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Pharmacognostic study and physiochemical assessment on the leaves of *Melastomastrum capitatum* fern. anticancer plant in Nigeria

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ABSTRACT

Background: This paper provides vital information about the pharmacognostic and physiochemical parameters of *Melastomastrum capitatum* leaf. The leaf has been used to treat ovarian cancer by the Fulani people in Nguroje, Taraba State, Nigeria.

Objectives: To evaluate the pharmacognostic and physiochemical parameters of *M. capitatum* leaf.

Methods: Pharmacognostic parameters such as leaf surface data, cell inclusions, and macroscopic data as well phytochemical screening of leaf extract were determined using standard procedures.

Results: Phytochemical screening of n-hexane, ethyl acetate, n-butanol, and aqueous fractions of leaf methanol extract of leaf showed the presence of alkaloids, flavonoids, gums and mucilage, phytosterols, carbohydrates, proteins, phytosterols, and saponins with no anthracene detected. The n-hexane fraction showed only tannins and fixed oils while n-butanol and aqueous fractions also showed the presence of most metabolites indicated in ethyl acetate fraction. Paracytic type of stomata was observed on the upper surface of the leaf with a covering trichome. Prismatic calcium oxalate crystals were also observed on the leaves. Moisture contents and extractive values ranged from 0.41 to 11.04.

Conclusion: Information derived from this study will help in formulating pharmacopeia standards of drug and in taxonomic identification of the plant.

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Introduction

Plant materials have been a source of remedies in the healthcare delivery system for many decades. This knowledge of the ethnomedicinal use of medicinal plants has gained wider recognition in both developed and developing nations of the world. It has been revealed that more than millions of native peoples in Africa and Asia still rely on medicinal plants for solving their health challenges. Besides, in Europe, many people are now patronizing herbal products and medicinal plants, and the consumption of herbal products and medicinal plants is estimated to have doubled [1]. In Nigeria, there are many cultural heritages of traditional medicines, which chiefly comprised the use of medicinal plants and animal parts. These crude drugs have global advantages such as easy accessibility, cost-effectiveness, and easy to use technology. The multiple therapeutic actions, histological features, and the use of most of these medicinal plants have not been sufficiently described in official books such as pharmacopeia and drug index.

Melastomastrum capitatum is a member of the Melastomataceae family. It is a dicotyledonous herbaceous plant or shrub found mostly in the swamps and tropical rainforest of Eastern and Southern Nigeria as well as some parts of Nigeria with cold climatic conditions throughout the year. It is locally called "*Belkon*" in "*Fulfulde*" language by the Fulanis in Nigeria. It is an erect or sub-erect perennial herb

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or a shrub of about 1.25 m high. In Nigeria, the plant is found mainly in swampy areas of Mambila Plateau in Taraba State, the edges of ponds in Ogurugu Enugu State, and parts of Edo State. The plant is well distributed in other African countries like Uganda, Zaire, Ghana, and Sierra Leone, where it occurs on fringes of riparian woodland, semi-evergreen rain forests, and swamps in cold climates [2–3].

The leaf is ovate in shape with an entire margin containing green and pink colors, and this very unique. A large part of the plant has sweet to sour taste. The leaves have previously been as anticancer agents [4] and anti-rheumatic agents for curing stomach aches, purification of blood vesicles and blood, for alleviating diuresis (leaf sap), and as sedatives [5]. The leaf methanol extracts showed analgesic and anti-inflammatory activities as well as antihypercholesterolemic activity in mice [6,7].

In traditional medicine, the leaf decoction is used to treat stomach ache, rheumatism, blood vessel problems, diabetes, and sedatives. The leaf is rounded with pubescence [2]. The leaf methanol extract has been reported to contain six types of fatty acids, namely, methyl tetradecanoate, 9-dodeoenoic acid methyl ester, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, methyl stearate, and methyl 18-nonadecanoate [4]. Literature on this plant is still very scant since much research has not been done in this field.

Therefore, this study was carried out in order to evaluate the pharmacognostic and physiochemical parameters of *M. capitatum* (Plate 1) leaves with a view to providing more information on the plant and for easy identification of the plant.

Plate 1. Pictorial view of *M. capitatum* plant in its natural habitat: Source: Field trip to Mambila Plateau.

Materials and Methods

Collection and identification of plant

Fresh leaves of *M. capitatum* were collected in the morning hours in Mambila Plateau Sarduana (a L.G.A) in Taraba State of Nigeria. The identification of the plant was done in the Department of Botany, Ahmadu Bello University Zaria, Nigeria, by Mr. Namadi Sunusi, where a voucher specimen number was deposited for the plant.

