

Full Length Research Paper

Pharmacognostic, physicochemical and phytochemical evaluation of the leaves of *Fadogia cienkowski Schweinf* (Rubiaceae)

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***Fadogia cienkowski* is a shrub used in folklore medicine. Evaluations of the leaves were carried out to determine the macroscopic, microscopic, chemomicroscopic, physicochemical and phytochemical profiles using standard methods. The macroscopic examination revealed fresh leaves are green, odourless with a bitter taste. The leaf is oblong-elliptic in shape and sub-acute at apex; rounded at the base with entire margin. Microscopic examination indicated the presence of calcium oxalate crystals, starch grains, xylem, phloem, trichomes, epidermal cells, collenchyma cells, paracytic stomata and reticulate vessels. Chemomicroscopic characters present are lignin, starch, cellulose, mucilage and calcium oxalate crystals. The physicochemical evaluation indicated 4.6% moisture content, 1.4% total ash value, 0.8% acid insoluble ash value, 0.4% water soluble ash value, 7.8% water soluble extractive value and 9.0% alcohol soluble extractive value. The phytochemical evaluation revealed the presence of tannins (17.6%), saponins (1%), glycosides (2.5%), alkaloids (3.3%), steroids (1.1%), terpenoids (6.6%), phenols (8.8%), flavonoids (17.7%) and the absence of hydrogen cyanide. This study is useful in pharmacognostic standardization of this plant. The parameters laid down will be useful and suitable for compilation of a monograph and help in identifying this plant in its crude form and prevent it from adulteration and ensure its therapeutic efficacy.**

Key words: *Fadogia cienkowski*, pharmacognostic, phytochemical, physicochemical, macroscopic, microscopic, chemomicroscopic.

INTRODUCTION

Medicinal plants are important in healthcare system throughout the world for their proven and effective

therapeutic properties (Helmstädter and Staiger, 2014). An estimated 80% of the world's population is relying on

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medicines that contain compounds of herbal origin (Ekor, 2013). The International Union for Conservation of Nature has suggested that approximately 50,000 to 80,000 flowering plants are used for medicinal purposes (Chen et al., 2016). Although medicinal plants have been used globally, their wider usage is limited to a few countries like Japan, India, China, Pakistan, Thailand, Iran, and some African countries (Bahmani et al., 2014; Iwu, 2014; Li, 2016; Sivasankari et al., 2014). Other countries are also encouraging the use of plant-based medicinal products in their healthcare systems. For example, Natural Health Product Regulations of Canada for the plant-based product in healthcare encourages usage of modern technology and evidence-based scientific support towards promoting medicinal plants and the associated products (Tomlinson and Akerele, 2015).

However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is the need for documentation of research work carried out on traditional medicines (Dahanukar et al., 2000). With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies (Ozarkar, 2005). These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Anonymous, 1998).

Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensure reproducible quality of herbal products which will lead to safety and efficacy of natural products (Sumitra, 2014).

Pharmacognostic standardization of crude drugs is a series of laboratory experiment which reveals and assembles a set of inherent peculiar characteristics such as constant parameter, definite, qualitative and quantitative values or specific and unique features on the basis of which similar herbal medicine claim to be the same, can be compared for the purpose of authenticity, efficacy, genuiness, purity, reproducibility and overall quality assurance. The broad use of herbal drugs in conventional medicines, standardization becomes an important measure for ensuring quality, purity and authenticity of the crude drugs. First step in this context is authentication of plant species which can be done by morphological and anatomical analysis or pharmacognostic analysis. It is one of the simplest and cheapest methods for establishing the correct

identification of the source materials (Nirmal et al., 2012; Kumar et al., 2012a).

Fadogia cienkowskii belongs to the family Rubiaceae. It is locally called 'Ogwu-agu' in Igbo and 'Ufu-ewureje' in Igede tribe of Benue State within the middle belt of Nigeria. The leaves were highly acknowledged for their wide therapeutic efficacy in the relief of headache, general body debility, inflammation, diarrhoea and other ailments especially in infants. The plant is a shrub of less than 1 m high usually found in the savannah region and found to be widely dispersed into the drier parts of tropical Africa (Emeline et al., 2012).

