



Pharmacognostic Evaluation of the Leaves of *Mitracarpus Scaber* Zucc

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Abstract

Mitracarpus scaber is a small herbaceous plant found ubiquitously in the Sahel Savannah region with great utility by the locals for its therapeutic activity. Earlier studies have reported its inhibitory actions as antibacterial and antifungal agents. On these bases the plant was evaluated firstly, to establish its pharmacognostic profile and secondly to reinvestigate its phytoconstituents, in inline with its antimicrobial records, so as to furnish information that will assist in the establishment of a monograph for the plant. Fresh powdered and anatomical sections of the leaves were examined macroscopically and microscopically under the microscope followed by physicochemical investigations using standard established methods. Phytoconstituents chemicals of the plant was determined by the wet test method on three extract fractions of pet ether, chloroform, and methanol as well as their TLC profiles. Pharmacognostic studies revealed the presence of anomocytic type of stomata, non-glandular trichoms and cork cells. Physicochemical study gave moisture content, total ash, acid-insoluble ash, and alcohol extractive and water extractive values of $8\% \pm 0.313$, $27.5\% \pm 0.550$, $19.5\% \pm 0.523$, $3.4\% \pm 0.134$ and $6.8\% \pm 2.221$, respectively. The TLC profile also revealed a consistent number of spots within an Rf value of 0.2-0.8. These findings can serve as a relevant reference in the proper identification and establishment of standards, and also the provision of additional scientific information to validate the folkloric and other therapeutic claims of *M. scaber*.

Key words: *Phytoconstituents; Anomocytic; Physicochemical; Monograph; Microscopical.*

1.0 INTRODUCTION

Focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Thus, in recent times there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment (Joy *et al.*, 2001). *Mitracapus scaber* (*M. scaber*) is a common herbaceous plant from the family Rubiaceae and abound ubiquitously in northern tropical Africa with great utility by the locals for its therapeutic activity, and is popularly known as 'madder'. The family belongs to the Gentianales order recently called Rubiales consisting of about 500 genera and 6000 species distributed all over the world with highest concentration in tropical Africa (Olorode, 1984). In Nigeria, the species is mainly found in the Sahel savannah, and rarely in tropical rain forest.

M. scaber is known to contain a variety of phytochemicals: flavonoids, tannins, glycosides, saponin, steroid anthraquinone, triterpenoids—have been detected from the plant (Abere, *et al.*, 2007), while isolated compounds include Gallic acid, 3,4, 5-trimethoxy benzoic acid, methoxyacetophenone, trimethoxy acetophenone, kaempferol, rutinoside, rutin and psoralin (Bisignano *et al.*, 2000). Pentalosin was isolated from fresh aerial parts of *M. scaber* which demonstrated a potent antifungal activity against *Candida albicans* and *Trichophyllum soudanense* (Moulis, *et al.*, 1992). Oleanolic acid (OA) and ursolic acids (UA) were isolated for the first time from the alcoholic extract of *M. scaber* possessing anti-microbial effect on *Dermatophilus congolenses*. This two tripterpenoid acids (OA and UA) were established active (MIC 15ug/ml) on this ambiguous disease causative agent of *dermatophilosis* in African animals. To quantify OA & UA in *M. scaber*, a new, simple and rapid high performance liquid chromatography (HPLC) method compatible with mass spectroscopy was utilized, which detected and validated the isolates (Gbaguidi *et al.*, 2005).

Pharmacological investigation also showed that different extract of *M. scaber* exhibited broad antibacterial and antifungal activity against standard strains of clinical isolates of *Staphylococcus aureus* and *C. albicans* responsible for common skin infection (Sanogoet *et al.*, 1996). The hepatoprotective effects of *M. scaber* decoction on carbon tetrachloride (CCl₄) induced hepatotoxicity using isolated hepatocytes has been reported (Germano *et al.*, 1999). Previous works have compared the antibacterial activity of an aqueous and alcoholic extract of *M. scaber*, *Lantana camara* and *Senna alata* against *Dermatophilus congolenses* that caused dermatophilus infection in cattle of which *M. scaber* extract were the most active (Ali *et al.*, 2003).