Preparation and extraction of plant materials

Five hundred grams (500 g) of the powdered dried leaves was weighed using an electronic scale balance into a 1,000 mL capacity separating funnel while other powders were kept for further use. The leaf powder was extracted in 1,000 mL methanol (analytical grade) by cold maceration technique, and concentrated *in vacuo* using a rotary evaporator. The final weight of the extract was noted, and the percentage yield was calculated with reference to the initial weight of leaf powder. The crude leaf methanol extract was then stored in a desiccator for further use.

Liquid to liquid partitioning of M. capitatum leaf methanol extract

M. capitatum leaf methanol extract weighing 78 g was successively partitioned in n-hexane, ethyl acetate, n-butanol, and aqueous fractions to obtain various crude extracts of n-hexane, ethyl acetate, n-butanol, and aqueous fractions in the order of their polarities.

Phytochemical screening of various extracts of M. capitatum leaf

The partitioned fractions obtained were subjected to phytochemical screening adopting the methods of by Evans [8] and Kokate [9] to determine the presence of secondary metabolites. Briefly, these tests were outlined in the following sections:

Test for saponins

Frothing test: Small quantities of the extracts were each dissolved in 10 mL of distilled water and shaken vigorously for 30 seconds and allowed to stand for about 30 minutes. The foam, which persisted for more than 30 minutes, indicates saponins.

Test for flavonoids

Sodium hydroxide test: 0.5 g of the extracts was added with two drops of aqueous NaOH solution. Yellow coloration indicated the presence of flavonoids.

Shinoda's test: This was carried out by heating the extract in 2 mL of 50% methanol and then adding metallic magnesium plus four drops of concentrated HCl solution. An orange color indicated the presence of flavonoids.

Test for tannins

Ferric chloride test: 0.5 g of the extracts were each stirred with 10 mL of distilled water and then filtered. Two drops of 5% $FeCl_3$ was then added. A green ppt. was taken for the presence of condensed tannins.

Test for alkaloids

Dragendorff's test: Few drops of *Dragendorff's reagent* were added to 0.5 g of the extracts. A rose-red precipitate was taken to indicate the presence of alkaloids.

Meyer's test: Few drops of this reagent were added to the sample extract in the test tube; cloudy or creamy precipitate indicates alkaloids.

Wagner's test: Few drops of this reagent were added to the sample extract in the test tube; whitish precipitate indicates alkaloids.

Picric acid test: Few drops of 1% picric acid solution were added to the extract; yellow-colored solution indicates alkaloids.

Test for anthraquinones

Borntrager's test (for free anthracenes): 0.5 g of the extracts was shaken with 10 mL of chloroform and filtered; 5 mL of 10% ammonia solution was added to the filtrate and stirred. The presence of pink-red color indicates the presence of free anthraquinones.

Modified Borntrager's test (for combined anthracenes): Combined anthracenes were also tested for by boiling the extracts with 5 mL of 10% HCl solution for 3 minutes, this will hydrolyze the glycosides to yield aglycones that are soluble in hot water only. The solution was filtered hot, and filtrates were allowed to cool. The filtrates were extracted with 5 mL of benzene. The benzene layer was filtered off and shaken gently with half its volume with 10% ammonia solution. The cherry-red color indicates combined anthracene.

Test for cardiac glycosides

Keller-Kiliani: A portion of the extract was dissolved in 1 mL of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube; 1 mL concentrated sulfuric acid was then added down the side of the tube to form a lower layer at the bottom. A presence of a purple-brown ring at the interphase indicates the presence of deoxy sugars, and a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides.

Test for phytosterols

Liebermann's test: To a portion of the extract, an equal volume of acetic acid anhydride was added and mixed gently; 1 mL of concentrated sulfuric acid was added down the side of the test tube to form a lower layer. Color changes were observed immediately and over a period of one hour. Blue to blue-green color in the upper layer and a reddish, pink, or purple color indicate the presence of triterpenes.

Test for carbohydrates

Molisch test: A small portion of the extract was dissolved in a test tube with distilled water. A few drops of Molisch reagent were then added followed by the addition of 1 mL concentrated sulfuric acid to the downside of the test tube to form a lower layer. A reddish ring at the interphase indicates the presence of carbohydrates.

Test for fixed oils

Spot test (paper test): 0.2 g of the leaf extract was stained on a clean white paper. A translucent grease spot indicates the presence of fixed oils. From the test, no spot was seen in other fractions except trace spots in n-hexane leaf extract.