The central and peripherally mediated nervous effects, acute toxicity studies, effect on phenobarbitone-induced sleeping time, local anaesthetic effects, analgesic activity and muscle relaxant effects were reported by Ode et al. (2015). As there are incomplete pharmacognostic work recorded on the leaves of *F. cienkowskii* by Chukwube et al. (2018). The present study reports the detailed pharmacognostic, physicochemical and phytochemical evaluation of the leaves of *F. cienkowskii* Schweinf (Rubiaceae). These parameters will be useful in complete authentication and standardization of the crude extract, which can guarantee the quality and purity of the drug and maintain its therapeutic efficacy (Figure 1).

MATERIALS AND METHODS

Plant materials

F. cienkowskii leaves were collected in July 2018 from Enugu state, Nigeria. The plant was identified and authenticated by a taxonomist in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria. Herbarium number PCG474/A/005.

Equipments

Microscope (Finlab, Nig), Hot air oven (Genlab, UK), Electronic weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex; 10, 50, 100 and 1000 ml), measuring cylinders, hand grinding machine (Ohaus Corp, USA), syringes and needles (1, 2 and 10 ml capacity), refrigerator (Thermocol, England), cotton wool (Pyrex).

Reagents and chemicals

Concentrated sulphuric acid (Versha Chemicals, Belgaum), naphthol solution in ethanol (Molisch reagents) (Nalco Chemicals, USA), Ammonium solution (Shakti Chemicals, India), Aluminum chloride (Neel Chemicals, India), Fehling solution A and B (Alpha Chemika, India), Hager's reagent (saturated solution of picric acid) (Alpha Chemika, India), Wagner's reagent (iodine and potassium iodide) (Alpha Chemika, India).

Preparation of plant material

The leaves were dipped in water to remove dust and unwanted particle. They were air dried at room temperature for two weeks.

The dried leaves were pulverized with an analytical milling



Figure 1. Habit photograph of *F. cienkowskii*.

machine and sieved to control the particle size. Then it was stored in an airtight container for further analysis (Bruce et al., 2016).

Extraction

A quantity (600 g) of the powdered leaves was extracted using ethanol (2500 ml) with occasional stirring for 72 h by cold maceration. The mixture was sieved using porcelain cloth and filtered with a filter paper. The filtrate was dried *in vacuo* at 40°C. The extract was stored in a refrigerator for use (Onyegbule et al., 2019).

Pharmacognostic studies

Macroscopic examination

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of the leaf of plant were observed (Evans, 2002).

Microscopic examination

Microscopic studies were carried out by preparing thin sections of leaf. The thin sections were further washed with water, staining was done by clearing in chloral hydrate solution then heat fixed and allowed to cool, then mounted using glycerine. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (10x, 40x) (Khandelwal, 2008).

Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, and vein – islet number and vein let termination number were carried out on epidermal strips (Evans, 2002).

Chemomicroscopic examination

Examination of the powder for lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein were carried out using standard techniques (Evans, 2002).

Physicochemical analysis

The parameters which were studied are moisture content, ash values and extractive values (Eleazu and Eleazu, 2012; AOAC, 2005; Tatiya et al., 2012).

Phytochemical analysis

Qualitative phytochemical analysis

The plant crude extracts were tested for the presence Reducing sugar, Hydrogen cyanide, Soluble carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides using standard methods (Evans, 2002).

Quantitative phytochemical analysis

The coarse powder of the plant material were tested to determine the quantity of Reducing sugar, Hydrogen cyanide, Soluble carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides present (Edeoga and Gomina, 2000).