M. scaber is widely use in traditional medicine practice in west Africa for the treatment of headaches, toothaches, amenorrhea, dyspepsia, hepatic disease, venereal disease, and leprosy. It was claimed that the plant has both antibacterial and antifungal activity thus. In Nigeria, the juice from the crushed plant is known to be applied topically for the treatment of skin diseases such as ringworm, lice, itching, 'craw-craw' and other diseases; it can as well be applied to dress for fresh cuts, wounds and ulcers. It is also use as ingredients of fish poison by some pagan tribes (Gbile, 1984; Abere *et al.*, 2007). It can be easily identified with the following colloquial names in Nigeria: Harwatsi (Hausa), Ikuwoile (Yoruba) (Abere *et al.*, 2007), and Obuobwa (Igbo). Other species of *M. scaber* include *M. senegalensis*, *M. verticillatus*, *M. hirtus* and *M. villosus* (Flora Zambesiaca, 1989).

Inadequate scientific information that can aid in the collection of the right specie of an experimental plant can pose a great danger. There are indications that owing to the great therapeutic activity of *M. scaber* and the availability of other closely related species of same plant, it can be easily substituted for the wrong specie during collection or can even be adulterated with low grade material if the supply of crude drug is inadequate (Kare *et al.*, 2011).

This adulteration can be prevented by means of various evaluation parameters like microscopic and macroscopic study. Microscopy is an important tool for authentication of crude drugs and study of powdered drugs (Rangari, 2006). It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance (Evans, 2009). Therefore, in a related effort toward the continuation of pharmacognostic research on the

candidate plant, this paper present a re-evaluation of both the macroscopic and microscopic study of the leaves of *M. scaber* and its preliminary phytochemical study so as to establish an enriched data findings on morphological and microscopical characters of leaves and stems of the plant, which will assist in its standardization and hence, can guarantee quality, purity and identification of samples. These perspectives are in line with those of Kumar *et al.*, (2011).

2.0 MATERIALS AND METHODS

2.1 Collection

The plant *M. scaber* was collected from the wild around Bukkuyum town of Bukkuyum L.G.A. of Zamfara State Northwest Sahel Savanna region of Nigeria in August, 2012.

2.2 Identification

The plant was preliminary identified using monograph of Hausa Plant collection, and was subsequently authenticated by taxonomist Gummi A.M of botany unit, of the Biological Sciences Department of Usmanu Danfodiyo University, Sokoto. The specimens were deposited at the Herbarium of Pharmacognosy Department of Usmanu Danfodiyo University, Sokoto for feature referencing.

2.3 Plant Preparation

The leaves of *M. scaber* were air dried. The dried plant material was then size reduced and sieved to obtained fine powdered drug materials. Coarse powder (60#) of dried plant was stored for macroscopical and microscopical evaluation, physicochemical analysis and phytochemical investigations.

2.4 Plant Extraction Processes

Extraction and fractionation of crude plant powdered materials was carried out by exhaustive gradient maceration techniques; which implies a gradient form of extraction of phytoconstituents into lower, moderate and higher polar solvents. This was done firstly, by macerating 150grams of crude drug in Petroleum ether for 18hours, after which it was filtered and the marc (after drying) was further macerated in chloroform and lastly methanol in a similar fashion. Consequently, petroleum ether fraction (F1), chloroform fraction (F2) and a

methanol fraction (F3) were obtain and concentrated.

2.5 Macromorphology

The entire herb *M. scaber* and its powder were evaluated for its sensory profile by observing of its colour, odour and taste alongside some extra macroscopical characters as per standard WHO guidelines (WHO, 1998).

2.6 Microscopy/Cytomorphology

Microscopy of herb was studied by evaluating various features present in the powder as well as fresh leaf sample for the presence or absence of the following: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). These detail cytomorphological characters were observed under a microscope fitted with a digital eyepiece. Furthermore, the latter cytomorphological determinations on both fresh leaf and thin powdered drug sessions was separately done as below:

2.6.1 Examination of fresh leaf: an epidermal layer from fresh *M. scaber* leaf was peeled with the hand technique. It was cleared of chlorophyllous pigment, firstly in alcohol and finally in chloral hydrates. The cleared sample was then mounted on glass slide using glycerin as the mountant and covered with slip before viewing to observe through the microscope under low and high objective powers.

2.6.1 Examination of powdered *M. scaber*: a sample specimen of thin powdered drug session was prepared by clearing with chloral hydrate and mounting with glycerin on a glass slide covered with slip. This was then observed under low and high power of the objective for

histological features. All evaluations of microscopical features were reported according to prescribed method in literature by Kokate (1997).