Test for proteins

Millon's test: 0.5 g of the leaf extracts from various fractions were each dissolved in 5 mL distilled water in test tubes and shacked gently. A few drops of Millon's reagent were then added; yellow color indicates the presence of proteins, ethyl acetate, and aqueous fractions.

Chemo-microscopy of powdered leaf

Chemo-microscopic studies of the powdered leaves were done with the aim of determining the presence of cell wall contents using the reported method [9].

Determination of leaf surface parameters

Leaf surface data, such as stomata type and number, stomatal index, vein let termination, vein islet number, and palisade ratio, were determined following the methods described by Evans [8] and Kokate [9].

Physiochemical evaluation of powdered M. capitatum leaves

The methods as described by Brain and Turner [10] were used to evaluate the following physical constants and extractive values: moisture content, ash value, loss on drying, dry weight, fresh weight, acid insoluble ash value, water-soluble ash, as well as n-hexane, ethyl acetate, n-butanol, and water (aqueous) extractive values. A replicate of three experiments was carried out.

Statistical analysis

Raw data obtained from the experiment were expressed as mean ± standard error of three consecutive readings.

Results

The leaf extracts were observed in daylight and under ultraviolet (UV) light in an attempt to detect the fluorescence compounds. The rate of absorption of each portion was also investigated. From the results shown in Table 1, the ethyl acetate portion of extract has the highest with 2.443 nm absorption followed by n-butanol portion with 2.083 nm. In Table 2, more secondary metabolites were detected in ethyl acetate fraction of the extract than the rest of the extracts. Table 3 showed the leaf surface data indicating that the leaf possessed a covering trichome with paracytic stomata. Similarly, in Table 4, more stomata were observed on the upper surface (adaxial) of the leaf with 45-39 – 64 mm^2 when compared with the lower surface (abaxial). Also, the palisade ratio of the

Table 1. Fluorescence character and UV-vis absorption of *M. capitatum* leaf extract

Parameter		Extract (% w/v)		
n-hexane		**ethyl acetate**	**n-butanol**	**aqueous**
Consistency	creamy	sticky	sticky	creamy
Daylight colour	green	green	brown	reddish brown
UV-light colour	black	dark green	pink	black
UV absorption (nm)	0.307	2.443	2.083	1.136

UV wavelength was 570 nm, results of UV- vis were means of three consecutive readings.

Table 2. Qualitative phytochemical analysis of *M. capitatum* leaf extract

Test	Constituent	n-hexane	Ethyl acetate	n-butanol	Aqueous
Dragendorff's	Alkaloids	-	+ +	+	-
Wagner's	''	-	+	+	-
Picric acid	''	-	+ +	+	-
Meyer's	''	-	+ +	+	-
Shinoda's	Flavonoids	-	+ +	-	+
NaOH	''	-	+ +	-	+
FeCl3	Tannins	-	-	+ +	+ +
Borntrager's	Anthraquinones	-	-	-	+
Keller Killiani's	Cardiac glycosides	-	-	+	+ +
Liebermann's	Phytosterols	-	+ +	+	-
Frothing	Saponins	-	+	+ +	+ +
Molisch	Carbohydrates	-	+	+ +	+ +
Spot test	Fixed oils	+	-	-	-
Millon's	Proteins	-	+	-	+ +

+ + = detected in high amount, + = detected in small amount, - = not detected.

Table 3. Leaf surface data of *M. capitatum*

Features	Description
Shape	ovate
Anticlinal walls	slightly wavy
Thickenings	absent
Papillae	absent
Cuticle	striated
Type	simple
Frequency	moderate
Venation	pinnate
Texture	brittle
Surface	pubescence
Leaf colour	green-purple
Apex	acuminate
Incision	pinnatifid
Leaf base	asymmetric
Petiole	stipulate (3-6 cm long)
Margin	serrate
Taste	sweet to sour
Stomata	paracytic
Trichome	covering

Table 4. Quantitative microscopic data of *M. capitatum* fresh Leaf; mean±SE

Parameter (mm2)	Upper surface (adaxial)	Lower surface (abaxial)
Stomatal number	45- **39** - 64	24 - **29**-31
Stomatal index	8. 12 **-10** - 13.7 %	15.2 - **24** - 28 %
Vein islet number	22-**16**-32	_
Vein termination	31 **-27**- 42	_
Palisade ratio	12 **-10** -22	8 - **12** -14

Results are mean±SE, n= 3, numbers in bold are means of three counting.

upper leaf surface was the highest with 12 -10 -22%. In Table 5, the cell inclusions revealed the presence of starch with eccentric hilum and calcium oxalate crystals. Table 6 showed that the leaf powder contained only cellulose and hemicellulose, while pectin, lignin, gums, and mucilages were chemo-microscopically absent. Similarly, in Table 7, the physiochemical parameters showed that water extractive value was the highest, while n-hexane extractive value was the lowest.