RESULTS

Pharmacognostic evaluation

Macroscopic characteristics of *F. cienkowskii*

Macroscopic characteristics of *F. cienkowskii* leaf are given in Table 1. The fresh leaves are green in colour, odourless with a bitter taste. The leaf is oblong-elliptic in shape and sub-acute at apex; rounded at the base with entire margin. The leaves are arranged in whorls of 3 at each node or rarely opposite. Their surface is pubescent. They measure up to 8 cm in length and 2.5 cm in breadth (Table 1).

Microscopic examination

The result of microscopic examination is presented in Figure 2. Microscopic examination indicate the presence

Table 1. Macroscopic characteristics of *F. cienkowskii*.

Feature	Observation
Shape	Oblong-elliptic
Size	8 cm in length and 2.5 cm in breath
Colour	Green
Taste	Bitter
Texture	Rough
Apex	Sub- acute
Base	Round
Margin	Entire
Surface	Pubescent

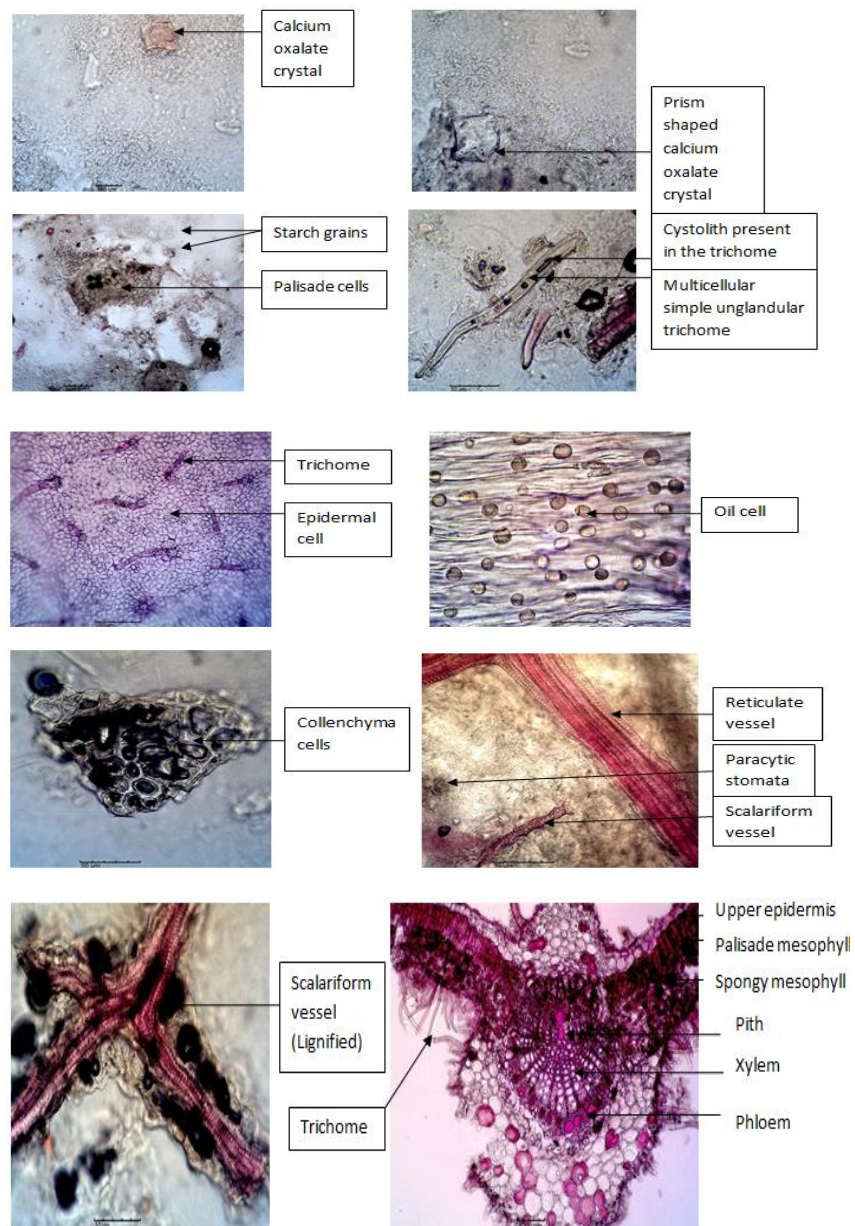


Figure 2. Transverse section of the leaf of *F. cienkowskii* X100.