2.7 Physicochemical Evaluation

The various physicochemical parameters viz. ash values, acid insoluble ash, extractive values and ‘loss on drying’ were carried out on the powdered drug. Physicochemical constants were determined based on the procedures mentioned in the WHO guidelines (WHO, 1992).

2.8 Preliminary Phytochemical Screening

The extract fractions were subject to phytochemical screening so as to identify

generally, the phytochemical constituents present in the plants by means of qualitative chemical (wet) tests as using standard methods as described in relevant literatures. These, so as to validate and authenticate earlier detected classes of secondary metabolite in the plant *M. scaber*.

2.9 Thin Layer Chromatography

TLC of the fractions (Pet ether, chloroform and ethanol extractions) was carried out on pre-coated silica plates using the ascending TLC technique with suitable solvent systems.

3.0 RESULTS

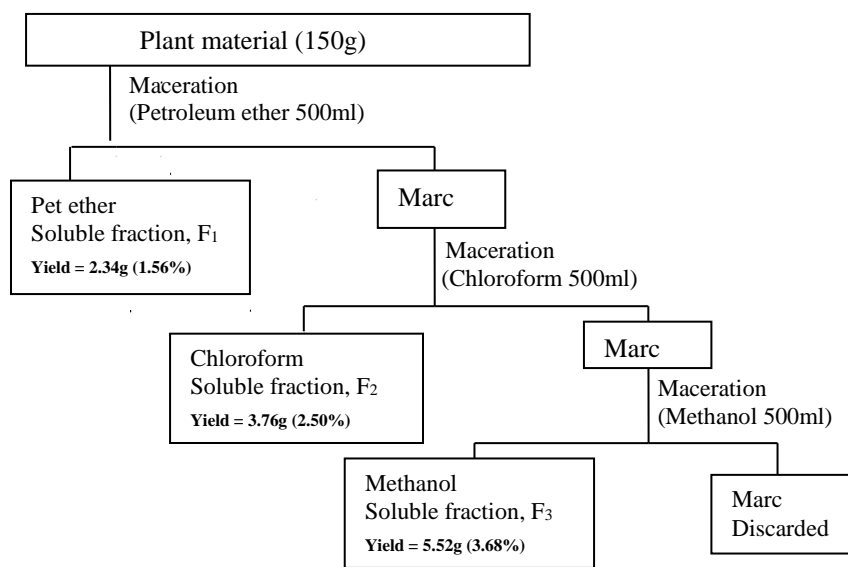


Figure1. Flow chart indicating % yield of extracts for the gradient extraction of *M. scaber* crude powdered plant drug

Table 1. Macroscopic characters of plant parts of *M. scaber*

Sr No.	Characters	Observations		
		Leaves	Stem	Roots
Organoleptic Characters				
1.	Colour	Green	Greenish brown	Light brown
2.	Odour	Characteristic	-	-
3.	Taste	Bitter	-	-

Table 2: Microscopical examination of *M. scaber*

Specimen used	Histological feature observed	Type of histological feature
Epidermal layer of leaf	Stomata	Anomocytic stomata
Thin powdered section	Stomata	Anomocytic stomata
Thin powder section	Trichomes	Non glandular hair trichomes
Thin powder section	Cork cell	Cork cells

