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Chukwube VO

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Ezugwu CO

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Odoh UE

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Inya-Agha SI

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Ugwuja CO

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Correspondence**Chukwube VO**

Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu, Nigeria

Pharmacognostic standardization of the leaf of *Fadogia cienkowskii* Shein fam. *Rubiaceae*

Chukwube VO, Ezugwu CO, Odoh UE, Inya-Agha SI and Ugwuja CO

Abstract

Background: Mother Earth has endowed mankind with numerous resources of plants and animals of terrestrial, aquatic and marine origins. A large species of these plants and animals have been screened for their phytochemical constituents, bioactivity, nutraceutical significance and use as Pharmaceutical intermediates. Plants are Pharmacognostical standardized in order to establish their profiles which can further be developed into Pharmacopoeia monographs. *Fadogia cienkowskii* is a rapidly developing perennial plant predominantly found in the savanna regions and widely used in the treatment of variety of abnormal health conditions. The recent quest for the use of medicinal plants in the treatment of diseases in man is predicated upon the incidence of adverse reaction and untoward effects often resulting from the use of orthodox medicines. Aside from safety, medicinal plants are also cost effective although they may be liable to adulteration, substitution and sophistication. Pharmacognostic standardization must be carried out to ensure identity, authenticity, purity, quality, and genuineness of the medicinal plant material. The result of such standards must be reproducible and predictable based on monographic profile of the given plant.

Methods: This involves macroscopic and microscopic evaluations as well as preliminary phytochemical analysis.

Results: The anatomical sections revealed the presence of numerous multicellular trichomes, calcium oxalate crystals, stomata, palisade cells, and xylem and phloem vessels. Quantitative microscopical analysis gave vein islet number of 5.46mm, stomata index 18.92 while phytomedicinal evaluation yielded total ash 3.85%, moisture content 3.17%, water soluble extractive 3.4% and alcohol soluble extractive 4.4%. Phytochemical screening of the methanol extract of the powdered leaf of plant revealed the presence of saponins, alkaloids, flavonoids, carbohydrates, glycosides, terpenes, tannins and resins.

Conclusion: In the light of this result, *Fadogia cienkowskii* has been observed to possess characteristics that have made its use in ethno medicine inevitable.

Keywords: *Fadogia*, standardization, pharmacognostic, monograph, profile

Introduction

Ever since the ancient times, plants have been associated with health of mankind (Soforowa 1982^[12]). The use of plants in traditional medicine practice has a long-drawn history and remains the mainstay of primary healthcare in most of the third world. A Plant becomes a medicinal plant only when its biological activity has been ethno pharmacologically reported or scientifically established. (Elujoba, 1977)^[4].

The World health organization (WHO 1997)^[13] has estimated that up to 80% of the world population rely on plants for their primary healthcare (BCGI 1995)^[2], while in Nigeria a WHO survey estimated that up to 75% of the population patronize traditional medicines.

More importantly plants have been the main source of medicine for man before the advancement of science and technology. The 21st century is the century of Biology powered and propelled by scientific knowledge and technical expertise. Three technologies namely Biotechnology, Herbal technology and information technology (Bioinformatics) are going to be the most powerful elements that are crucial for the welfare and prosperity for the people of nations. Various diseases treated with medicinal plants include; respiratory infections, diarrhea, fever, hypertension, obesity, diabetes mellitus among others. Standardization is a process of establishing certain criteria to be employed as a basis in the determination of identity, purity, and strength of a given crude drug and nature of its adulterants. *Fadogia cienkowskii* belongs to the *Rubiaceae* family. The *Rubiaceae* family is a family of about 500 genera and 6000 species` most of which are tropical trees and shrubs (Trease and Evans, 2005)^[5] p. 32-33. *Fadogia cienkowskii* is an erect underground shrub that forms stout bases. It is about 3ft (1m) high with pale undersurface leaf.