of the following features: calcium oxalate crystals, starch grains, lignified tissues, cystolith, xylem phloem, scalariform vessels, pith, trichomes, spongy and palisade mesophyll, oil cells, epidermal cells, collenchyma cells, paracytic stomata and reticulate vessels (Figure 2). The diagnostic characteristics are:

(i) The upper epidermis is composed of polygonal cells with very slightly wavy walls which are irregularly thickened and beaded, in the areas over the veins the cells are more elongated; covering trichomes or cicatrices where the trichomes have been attached and some of these give a faint reaction for lignin.

(ii) Oil cells which are usually fragmented, large and spherical surrounded by moderately thick walled parenchymatous cells.

(iii) The cluster crystals of calcium oxalate which occur in a layer of cells in the spongy mesophyll immediately below the palisade.

(iv) The prisms of calcium oxalate, which are found scattered and frequently associated with the groups of fibres; they vary considerably in size and are occasionally quite large and irregularly shaped.

(v) The numerous vessels from the stem, which usually occur in small groups; they are fairly large, lignified and reticulately thickened or bordered pitted; they are frequently associated with thin-walled, lignified fibres and lignified parenchymatous cells; paracytic stomata are fairly numerous but rather faint and distinct.

(vi) The occasional fragments of collenchyma from the midrib composed of fairly large cells.

(vii) Long warty, cystolith covering trichomes are present with cellular simple unglanular trichomes.

(viii) Starch grains which are simple and frequently found massed together in groups, a small number of compound grains occur in two or three components; the underlying palisade cells are fairly large, thin walled and loosely packed.

(ix) The numerous vessels from the stem, which usually occur in small groups; they are fairly large, lignified and reticulately thickened or bordered pitted.

(x) The transverse section of the leaf showed the presence of the outermost covering tissues- the upper and the lower epidermises, which are multiseriate and lack chloroplasts. There was presence of closely packed palisade mesophyll cells with numerous chloroplasts (the main photosynthetic organ) and scattered spongy mesophyll cells that are loosely fitted to leave air spaces. The midrib bears the vascular bundle which comprises the phloem (exteriorly located) and the xylem (interiorly located)- the main conducting organs. Some mass of parenchymatous cells formed the pith at the centre (Jackson and Snowdon, 1974).

Quantitative leaf microscopy of *F. cienkowskii*

The result of quantitative leaf microscopy is presented on

Table 2. The quantitative leaf microscopy is to determine palisade ratio, stomata number, stomata index, vein-islet number and vein let termination number were carried out on epidermal strips (Table 2).

Chemomicroscopic examination of the leaves of *F. cienkowskii*

The result of chemomicroscopic examination is presented on Table 3. The chemomicroscopic examination of the leaves revealed the presence of lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein (Table 3).

Physicochemical analysis

The result of phytochemical analysis is presented on Table 4. The physicochemical analysis of *F. cienkowskii* powdered leaves reveals the parameters such as moisture content, total ash values, acid insoluble ash values, water soluble ash values, alcohol soluble extractive value and water soluble extractive values (Table 4).

Phytochemical analysis

Qualitative phytochemical analysis

The result of qualitative phytochemical analysis is presented on Table 5. The qualitative phytochemical analysis of *Fadogia cienkowskii* leaf extract reveals the presence of tannins, saponins, glycosides, reducing sugars, alkaloid, steroids, terpenoids, phenols, flavonoids and the absence of hydrogen cyanide (Table 5).

Quantitative phytochemical test

The result of quantitative phytochemical test is presented on Table 6. The quantitative phytochemical test in *F. cienkowskii* leaf extract revealed that flavonoids (17.7%) and tannins (17.6%) as the highest phytoconstituents; while steroids and saponins are the lowest phytochemical constituents (Table 6).

