

Research Article

Antimicrobial Activities and Phytochemical Screening of the Leaf Extract of *Mitracarpus Scaber*

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Abstract: An evaluation of the anti-microbial activities of the leaf extract of *Mitracarpus scaber* shows that it has inhibitory effects on *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus* and *Trychophyton mentagraphytes* at various concentrations. The phytochemical screening carried out also shows the presence of secondary metabolites such as steroids, reducing sugars, alkaloids, tannins, tepenoids, saponins, cardiac glycosides and general glycosides. This shows that *Mitracarpus scaber* can be used as herbal mixture for the treatment of skin infections caused by some bacteria and fungi.

Keywords: *Mitracarpus scaber*, Antimicrobial activities, Traditional medicine, Extract, Inhibition

INTRODUCTION

Nigeria is richly blessed with many varieties of plants whose leaves, roots, fruits, seeds and barks are useful in traditional medicine. Among the numerous varieties of plants is *Mitracarpus scaber*.

Mitracarpus scaber is a widely used plant traditionally in the treatment of skin diseases, particularly infectious dermatitis, eczema, ringworm and scabies [1, 2].

In the Urhobo region of Delta State, Nigeria, the leaves of *Mitracarpus scaber* are macerated and mixed with palm kernel oil to be used topically for skin diseases and infections. It has been reported to be an effective antifungal agent against *Candida albicans* and *Trychophyton soudanense* [3].

The young leaves are squeezed and rubbed on the affected corporal parts several times a day. It has also been reported [4, 5] that the plant can be used for infantile toothache, anti-parasitic and venereal infections. It is also reported [6] to have some anti-bacterial effects on some limited bacteria.

Mitracarpus scaber is of the Rubiaceae family often found in the Tropics and Orients. It is an annual plant with erect stems of about 53cm high and branches that are about 8cm long. The plant has lanceolate leaves of about 4.5cm long with an upper scabrous surface. Its inflorescence consists of clusters of small white flowers which turn yellowish as the plant matures. It also has dehiscent capsulate fruits of about 1mm long. The tap root grows to about 9.5cm from the surface of the soil down. It is perennial and grows where there is much water during the rainy season (March to September.)

Pharmacognosical evaluation of *Mitracarpus scaber* [7] indicated the presence of simple leaf whorled arrangement, an entire margin with lanceolate shape, acute apex and base, parallel venation, thin, waxy anticlinal walls and numerous calcium oxalate crystals. It also has anomocytic stomatal arrangement with numerous covering trichomes on both surfaces. It was also shown to have chemomicroscopic characters that include lignin, starch, mucilage and calcium oxalate crystals.

Phytochemical analysis [8] reveals the presence of tannins, saponins, and antraquinones. However, alkaloids and cardiac glycosides were found to be present when further investigated [9].

This study therefore focuses on the anti-microbial activities of the leaf extract of *Mitracarpus scaber* using n-propanol and its phytochemical composition and the possible application of the extract for anti-bacterial and anti-fungal activities.

MATERIALS AND METHODS

The young fresh leaves of *Mitracarpus scaber* were collected in Orerokpe, Headquarters of Okpe Local Government Area of Delta State, Nigeria, in July, 2013. The plant was identified in the Department of Pharmacognosy and Traditional Medicine of the faculty of Pharmacy, Delta State University Abraka.

The leaves were air dried and powdered using a properly disinfected mixer (electric blender.) 120g of the dried powder was extracted with n-propanol (50%) using soxhlet. The crude extract was concentrated to dryness by evaporation using a hot air oven maintained at a temperature of 40°C. The extract was placed in a universal bottle, labeled and refrigerated at 4°C.

Preparation of Medium

The pure culture of the test organisms *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* were obtained from the Delta State University Teaching Hospital (DELSUTH) Oghara while the fungus *Trichophyton mentagraphytes* was obtained from the Delta State University Health Centre in Abraka. The bacteria were grown in Nutrient agar (Fluka) while the fungus was grown in Sabourad Dextrose Agar (SDA-Himedia). The media were prepared according to the method of Wolfgang and Hilda [10.]

Phytochemical Investigation

Preliminary phytochemical screening were carried out on the crude extract using standard methods described by Trease and Evans [11] to determine steroids, reducing sugars, alkaloids, flavonoids, tannin, terpenoid, saponins, cardiac glycosides and anthraquinones. The activity and quantitative results of these secondary metabolites could vary with geographical location of the plant [12.]

Anti-Microbial Sensitivity Test (Zone of Inhibition)

The zone of inhibition is the diameter over the growth of microorganism inhibited due to the presence of an antimicrobial agent. Zone of inhibition is carried out on solid media using a freshly prepared inoculated agar plate. The test for zones of inhibition was carried out in duplicates and their mean diameter recorded in millimeters.

A 5mm diameter cork borer was used to bore four wells in the agar plate into which appropriate concentrations (8.75, 17.5, 35 and 70 mg/ml) of the n-propanol leaf extract of *Mitracarpus scaber* were added. The plates of the bacteria were incubated at 37⁰c for 24hours while those of the fungus were left at room temperature for seven days. The zones of inhibition were measured using a transparent meter rule. The organic solvent, n- propanol was used as control.

RESULTS

Phytochemical screening revealed the presence of steroid, reducing sugar, cardiac glycosides and general glycosides.