Flowers are greenish yellow in color and plant grows in the savannah. It is a perennial plant with stems up to 1m long that can become woody rootstock. The fruit is much appreciated by children and people traveling through the bush who often harvest it from the wild. The plant is sometime grown as an ornamental plant. *Fadogia* is usually found in tropical Africa. *F. cienkowskii* and *F. tetraquetra* have the largest distribution and occur from Guinea to the Transvaal Province. Ethnobotanically the plant has been used among Igede tribe of Benue state within middle belt of Nigeria to cure diseases such as general body debility, inflammation, diarrhea, fever and other ailments especially in infants. In Enugu state of Nigeria, the plant was first found in Ngwo Udi district Milken hill by Coal miners. The people of Ede Oballa in Nsukka local government District use the plant for the treatment of acute malaria and male impotency. The aqueous leaf macerate of the plant radically clears the parasite as the hitherto brownish colored urine of the patient becomes pure white after a short period of gulping the water extract. Hence the name by the Nsukka people ogwu-agu (Lions drug).



Fig. 1: *Fadogia cienkowskii* in its natural habitat

Materials and methods

Collection, identification and preparation of the plant material

Leaves of *Fadogia cienkowskii* were collected and identified from Ede – Oballa in Nsukka local district of Enugu state of Nigeria by 1600 h on May 20, 2017. The plant material was authenticated by Mr. Felix Nwafor, a taxonomist with the Department of Pharmacognosy and Environmental Medicines, University of Nigeria Nsukka Enugu state of Nigeria and deposited in the herbarium of the same department under the voucher specimen no PCG/UNN/0092.

Microscopy and Microchemical evaluations

Microscopical evaluations were carried out using standard methods.

Extraction of the plant material

The leaves of *Fadogia cienkowskii* were dried under shade and was then coarsely pulverized using hammer mill (Manesty U.K). The powder was stored in an air tight container to avoid exposure to moisture.

A 1 kg of the leaf powder was placed in a conical flask and soaked with 4 liters of methanol, corked and shaken thoroughly at intervals of 6h for 24h using a mechanical shaker. At the end of this time period the sample was filtered using what man no 1 filter paper. The filtrate was concentrated using rotatory evaporator.

Preliminary Phytochemical tests

A 500gm of the crude extract was used for preliminary phytochemical analysis. Tests were carried out for the presence of the various secondary metabolites using standards.

Phytomedicinal evaluation was carried using standard methods outlined by the Pharmacopoeia.

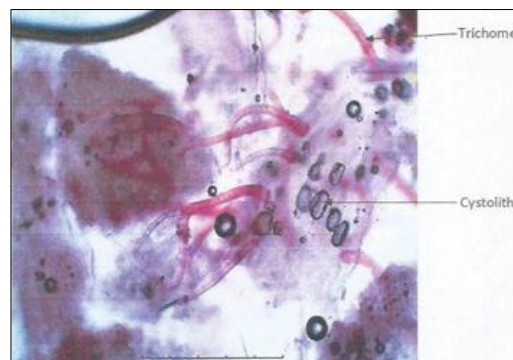


Fig 2: Photomicrograph of *Fadogia* leaf powder x 100

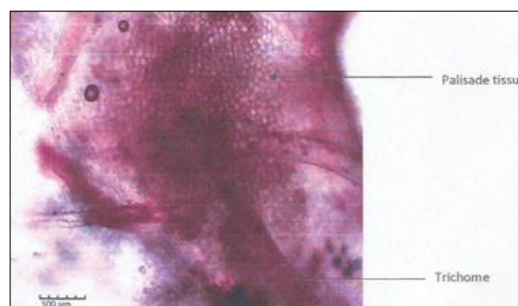


Fig 3: Photomicrograph of *Fadogia* leaf powder x400

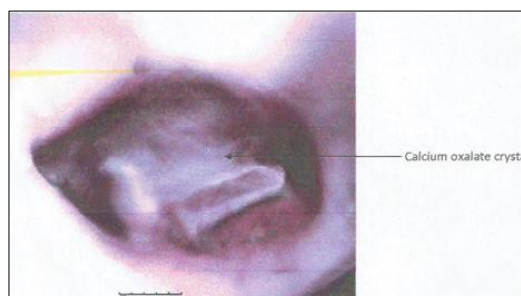


Fig 4: Powder microscopy of the leaf of *Fadogia cienkowskii* showing a crystal of calcium oxalate x400