DISCUSSION

Macroscopic characteristics reveal that the leaves are green in colour, odourless with a bitter taste. The leaf is oblong-elliptic in shape and sub-acute at apex; rounded at the base with entire margin. The leaves are arranged in whorls of 3 at each node or rarely opposite. There surface is pubescent. They measure up to 8 cm in length and 2.5 cm in breadth. This will aid in the physical or phenotypic identification of the plant.

Microscopic examination indicate the presence of calcium

Table 2. Quantitative leaf microscopy of *F. cienkowskii*.

Parameter	Range (mm ⁻²)
Palisade ratio	8.50-9.50
Stomata number	17.50-21.45
Stomata index	7.60-10.66
Vein islet number	4.50-5.23
Vein let termination number	3.40-4.34

Table 3. Chemomicroscopic characteristics of *F. cienkowskii*.

Test reagent	Observation	Inference
Sample+Phloroglucinol + Conc. HCl	Red colour observed	Lignin present
Sample+Iodine	Blue colour observed	Starch present
Sample+ Ruthenium red	Red or dark pink colour observed	Mucilage present
Sample+Hydrochloric acid	Bright crystals dissolved	Calcium oxalates
Crystal present		
Sample+ Chlor-Zinc Iodine or N/50 iodine + 66% H₂SO₄	Blue colour observed	Cellulose present
Sample+ Sudan IV reagent	Pink colour observed	Fatty oils Present
Sample+ 1% Picric acid and Million's reagent	Red colour observed	Protein present

Table 4. Physicochemical analysis of *F. cienkowskii* leaves.

Parameter	% composition
Moisture content value	4.6
Total ash value	1.4
Acid insoluble ash value	0.8
Water soluble ash value	0.4
Alcohol soluble extractive value	9.0
Water soluble extractive value	7.8

vessels, when compared with Chukwube et al. (2018), which reported the presence of calcium oxalate crystals of various configurations, starches of various oxalate crystals, starch grains, lignified tissues, cystolith, xylem phloem, scalariform vessels, pith, trichomes, spongy and palisade mesophyll, oil cells, epidermal cells, collenchyma cells, paracytic stomata and reticulate shapes, trichomes, stomata of various types and their quantitative values and of course the vessels and fibers that confer rigidity to the plant tissues.

The quantitative leaf microscopy contains palisade ratio (8.50-9.50 mm⁻²), stomata number (17.50-21.45 mm⁻²), stomata index (7.60-10.66 mm⁻²), vein - islet number (4.50-5.23 mm⁻²) and vein let termination number (3.40-4.34 mm⁻²) on epidermal strips. The chemomicroscopic examination of the leaves revealed the presence of lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein. Aberé et al. (2007) reported the presence of lignin, starch, mucilage, calcium oxalate crystals and

cellulose on the chemomicroscopic examination of the leaves of *Mitracarpus scaber* Zucc (Rubiaceae).

Pharmacognostic and physicochemical studies of whole plant act as a reliable tool for plant identification and detecting adulteration (Desai and Chanda, 2014; Zhao et al., 2011; Raj and Radhamany, 2012). Studies of macroscopic and microscopic study can be valuable source of information which is usually and helpful in evaluation of purity and quality of a crude drugs. The pharmacognostic evaluation indicates that *F. cienkowskii* leaves contains the moisture content value (4.6%) as compared with Chukwube et al. (2018), which reported the moisture content value (2.33%). Therefore the moisture content of the plant is not too high (falls within the limit of the general requirement of 8-14%), indicating less probability of microbial degradation. Excess moisture in crude drug may lead to the breakdown of important constituent and the growth of microorganisms especially during storage of drug (Adesina et al., 2008).

Table 5. Qualitative Phytochemical Analysis of *F. cienkowskii* leaf extract.