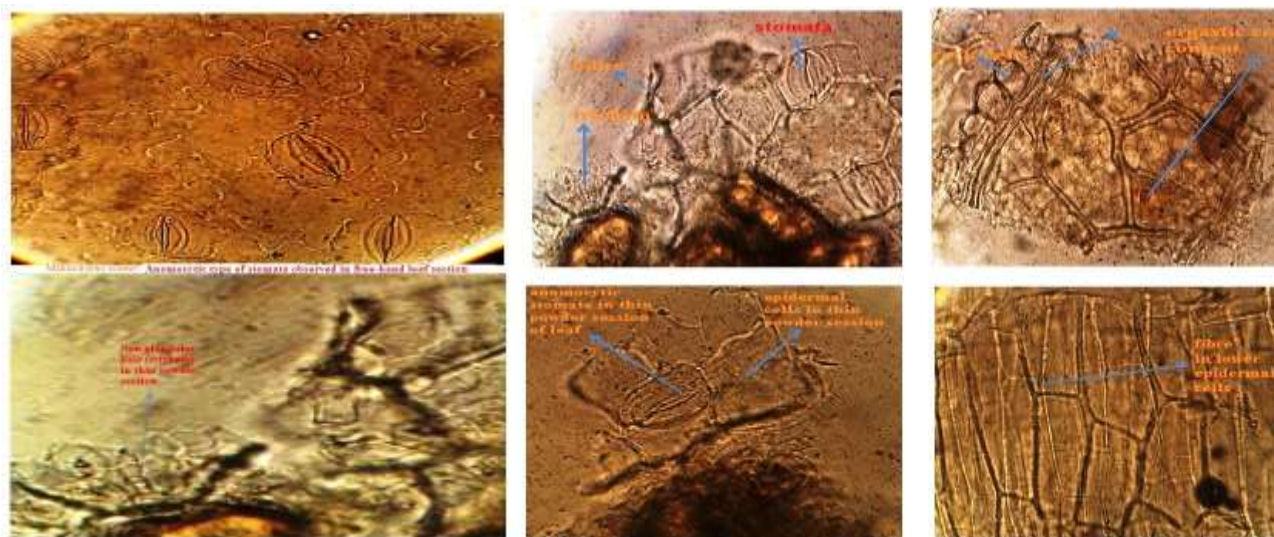


Figure 2. Microscopic observation of stomata, epidermis, fibres, trichomes, cork cells and ergastic cell contents cells (mag.400X)

Table 3: Physicochemical study of *M. scaber*

<i>Physical constants</i>	<i>Weight (w/w% with reference to *IWPD)</i>
Moisture-content	8 ±0.313
Ash value	27.5 ±0.550
Acid-insoluble ash value	19.5 ±0.523
Alcohol-soluble extractive value	3.4 ±0.134
Water-soluble extractive value	6.8 ±2.221

*IWPD=Initial weight of the powdered drug

Table 4: Preliminary phytochemical analysis of extract fractions from leave of *M. scaber*.

<i>Component Test Type</i>	<i>Result (observation)</i>			<i>Remarks</i>
	<i>F1</i>	<i>F2</i>	<i>F3</i>	
Tannins	-	-	+	(Agreed with Bisignano, 2000 & Abere, 2007)
Alkaloids	-	-	-	Not detected
Saponins	-	-	+	(Agreed with Bisignano, 2000 & Abere, 2007)
Flavonoids	-	-	+	„
Steroid & Triterpenes	-	-	+	„
Carbohydrate	-	-	+	(Agreed with Bisignano, 2000 & Abere, 2007)
Anthraquinones	+	+	+	„
Phenol	-	-	+	„
Protein	-	-	-	Not detected
Cardiac glycosides	-	-	+	(Agreed with Bisignano, 2000 & Abere, 2007)

+ = detected

- = Not detected

Table 5: TLC profile analysis of extract fraction of *M. scaber*.

	Solvent systems (ml)	Visualization technique	Component-spots (no. of spot)	R _f values obtained (cm)
1	Pet. ether: ethanol. (49:1) [Pet. ether extract, F ₁]	Coloured separation; Physically viewed	7	(0.14),(0.10),(0.22),(0.40),(0.51),(0.70),(0.9)
2	Pet. ether :chloroform (1:1) [Pet. ether extract, F ₁]	Coloured separation; physically viewed	5	(0.01),(0.15),(0.88),(0.95),(0.98),
3	Chloroform: ethanol. (49:1) [Chloroform extract, F ₂]	Coloured separation; physically viewed	8	(0.1), (0.5), (0.8), (0.8).
4	Chloroform: Pet. ether (2:3) [chloroform extract, F ₂]	Coloured separation; physically viewed	5	(0.01),(0.15),(0.88),(0.95),(0.98)
5	n-hexane: ethanol: Ethyl ac (1:3:6) [Ethanol extract, F ₃]	Sprayed with 5% H ₂ SO ₄ , heated in an oven at 105 ^o C for 5min	8	(0.11),(0.28),(0.35),(0.40)(0.55).(0.63),(0.75),(0.85)

4.0 DISCUSSIONS

Mitracarpus scaber has proved to be highly important and versatile herb employ to manage most ailments that are of antimicrobial and antifungal etiology. Reported literature has shown extensive study on the later (Moulis *et al.*, 1992; Sanogo *et al.*, 1996 and Ali *et al.*, 2003), which may serve as a validation of therapeutic claims (offering scientific rationale) to the many folkloric used of the plant herb by local and unlettered societies. The need for standardization of the drug is therefore important, as this will established an official monograph for a global application of the plant use as a drug. Standardization of a crude drug is an integral part of establishing its correct identity, made possible from results of findings of evaluations of various pharmacognostic parameters and thereafter, documenting the data as standards in the pharmacopoeias (Abere, 2007).