Table 1: Phytochemical screening of the leaf extract of *Mitracarpus scaber*

Constituents	Inference
Carbohydrates	+
Glycosides	+
Free anthraquinones	-
Combined anthracene	-
Cardiac glycosides	+
Steroids	+
Terpenes	+
Flavonoids	-
Tannins	+
Alkaloids	+
Saponins	+

N.B.: + Present; - Absent

Anti-Bacterial and Anti-Fungal Activities

The anti-microbial activities of *Mitracarpus scaber* was evaluated using Mueller-Hinton Agar (Oxoid) for the bacteria and Sabourad Dextrose Agar (Hi media) for the fungus. The pour plate method was used. An overnight broth culture of the bacteria was used to prepare the plates and the agar diffusion (well) method was used for the sensitivity.

The MIC being the lowest concentration or highest dilution of the sample that prevented visible growth of microorganism was also determined as shown in the Table 2 below.

DISCUSSION

The anti-bacterial and anti-fungal activities of the leaf extract of *Mitracarpus scaber* using n-propanol show that the extract has inhibitory effect on *Pseudomonas aeruginosa*, *Proteus vulgaris*,

Escherichia coli, *Staphylococcus aureus* and *Trichophyton mentagraphytes* at the various concentrations used. The minimal inhibitory concentrations carried out reveal that *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* have the same MIC of 216.25ug/ml while that of *Pseudomonas aeruginosa* was 175ug/ml. That for *Trichophyton mentagraphytes* was 437.5ug/ml. These values are suggestive of the fairly good anti-bacterial and anti-fungal activities of the crude extract of *Mitracarpus scaber*.

The results of the phytochemical screening of the leaf extract establish what had been reported about the therapeutic effects of the leaf extract of *Mitracarpus scaber*.

Some alkaloids appear toxic but others show good health properties. Alkaloids have both anti-

bacterial and anti-fungal properties and have been used to prepare drugs [13.]

Tannins and flavonoids have been reported to have bacterial activities [14.] The anti-inflammatory properties of Tannin have also been reported [15.] The presence of some of these secondary metabolites in the leaf extract of *Mitracarpus scaber* could be responsible

for both the anti-bacterial and anti-fungal activities [16, 17].

It therefore means that *Mitracarpus scaber* leaf extract possesses both anti-bacterial and anti-fungal properties and hence it can be used to formulate herbal mixtures for the treatment of skin infections caused by some bacteria and fungi.

Table 2: Zone of inhibition and MIC of extract on microorganisms

<i>Microorganism/Extract concentration (mg/ml)</i>	<i>Zone of inhibition (mm)</i>	<i>Minimum inhibitory concentration (MIC) (ug/ml)</i>
<i>Pseudomonas aeruginosa</i>		
70	4	
35	6	175
17.5	2	
8.75	4	
<i>Proteus vulgaris</i>		
70	9	
35	6	216.25
17.5	5	
8.75	3	
<i>Escherichia coli</i>		
70	8	
35	6	216.25
17.5	7	
8.75	3	
<i>Staphylococcus aureus</i>		
70	10	
35	7	216.25
17.5	5	
8.75	5	
<i>Trichophyton mentagraphytes</i>		
70	8	
35	14	437.5
17.5	6	
8.75	4	

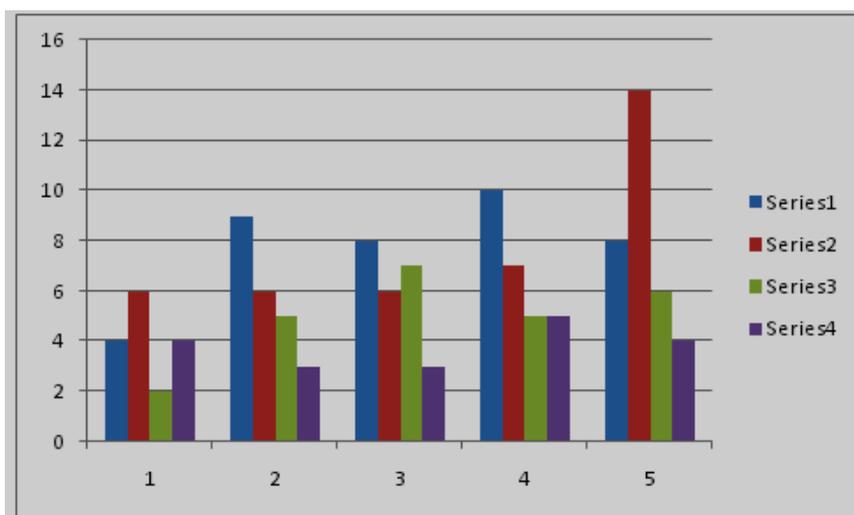


Fig 1: Graph showing zones of inhibition of the extract of different concentrations

Series 1: Extract concentration at 70mg/ml, Series 2: Extract concentration at 35mg/ml, Series 3: Extract concentration at 17.5mg/ml, Series 4: Extract concentration at 8.75mg/ml, 1—*Pseudomonas aeruginosa*, 2—*Proteus vulgaris*, 3—*Escherichia coli*, 4—*Staphylococcus aureus*, 5—*Trichophyton mentagraphytes*

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