Table 5. Cellular inclusions of *M. capitatum* leaf powder

Inclusions		
Feature	**Starch**	**Calcium oxalate crystal**
Shape	elliptical	single prism
Size	32-35 µm (Large)	12 -16 µm (Large)
	2-4 µm (small)	7-9 µm (Small)
Hilum	eccentric	-
Striation	present	-
Frequency	many	numerous
Aggregation	simple grains	-

- (not applicable).

Table 6. Chemo-microscopic features of powdered *M. capitatum* leaves

Cell wall material	Inference
Cellulose	+
Hemicelluloses	+
Gum/Mucilage	-
Lignin	-
Pectin	-
CaCO3	-

+ (present), - (absent).

Table 7. Physiochemical values of *M. capitatum* leaf extract

Constant	Mean ± SE (%w/w)
Water extractive	16.04±0.40
n-butanol extractive	9.10±0.21
Ethyl acetate extractive	12.22±0.31
n-hexane extractive	4.14±0.20
Total ash	4.02±0.01
Acid soluble ash	1.11±0.01
Acid insoluble ash	0.56±0.01
Water soluble ash	1.83±0.21
Loss on drying	0.41±0.01
Moisture content	5.36±0.20
Fresh weight	3.60±0.10
Dry weight	1.32±0.01

Results are mean±SE, n = 3.

Discussion

The pharmacognostic examination of crude drugs provides vital information that helps in establishing

their identity. It is a major pre-requisite that provides information leading to the identification of plant materials. This reveals why the pharmacognostic data and standards of a particular crude drug are usually established before incorporating in an herbal pharmacopeia.

Pharmacognostic studies apart from ensuring reproducibility of quality and purity of crude drugs, it is also accurate, reliable, and cheap (reference). As a statement of fact, the WHO has stressed the importance of macroscopic and microscopic examinations of medicinal plants as a fundamental measure which must be taken for proper identification and should be performed before any other test [11].

Most crude drugs exhibit fluorescence characteristics when they are exposed to colored light such as the UV light, and this is very essential in their evaluation pharmacognostically. Fluorescence study, which is a qualitative evaluation, provides reference data that could help in the identification of adulterants. In this study, various leaf extracts of *M. capitatum* showed green and brown colors under the UV light. For instance, the ethyl acetate and n-hexane extracts showed green color under the UV light, whereas they were brownish in daylight; n-butanol and aqueous extract were dark green in daylight and brownish under the UV light. This is very crucial in the identification of the crude extract from these solvents. Besides, UV-vis absorption by the extracts showed that ethyl acetate extract absorbed more light than the other extract (Table 1). This probably confirms the statement by Ukwubile et al*.* [4] that the ethyl acetate fraction of *M. capitatum* showed more biological activity against cancer cell line *in vitro*. This assertion will further give an insight into the extract of plants with more biological activity.

From the results of the phytochemical screening (Table 2), these secondary metabolites were present mostly in the ethyl acetate and aqueous extract are responsible for some of the biological activities of the plant's leaf such as anticancer, anti-tumor, analgesic, and anti-cholesterol. For instance, tannins, glycosides, and alkaloids methanol leaf extracts of *Moringa olifera* were reported to be active against some resistant strains such as Escherichia *coli*, *Pseudomonas vulgaris, klebsiela pneumonia*, and *Aspergillus niger* [12]. Similarly, saponins are a special class of glycosides with soapy characteristics. It has also been shown that saponins are active antifungal agents, while tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them [13]. In a similar scenario, alkaloids are among the major powerful poisons; some of them have been reported to be useful in correcting renal disorders and exhibit antibacterial activities no matter how small they occur in the plant [14]. The biological roles of these aforementioned metabolites were not different from those exhibited in *M. capitatum*. Ukwubile et al. [15] had shown that *M. capitatum* leaf methanol extract contains mainly different types of saturated fatty acids. Their result showed oleic acid isolated and analyzed by the gas chromatography-Mass spectroscopy when tested against various cancer cells line *in vitro* was potent on ovarian cancer cell line OV-7. This study was the first attempt made in isolating pure compounds from the leaf extract of *M. capitatum*.