Phytochemical	Crude extract
Saponins	+
Tannins	++++
Flavonoids	++++
Steroids	+
Terpenoids	+++
Alkaloids	++
Phenol	+++
Hydrogen cyanide	-
Reducing sugars	++
Glycosides	++

(+) = Present in small concentration, (++) = Present in moderately high concentration, (+++) = Present in high concentration, (++++) = Abundantly Present, (-) = Not Present.

Table 6. Quantitative phytochemical test of *F. cienkowskii* leaf extract.

Phytochemical	Crude extract
Saponins	1.0
Tannins	17.6
Flavonoids	17.7
Steroids	1.1
Terpenoids	6.6
Alkaloids	3.3
Phenol	8.8
Reducing sugars	4.6
Glycosides	2.5

Total ash value is (1.4%) as compared with Chukwube et al. (2018), which reveals the total ash value (3.85%), which can also be used to detect foreign organic matter and adulteration of sand or earth (Kunle et al., 2002). Acid insoluble ash value is (0.8%) as compared with Chukwube et al. (2018), which reported the acid insoluble value of (1.0%), and also compared to that of *Atropa belladonna* L. leaves which is not more than 4% (British Pharmacopoeia, 2011), water soluble ash value is (0.4%), as compared with Chukwube et al. (2018), reveals the water soluble ash value of (0.50%). The water soluble ash is used to estimate the amount of inorganic compound present in drugs (Tatiya et al., 2012).

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Ozarkar, 2005). Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions

of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya et al. 2012).

Water soluble extractive value of *F. cienkowskii* leaves is 7.8%, as compared with Chukwube et al. (2018), which reported the water soluble extractive value of (3.40%), and also compared to that of *Azadirachta indica* A. Juss. leaves which is less than 20% (British Pharmacopoeia, 2011). The alcohol soluble extractive value of *F. cienkowskii* leaves is 9.0%, compared with Chukwube et al. (2018), which reported the alcohol soluble extractive value of 4.40%. This suggests that the use of alcohol as an extractive solvent is a better choice for the polar metabolites present in the plant. The qualitative phytochemical analysis of *F. cienkowskii* leaf extract reveals the presence of tannins, saponins, glycosides, reducing sugars, alkaloid, steroids, terpenoids, phenols, flavonoids and the absence of hydrogen cyanide, when compared with Chukwube et al. (2018), reported that the phytochemical analysis contains high amounts of alkaloids, tannins, saponins, flavonoids and moderate

amounts of carbohydrates, glycosides, saponins, resins and terpenoids with low concentrations of proteins and steroids. Alkaloids are known to be the largest group of secondary metabolites found in plants. They are claimed to have powerful effects on humans and animals and hence can be used as analgesics (Kam and Lie, 2002). Alkaloids are found to have antimicrobial activity by inhibiting DNA topoisomerase (Bonjean and De Paw-Gillet, 1998).

The quantitative phytochemical test in *F. cienkowskii* leaf extract revealed that flavonoids (17.7%) and tannins (17.6%) are the highest phytochemical constituents. Tannins reduce the risk of coronary heart disease (Ranjith, 2010). Saponins, present in plants have been suggested as possible ant carcinogens. Flavonoids and phenols are excellent sources of natural antioxidants (Ali et al., 2008). Steroids have been reported in clinical studies as anti-inflammatory and analgesic agents and also used in the treatment of congestive heart failure (Saidu et al., 2012). Tannins are also suggested to have anticancer activities (Liq et al., 2006) and hence could be used for cancer prevention.

Conclusion

The current investigation reveals the pharmacognostic features, physicochemical and phytochemical properties of *F. cienkowskii*. These parameters could be useful in the preparation of the herbal section of proposed Nigerian Pharmacopoeia. Any crude drug which is claimed to be *F. cienkowskii* but whose characters significantly deviate from the accepted standard above would then be rejected as contaminated, adulterated or fake. The high content of poly-phenolic secondary metabolites (alkaloids and flavonoids) in *F. cienkowskii*, and its used in complementary medicine are indications that the plant is of great potential for wide range of applications in ethnomedicine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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