physiological and non-physiological ash

from the plant. The acid insoluble ash is part of total ash that is soluble in dilute acid and indicative of mainly silica, especially siliceous earth while the water-soluble ash is the water soluble portion of the total ash [23]. In this study, the total ash value determined to be 5.67 % w/w, acid insoluble ash value 2.00 % w/w and water soluble ash value 1.33 % w/w (Table 4). The acid insoluble acid value indicates that about 3.67 % w/w of the total ash will be physiologically present when the plant drug is ingested. Total ash value is a reliable aid for detecting adulteration in drugs [10]. This was compared with Bruce *et al*. [13] report that reveal a total value to be 1.4 % w/w, acid insoluble ash value 0.8 % w/w, water soluble ash value 0.4 % w/w.

Extractive values are useful to estimate the chemical constituents present in the crude drug and are a measure to determine the solubility of phytoconstituents from the crude drug in a given solvent [24]. The results of water and alcohol extractive value of powdered leaves of *F. andersonii* reveals that water has a high extractive yield (13.33 %w/w) compared to alcohol extractive (10.67 % w/w.) which implies more constituents of the plant to be soluble in water than in alcohol. Water and alcohol soluble extractive values van be used to detect exhausted and already utilized drugs to avoid substitution or adulteration [25]. The above parameters could serve as reference for identification and quality of the *F. andersonii* crude drug.

The elemental analysis carried out on the powdered sample of *F. andersonii* leaf revealed presence and concentration of Copper (286.6 ppm), Iron (36.3 ppm), Zinc (26.3 ppm), Manganese (21.31 ppm), Lead (14.4 ppm) and Nickel (5.0 ppm). The concentration of element analysed were compared with the permissible limit for edible plants established by FAO/WHO (Table 5). These trace elements are found in soil, plants and living organisms in small quantities. Some are of them are essential for the body's functions like catalysts in enzyme systems, energy metabolisms etc [26]. The plant material has iron (Fe) as the highest concentration (286.6 ppm). The level was found to be above limit when compared to permissible limit edible plants (20 ppm). Fe is part of haemoglobin and is responsible for oxygen transport, energy production, maintains healthy immune system and also an active site for several enzymes [27]. Excessive storage can lead to toxicity, cirrhosis, fibrosis and heart failure but however, this could sever as source of iron in treatment of anaemia as result of iron deficiency [28]. Manganese (Mn) a very essential trace element used for reproduction and normal ducting of the central nervous system [29]. The level of concentration (36.3 ppm) was found to be above permissible limit of edible plant (2.00 pm). Lead (Pb) is one of the non-essential trace elements found, having functions neither in human's body nor in plants but exposure might cause an adverse effect on the body [30]. However, lead (26.3 ppm) was found to be above permissible limit of edible plants (0.4 ppm). Zinc (Zn) is an essential component of many enzymes' participating in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrient [27]. it has a level of concentration (21.31 ppm) which was found below permissible limit of edible plant (27.40 ppm). It was reported that Zinc in *Microdermis keayan* plant is required for the production of the male sexual hormone (testerone) and to prevent male infertility [31]. Copper (Cu) functions in myelin formation, erythropoietin's, modulation of catecholamine metabolism, as antioxidant and regulation of immune functions [26]. Nickel is available in nature in abundance. Research studies show that every edible food item found contains amount of nickel up to some extent [32]. Although it may be beneficial as it can activate some enzyme systems [33]. There are number of trace elements that plays an important metabolic role to be used medicinally for their

therapeutic effect [34]. The plant material contained an obvious amount of Fe which could use as source of Fe supplement.

Conclusion. The present study has revealed the presence of some pharmacognostic standardization and elemental composition of *F. andersonii* leaves which will help in setting a suitable plant profile for the proper identification, quality control and compilation of a suitable monograph on the plant.

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