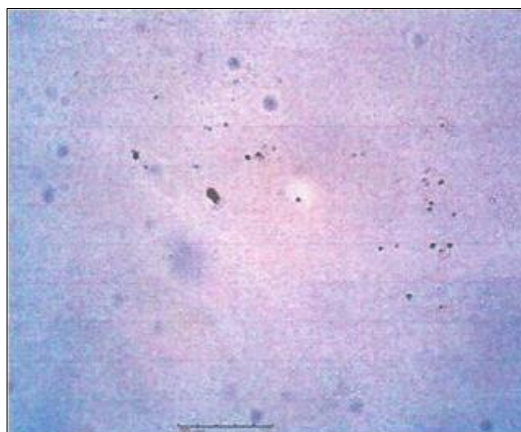


Fig 5: Powder microscopy of the leaf of *Fadogia cienkowskii* showing starch grains x400

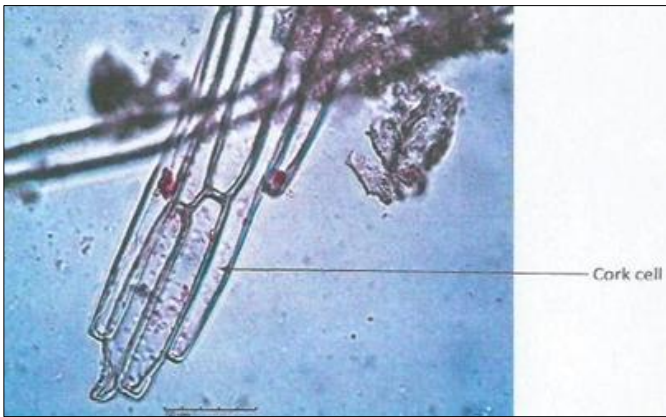


Fig 6: Powder microscopy of the leaf of *Fadogia Pienkowski* showing cork cells x400

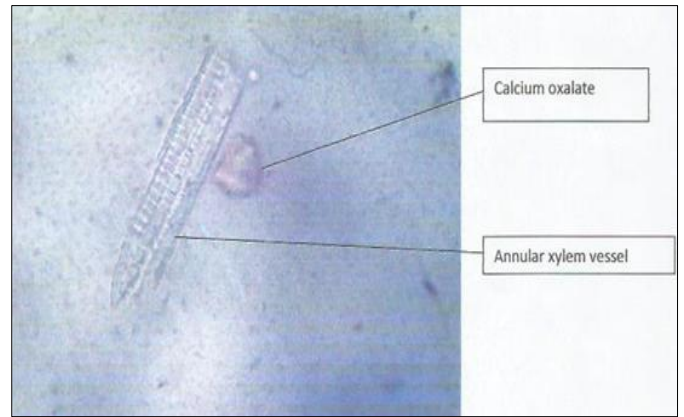


Fig 10: Annular xylem vessel with irregular shape calcium oxalate

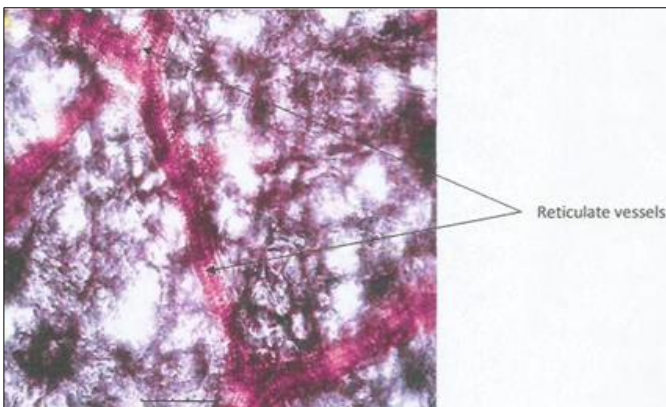


Fig 7: Photomicrograph of *Fadogia* leaf powder showing reticulate vessels.