Consequently, macroscopical characters observed (Table 1) revealed simple green leaves that are lanceolate and opposite in arrangement with cute apex and base and an entire margin of parallel venation. Organoleptic characteristics revealed fresh leaves that are odourless with slightly acrid taste. These findings are in agreement with those of Abere *et al.*, 2007 and Abere, (2007) and hence, further strengthens and

could validate information on macroscopical features of *M. scaber*.

Figure two reveals the transverse section of the leaf passing through midrib region, which showed the presence of two, three and sometimes four epidermal cells (which are not different from each other) surrounding the stoma of an anomocytic type of arrangement. It also shows presence of uniseriate (non-glandular hair) covering trichomes, while pericyclic fibres of parenchymatous cells containing prisms of calcium oxalate (ergastic cell content) was visibly present. Similarly, microscopy of the powdered plant shows the presence of epidermal cells, unicellular trichomes and phloem fibres. These data are unique for this plant and will add up with data from earlier works on both qualitative and quantitative microscopy to qualify as a rich source of information for the establishment of reference standards that are of Pharmacopeia value.

Physicochemical parameters presented in Table 3 signifies that the quality and purity of raw material could not be rated high enough; moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Deterioration time of the crude drugs depends upon the amount of water present in formulation. If the water content is high, the

crude drugs can be easily deteriorated due to fungus (British Pharmacopoeia, 1980), hence a moisture content value of $8 \pm 0.313\%$ fairly demands that the plant material should be properly dried for proper storage. The results of Ash values signify purity of drug in terms of presence or absence of foreign matter such as metallic salt or silica present in the raw material. The total ash usually consists of carbonates, phosphates; silicates and silica which include both physiological ash and non-physiological ash while acid insoluble ash particularly indicates contamination with siliceous materials e.g., earth and sand. Comparisons of the later with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Consequently, the values $27.5 \pm 0.283\%$ for total ash and $19.5 \pm 0.523\%$ for acid insoluble ash obtained may be used as a reference. The total soluble active constituents of a crude drug in any particular solvent or mixture of solvents is determined by extractive value. The water soluble extractive value found ($6.8 \pm 2.221\%$) and that of the alcohol soluble extractive value ($3.4 \pm 0.134\%$) found signifies the nature of the phytoconstituents present in the plant *M. scaber*.

5.0 CONCLUSION

The findings here-in showed some consistency of evaluated parameters, which compare favorably well with earlier research on same plant. Findings of such may serve as an additional information in the evaluation of standards for *M. scaber*. Consequently,

6.0 REFERENCE

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The extraction method employed (Figure 1 and Table 1) showed methanol to be the best extracting solvent with respect to the amount of plant material used. This is evident from the preliminary phytochemical investigations of the extracts which showed higher number of the metabolites detected in the methanol extract fraction. However, the findings of Bisignano *et al.*, (2000) on phytochemical constituents, showed alkaloid and protein as being absent in the methanolic leave extract of *M. scaber*. On a general note, the detected phytochemicals (Table 4 and 5) as well as the Rf values of component (phytochemicals) detected using the chosen solvent systems for all the three extracts fractions (of petroleum ether, chloroform and methanol) would serve as a guide for chemical assay and isolation of pure phytomolecules of *M. scaber*. The reproducibility of the process may be of usefulness in future investigations leading to additional established data. Hence with such available and useful information, evaluation of quality control can be achieved. Consequently,

Phytopharmacopoeial specification for the plant materials can serve as an end user reference for research and applications of the candidate plant. A typical area of importance will be for the proper verification and authentication of specimens.

- phytopharmacopoeial specification when established and documented will serve as an end user reference for the proper verification and authentication of specimens as well as for research and applications of *Mitracarpus scaber* Zucc.
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