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Research Article

Pharmacognostic evaluation of the leaves of *Mitracarpus scaber* Zucc (Rubiaceae)

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Abstract

Purpose: The methanolic extract and isolated constituents of *Mitracarpus scaber* Zucc have been reported to exhibit hepatoprotective, antibacterial and antimycotic activities. Establishment of Pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification.

Method: Evaluation of the fresh, powdered and anatomical sections of the leaves were carried out to determine the macromorphological, micromorphological, chemomicroscopic, numerical and phytochemical profiles.

Results: Macro - and microscopical studies indicated presence of simple leaf whorled arrangement, an entire margin with lanceolate shape, acute apex and base, parallel venation, thin and wavy anticlinal walls with numerous calcium oxalate crystals. Stomata arrangement was anomocytic with numerous covering trichomes on both surfaces. Chemomicroscopic characters present include lignin, starch, cellulose, mucilage and calcium oxalate crystals while phytochemical evaluation revealed the presence of alkaloids, tannins, cardiac glycosides and saponins. The investigations also included numerical and quantitative leaf microscopy.

Conclusion: These findings should be suitable for inclusion in the proposed Pharmacopoeia of Nigerian Medicinal plants.

Keywords: *Mitracarpus scaber*, Pharmacognostic evaluation, Pharmacopoeia.

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INTRODUCTION

The family Rubiaceae, popularly known as Madder family belongs to the Gentianales order, recently called Rubiales order. The family, consists of about 500 genera and 6,000 species distributed all over the world. Some of them are tropical trees and shrubs (erect, struggling or twining) while few members are herbs (erect or decumbent)¹. *Mitracarpus scaber* is a perennial annual herb of about 30cm tall or much smaller and possess rough leaves². In Nigeria, it is known as Obuobwa in Igbo language, Gududal in Hausa language³ and Irawo Ile in Yoruba language⁴.

The leaf extracts of *Mitracarpus scaber* is widely used in traditional medicine practices in West Africa for the treatment of headaches, toothaches, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy⁵. It is claimed that the plant has both antibacterial and antifungal activities^{6,7}. In Senegal, the plant is used for the treatment of sore throat and also for leprosy in the same way as *Cola cordifolia*⁸ and 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 fungi diseases or applied to dressings for fresh cuts, wounds and ulcers⁹. It is also used as an ingredient in fish poison by some pagan tribes⁸.

The methanolic extract and isolated constituents of the aerial parts of *Mitracarpus scaber* were reported to exhibit both antibacterial and antimycotic activities¹⁰. The methanolic extract was subsequently fractionated and monitored by bioassay leading to the isolation of seven compounds screened for antibacterial and antimycotic activities. The crude extract also compared favourably with 0.5% Hibitane[®] at concentrations of 30 % w/v and 100 % w/v against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Trichophyton rubrum* and *Trichophyton tonsurans*, though when a 35 % w/v of the extract was incorporated into a liquid soap formulation, the antimicrobial activity was reduced¹¹. Extracts from the leaves of *Mitracarpus scaber* have been successfully

formulated into a pleasantly tasting oral dosage form despite its taste for the treatment of sore throat and other upper respiratory diseases¹². Evaluation of the effect of *Mitracarpus scaber* on carbon tetrachloride–induced acute liver damage in rat showed significant hepatoprotection both *in vivo* and *in vitro*¹³.

Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized¹⁴. The World Health Organisation (WHO) has recommended the use of arthemisinin derivatives from *Artemisia annua* (Composite), a Chinese herb with established pharmacognostic data, as a first line drug in the treatment of malaria^{15,16}. Establishment of the pharmacognostic profile of the leaves of *Mitracarpus scaber* will assist in standardization, which can guarantee quality, purity and identification of samples.

Materials and Methods

Fresh leaves of *Mitracarpus scaber* were collected in Ugbowo area of Benin City. Identification and confirmation were done by Usang Felix of Forest Research Institute of Nigeria, Ibadan where voucher specimens were deposited with the number, FIH 107154.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste^{1,17}.

Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence / absence of the following were observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed¹⁸.

Table 1: Numerical data of leaves of *Mitracarpus scaber*

Parameter	Mean (% w/w)
Moisture content	13.6500 ± 0.1519
Total ash	13.3167 ± 0.2565
Acid – insoluble ash	6.1667 ± 0.1351
Water – soluble ash	2.1667 ± 0.0630
Alcohol – soluble extractive	0.1673 ± 0.0022
Water – soluble extractive	0.3440 ± 0.0017

Table 2: Quantitative leaf microscopy of *Mitracarpus scaber*

Parameter	Range	Mean
Palisade ratio	6.00 – 8.00	6.85 ± 0.3841
Stomata number Upper surface	8.00 – 10.00	8.80 ± 0.1864
Stomata number Lower surface	5.00 – 9.00	6.95 ± 0.2348
Stomata index Upper surface	17.02 – 23.26	20.2785 ± 0.4150
Stomata index Lower surface	13.95 – 17.65	16.0275 ± 0.2617
Vein islet number	3.00 – 4.50	3.35 ± 0.2915
Veinlet termination number	2.75 – 3.00	2.85 ± 0.0612

Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques.¹

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and Modified iron complex tests), cardiac glycosides (Keller-Killiani and Kedde tests), alkaloids (Mayer's; Dragendorff's; Wagner's and 1% picric acid reagents), Saponin glycosides (frothing and haemolysis tests), anthracene derivatives (Borntrager's test for combined and free Anthraquinones) and Cyanogenetic glycosides (sodium picrate paper test)^{1, 19, 20, 21}.

Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination

number were carried out on epidermal strips. Other parameters determined for the powdered leaves were moisture content, total ash, acid – insoluble ash, water – soluble ash, alcohol (90 % ethanol) and water soluble extractive values²².

RESULTS

Macroscopically, the leaf is simple in composition, opposite in arrangement, apex and base are acute, margin is entire, venation is parallel, shape lanceolate and average leaf size is 2.7cm ± 0.9 (length) and 1.5cm ± 0.2 (breadth). Fresh leaves are green in colour, odourless with a slightly acid taste.

Micromorphological features revealed that anticlinal walls are thin and wavy and contains numerous calcium oxalate crystals. There are two, three and sometimes four epidermal cells which are not different from each other surrounding the stoma (Anomocytic arrangement). Uniseriate covering trichomes are present on both surfaces. Transverse section of the leaf across the mid – rib shows an upper epidermis consisting of straight (5 -6 sided) polygonal cells and a lower epidermis of smaller cells (It has Isobilateral leaf arrangement). The

mid – rib bundle is surrounded by a zone of pericyclic fibres. On these pericyclic fibres are parenchymatous cells which contain prisms of calcium oxalate.

Chemomicroscopic examination of the leaves revealed the presence of lignin, starch, mucilage, calcium oxalate crystals and cellulose. Phytochemical evaluation revealed the presence of alkaloids, tannins, cardiac glycosides and saponins. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the various actions of *Mitracarpus scaber*. The numerical and quantitative values are presented in Tables 1 and 2.

DISCUSSION

Mitracarpus scaber “Zucc” is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established.

Mitracarpus scaber is a plant that has been confused with other species due to their relative similarities. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The macro – and micro – morphological features of the leaf described, distinguishes it from other members of the genera. Chemomicroscopy, numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization. Phytochemical evaluation revealed the presence of tannins which have been claimed to be responsible for its antimicrobial activity¹⁰.

CONCLUSION

These parameters, which are being reported for the first time, could be useful in the preparation of the herbal section of proposed Nigerian Pharmacopoeia. Any crude drug which is claimed to be *Mitracarpus scaber* but whose characters significantly deviate from the accepted standard above would then be rejected

as either contaminated, adulterated or downright fake.

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