Fig 11: Non-glandular trichome with oil cells

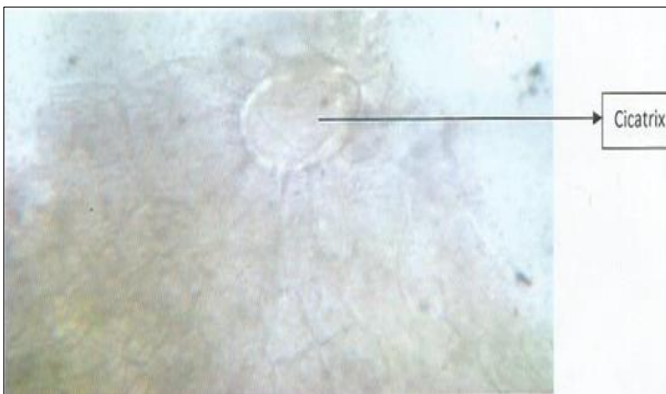


Fig 8: Photomicrograph of epidermal cell showing cicatrix

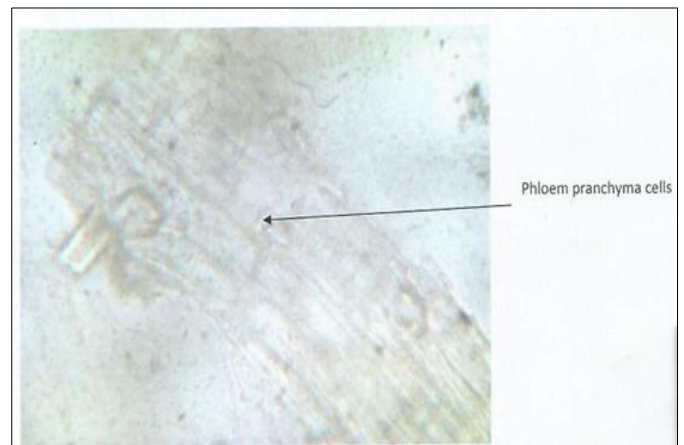


Fig 12: Phloem parenchyma cells

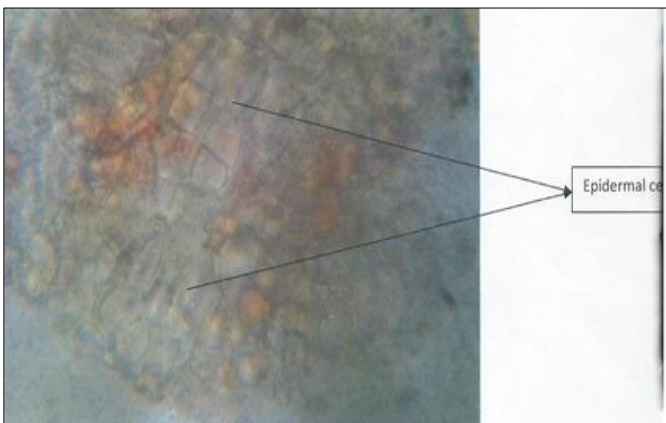


Fig 9: Epidermal cells from the midrib

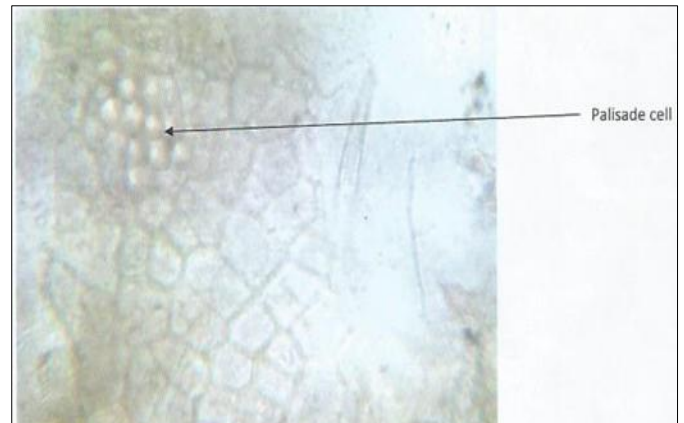


Fig 13: Upper epidermal cells with palisade cells

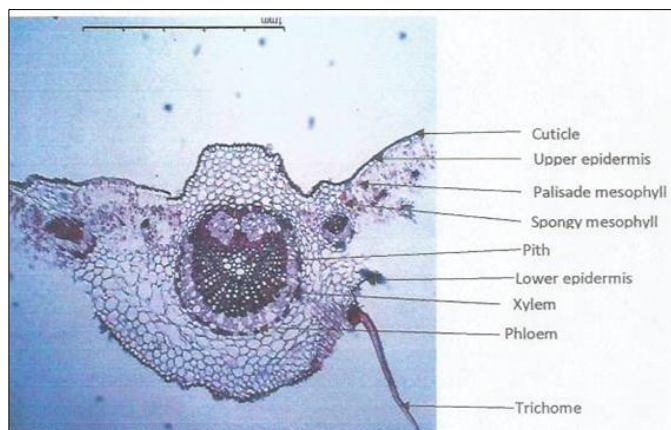


Fig 14: Transverse section of the leaf of *Fadogia cienkowskii* x 40

Results

Table 1: Preliminary phytochemical screening.