Flavonoids are known as water-soluble polyphenolic molecules containing 15 carbon atoms. They are found in most plant material occurring in fruits such as tea, soybean, apple (quercetin), and citrus (rutin and hesperidin). Flavonoids had been reported to have antioxidant activity, anti-allergy, anti-cancer, anti-inflammatory, anti-viral, and anti-microbial properties [16]. For instance, quercetin had been reported to possess the ability to reduce hay fever, eczema, sinusitis, and asthma. Red wine contains high levels of flavonoids, and the high intake by the French might explain why they suffer less from coronary heart disease than other Europeans, and this may be the reason why *M. capitatum* leaf is used to correct pulmonary vein and clean blood vessels in traditional medicine, especially in Mambila Plateau, Taraba State, Nigeria. It is possible that the flavonoids in this plant play some of these functions, especially anticancer activity [16]. Studies have also shown that flavonoids prevent the oxidation of low-density lipoprotein, thereby reducing the risk for the development of atherosclerosis [17]. Therefore, these phytochemicals in the crude leaf extract are responsible for the regular use of the leaf by traditional health practitioners in treating various diseases, since each of these constituents plays various roles in the biological activities of the plant as mentioned earlier.

The nature of the trichomes in the genus seems to be more reliable than their mere presence or absence. Three basic types of trichomes had been identified among the family: uniseriate (stalked with flat plate), covering, and glandular [18]. However, in this study, the leaf epidermis of showed one covering type of trichome which is covering with uniseriate stalk (Fig. 1a), and it is possible that the plant responds to its environment in specific

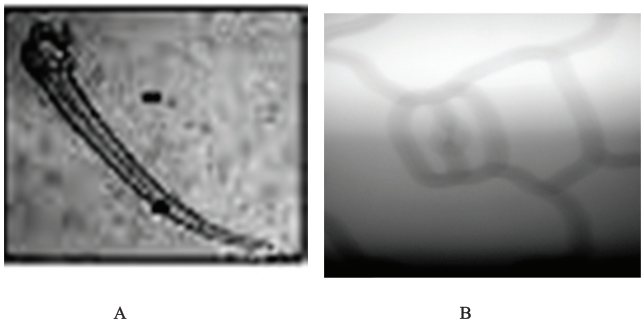
ways by modifying the basic plan of certain features to improve its adaptation (Table 3). The number of paracytic stomata (Fig. 1b) on the upper surface was more than the lower surface probably because the plant tends to avoid drought during the wet season as such more transpiration from leaves is required on the upper surface. Also, though the plant is situated in wet areas, the water may not be available in extreme dried conditions. This is an adaptation to reduce water loss from the stomata with a reduced number on the lower surface (Table 4).

Chemo-microscopic analysis of powdered leaves showed the absence of fats and oils. This was simply due to the natural habitat of the plant which is wet areas. Starch in powdered plant materials is an important diagnostic tool in the identification of the plant. In this study, the leaf contains spherical starch granules with hilum at eccentric position and striations (Tables 5 and 6). The position and form of hilum and the presence or absence of well-defined striations are important in the characterization of starches. This attribute will help to differentiate the plant with related features.

It has been reported that excess moisture in drugs not only suggests that the buyer could be paying a high price for unwanted water but also that the drug has been prepared incorrectly or subsequent to preparation, has been wrongly stored. This can also lead to the breakdown of important constituents due to enzyme activity and the other microbial attacks. The moisture content of the drug was recorded as 5.36% (less than 14%) which is low and safe for bacteria, fungi, or yeast attack [19]. From the study, the moisture value obtained is within an acceptable range.

The extractive value determines the nature of the chemical constituents of a drug. In this study, the aqueous extractive values were greater than alcohol extractive values (Table 7). This is due to the fact that water extracts more constituents than alcohol but alcohol had been reported to extract most of the plants' active constituents [20]. The fact that the water extractive value was greater than the water extractive value suggests the presence of highly polar constituents in the plant more than non-polar and semi-polar ones in the leaf extract. This was in agreement with the study of Ukwubile et al. [15] when it was reported that *M. capitatum* leaf extract possessed mainly polar compounds.

This study has set a standard that could serve as diagnostic tools in identifying the authentic drug of this plant as well as in the development of a monograph for the leaf of this plant.



A B

Figure 1. Epidermal characters from *M. capitatum* leaf; A(trichome), B(paracytic stomata).

Conclusions

From this study, phytochemical, macroscopical, microscopical, and physicochemical parameters of fresh and powdered leaves of *M. capitatum* plant will greatly help in the identification and authentication of this plant from closely related species. This study was the first attempt being made to characterize the leaf interests, and it will be very helpful in providing more literatures on the plant. Thus, the parameters obtained from this study can serve as a reference standard for the plant with a view to assisting in the preparation of the monograph.

Conflict of interest

We have no conflicting interest.

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