Research Article

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

TA Abere*1, DN Onwukaeme1 and CJ Eboka2

1Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

Abstract

Purpose: The methanolic extract and isolated constituents of Mitracarpus scaber Zucc have been reported to exhibit hepatoprotective, antibacterial and antimitcotic 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 macro-morphological, micro-morphological, 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.

*Corresponding Author: E-mail: eseabere@yahoo.com
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 leaves 2. In Nigeria, it is known as Obuobwa in Igbo language, Gududal in Hausa language 3 and Irawo ile in Yoruba language 4.

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, veneral diseases as well as leprosy 5. 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 ulcers 9. It is also used as an ingredient in fish poison by some pagan tribes 8.

The methanolic extract and isolated constituents of the aerial parts of *Mitracarpus scaber* were reported to exhibit both antibacterial and antimycotic activities 10. The methanolic extract was subsequently fractionated and monitored by bioassay leading to the isolation of seven compounds screened for antibacterial and antifungal activities. The crude extract also compared favourably with 0.5% Hibitane 8 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 reduced 11. 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 diseases 12. 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 standardized 14. The World Health Organisation (WHO) has recommended the use of artemisinin 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 observed 18.
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 acrid 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 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.

Table 1: Numerical data of leaves of Mitracarpus scaber

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>13.6500 ± 0.1519</td>
</tr>
<tr>
<td>Total ash</td>
<td>13.3167 ± 0.2565</td>
</tr>
<tr>
<td>Acid – insoluble ash</td>
<td>6.1667 ± 0.1351</td>
</tr>
<tr>
<td>Water – soluble ash</td>
<td>2.1667 ± 0.0630</td>
</tr>
<tr>
<td>Alcohol – soluble extractive</td>
<td>0.1673 ± 0.0022</td>
</tr>
<tr>
<td>Water – soluble extractive</td>
<td>0.3440 ± 0.0017</td>
</tr>
</tbody>
</table>

Table 2: Quantitative leaf microscopy of Mitracarpus scaber

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palisade ratio</td>
<td>6.00 – 8.00</td>
<td>6.85 ± 0.3841</td>
</tr>
<tr>
<td>Stomata number Upper surface</td>
<td>8.00 – 10.00</td>
<td>8.80 ± 0.1864</td>
</tr>
<tr>
<td>Stomata number Lower surface</td>
<td>5.00 – 9.00</td>
<td>6.95 ± 0.2348</td>
</tr>
<tr>
<td>Stomata index Upper surface</td>
<td>17.02 – 23.26</td>
<td>20.275 ± 0.4150</td>
</tr>
<tr>
<td>Stomata index Lower surface</td>
<td>13.95 – 17.65</td>
<td>16.025 ± 0.2617</td>
</tr>
<tr>
<td>Vein islet number</td>
<td>3.00 – 4.50</td>
<td>3.35 ± 0.2915</td>
</tr>
<tr>
<td>Veinlet termination number</td>
<td>2.75 – 3.00</td>
<td>2.85 ± 0.0612</td>
</tr>
</tbody>
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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 activity 10.

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.

REFERENCES

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