Test	Result
Carbohydrate	++
Reducing sugar	-
Alkaloids	+++
Glycosides	++
Saponins	++
Tannins	+++
Flavonoids	+++
Resins	++
Proteins	+
Oil	-
Steroids	+
Terpenoids	++
Acidic compound	-

Key

Absent

Low in abundance

Moderately abundant

Highly abundant

Table 2: Result of phytomedicinal Evaluation

Standards	Results (%)
Water soluble extractive	3.40
Alcohol soluble extractive	4.40
Total ash	3.85
Sulphated ash	3.00
Acid insoluble ash	1.00
Water soluble ash	0.50
Moisture content	2.33

Discussion

This study has revealed unique inherent qualitative and quantitative analytical features of the *Fadogia cienkowskii* leaf on the basis of which its purity and authenticity can be analyzed. The phase 1 standardization (microscopic and macroscopic properties) has provided the simplest and quickest means of identification and authentication of the plant. The diagnostic characters of a plant obtained by its microscopy is unique for that plant and therefore can be used as a parameter for the evaluation of the plant material for its proper identification. These include the presence of calcium oxalate crystals of various configurations, starches of various 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 endpoint of these evaluations is that they are used as standards for the inclusion

of the plant or its morphological part in the pharmacopoeial monograph. Phytochemical analysis showed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, terpenes, steroids, glycosides, proteins and resins using standard qualitative procedures. The microscopic anatomicals gave evidence of closer cellular relationship discrimination and differences in their intact natural arrangement. From the result of the phytochemical analysis it was discovered that the plant 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. The Biologically active components of a plant with hypoglycemic actions include flavonoids, alkaloids, glycosides, polysaccharides peptidoglycans, steroids and terpenoids (Rahman and Zaman, 1989) [8]. 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, 2002) [6]. Alkaloids are found to have antimicrobial activity by inhibiting DNA topoisomerase (Bonjean and De Pauw-Gillet, 1998) [3]. Tannins reduce the risk of coronary heart disease (Ranjthkumar *et al.*, 2010) [9]. Saponins, present in plants have been suggested as possible ant carcinogens. Flavonoids and phenols are excellent sources of natural antioxidants (Ali *et al.*, 2008) [1]. 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) [10].

The presence of tannins in the methanol extract of *Fadogia cienkowskii* is of therapeutic importance. This is because tannins have been found to form irreversible complexes with prolin-rich protein (Shimada, 2006) [11] and this results in the inhibition of cell protein synthesis. It has also been revealed that tannins react with proteins to provide tanning effect which helps in the treatment of inflamed or ulcerated tissue. Most herbs that contain tannins as major constituents are claimed to be astringent in nature and find use in the treatment of intestinal disorder like diarrhea and dysentery. These outcomes could be responsible for the use of *Fadogia* in complementary medicine. Tannins is also suggested to have anticancer activities (Liq and Wang, 2006) [7] and hence could be used for cancer prevention. The moisture content value (3.17%) is an indication of storage quality of the powdered sample. This is because it is within the recommended official range of maximum of 6%. Moisture content plays an importance role on the stability of crude drug sample as an excess content of it leads to biodegradation resulting from enzymatic actions and bacterial activities. This therefore implies that the leaf plant material of *Fadogia cienkowskii* can be stored for a long period without possible and significant level of biodegradation. The total ash value (3.85%) is moderate which also indicated that adequate care was taken during the collection and drying of the plant material. The total ash value and the acid insoluble value (3.85%) is also moderate and compares with the recommended standard value showing the amount of organic and inorganic materials in the powdered plant drug sample. The alcohol and water-soluble extractive values (4.4 % and 3.4 % respectively) suggested that the use of alcohol as an extractive solvent is a better choice for the polar metabolites present in the plant. This is novel as search in the literature did not show any previous work carried out on its standardization.

Conclusion

The plant *Fadogia cienkowskii* has been identified, evaluated

as well as standardized and may upon further studies qualify to be included in official crude drug monograph. Its high content of polyphenolic secondary metabolites namely tannins, alkaloids and flavonoids and use in complementary medicine is an indication that the plant is of great potential for wide range of applications in medicine.

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