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# Antimicrobial Activities and Phytochemical Screening of The Ethanolic Leaves Extract of *Mitracarpus Scaber**

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## Abstract

An evaluation of the antimicrobial activities of the Ethanolic leaves extract of Mitracarpus scaber shows that it has inhibitory effects on Candida albicans, Aspergillus spp, Penicillium and Escherichia coli at various concentrations. Based on the zones of inhibition produced by the plant(M.scaber), the Ethanolic crude extracts showed a significant activity at concentrated and diluted solution of the extracts after 24hours of incubation on both C.albicans, Aspergillus spp, Penicillium and E.coli. The antifungal Fluconazole and antibacterial Amoxicillin served as positive control. The antimicrobial studies reveals that the highest zone of inhibition of growth was observed as **29.33±0.66mm** in the Ethanolic leaves extracts against C.albicans which shows a significant difference when compared with the standard drug Fluconazole (**27.50±0.28mm**) and the lowest zone of inhibition of growth was observed as **0.00±0.00mm** in the Ethanolic leaves extracts against all the tested organisms which also shows a significant difference when compared with the standard drugs. The lowest MIC of this plant part (Leaves) was observed at diluted concentration of the extracts against C.albicans and Aspergillus spp with corresponding MFC also at diluted concentration of the extracts against C. albicans and Aspergillus spp. The highest MIC and MFC was observed at concentrated solution of the leaves extracts against almost all the tested organisms. The phytochemical screening carried out also shows the presence of secondary metabolites such as alkaloids, tannins, and anthraquinones. This shows that the Mitracarpus scaber can be used as herbal mixture for the treatment of skin infections caused by some bacteria and fungi.

## Keywords

Antimicrobial activities, *Mitracarpus scaber*, Phytochemical screening, Ethanolic, Leaves.

## 1. Introduction

Resistance to antimicrobial agents is a major global health problem(Lagnika *et al*,2016).Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host(Akinpelu and Onakoya 2006).Many efforts have been made to discover new antimicrobial and antioxidant compounds from various kinds of sources such as microorganisms, animals, and plants. Systematic screening of folk remedies is another strategy in the discovery of novel effective compounds (Lagnika *et al*,2010).This situation has forced researchers to search for new antimicrobial substance from various sources including medicinal plants. Antimicrobials of plant origin have proved effective in the treatment of several infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Khatoon *et al*,2013). Microorganism or microbes are microscopic organisms that exist as unicellular, multicellular, or cell cluster. Microorganisms are widespread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types; bacteria, archea, fungi, protozoa, algae, and viruses (Gordon 2013). The World Health Organization in a number of resolutions emphasized the need to ensure the quality control of plant products by using modern techniques and applying suitable standards (WHO, 1992). Nigeria is richly blessed with many varieties of plants whose leaves, roots, seeds, fruits and barks are useful in traditional medicine. Among the numerous varieties of plants is *Mitracarpus scaber*. In Nigeria, the plant is known as (Gogamasi in Hausa), (Obuobwa in Igbo), and (Irawole in Yoruba). The medicinal plants are continually being utilized as therapeutic agents in formulation for treating disease in the traditional ethno-medicinal system in Northern Nigeria (Ajasa *et al*, 2004).

*Mitracarpus scaber* is widely used plant traditionally in the treatment of skin diseases, particularly infectious dermatitis, eczema, ringworm and scabies. 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 soudanease.* The young leaves are squeezed and rubbed on the affected corporal parts several times a day. It has also reported that the plant can be used for infantile toothache, anti-parasitic and venereal infections. It is also reported to have some antibacterial effects on some limited bacteria (0ghenejobo *et al., 2013*). Phytochemicals are non-nutritive plants chemicals that have protective or disease preventive properties. They are non-essential nutrients meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect human against diseases. According to the World Health Organization (WHO) about 60% of the World Health population exclusively relay on traditional medicine (plant extract) for their primary health care needs (Fans Worth, 1994). However, since most of the effective proprietary drugs are expensive and difficult to come by, the reach for cheaper, effective and easily available alternative is therefore necessary. Drug resistance to microbial infections have been reported since, therefore alternative treatment using herbals began to attract the attention of the medical profession and the community, because herbals are relatively safe and have minimal side effects. Thus microbial infections morbidity and mortality have been increasing as such, most of the proprietary drugs are expensive and difficult to come by. The reach for cheaper, effective and easily available alternatives like *Mitracarpus scaber* is therefore necessary (Karaye *et al.,* 2017). The main aim of this research is to carry out phytochemical screening and antimicrobial studies of Ethanolic extract of *Mitracarpus scaber* Leaves.while the specific objectives of this research is to carry out qualitative phytochemical screening of *Mitracarpus scaber* leaves extracts.To evaluate the antifungal activity of extract of *M.scaber.*To determine the Minimum Inhibitory Concentration (MIC) of *M.scaber* extracts.To determine the Minimum Fungicidal Concentration (MFC) of *M.scaber* extract..

## 2. Materials and Methods

### 2.1. Materials

Analytical weighing balance, pestle and mortar, water bath, dropper, Bunsen burner, Petri dish, refrigerator, incubator, volumetric flask(1000ml), measuring cylinder, filter paper, ultrasonic machine.

### 2.2. Chemicals

Ethanol (70%), distilled water, dragendorff's reagent, 1% Hcl and dilute Hcl, magnesium, dilute ammonium solution, Mueller-Hinton Agar, spirit, Fecl3, KOH, benzene, chloroform.

## 3. Sample Collection and Preparation

The *M.Scaber* leaves were collected from the rural area, near the river Komadugu of Gashua. The leaves of the plants were air-dried under shade at room temperature for one week. Thereafter the dried plant sample was pounded and grounded into powder using a mortar and pestle, the powder obtained was then used to prepare the extracts. One hundred grams (100g) of the plant sample weighed was transferred into large beaker. The 70% ethanol was gradually added to the sample. The sample was mixed thoroughly with the solvent. The mixture was covered with clean foil paper to avoid evaporation of the ethanol. The mixture was kept in a dark cupboard for 18 hours for extraction. The mixture was filtered using Whatman filter paper. The filtrate was evaporated for dryness. The extract was finally dried completely using oven. The dried extract was weighed and stored in an air tight container for subsequent analysis. Pure clinical isolates of *Candida albicans, Aspergillus, Penicillium* and *Escherichia coli* were obtained from the Laboratory Department (Parasitology), University of Maiduguri Teaching Hospital (UMTH), were grown on a Mueller-Hinton Agar in an incubator at $37^0$C for 24 hours. The antifungal and antibacterial activity of the plant was measured by culture media using Mueller-Hinton Agar and Blood (chocolate) Agar.

### 3.1. Phytochemical Screening

Standard laboratory procedures as described by Evans and Trease (2000) were used for the determination of the following bioactive components: Alkaloids, flavonoids, tannins, anthraquinones and carotenoids

### 3.1.1 Test for Alkaloids

About 2ml of the extract solution was mixed with 1ml of 1% Hcl, boiled on a water bath, and 6 drops of Dragendorff's reagent was added. The appearance of orange precipitate was taken as the presence of alkaloids in the extract.

### 3.1.2 Test for Flavonoids

The extract solution (2ml) was mixed with 1ml of concentrated Hcl and magnesium ribbon. No effervescence, orange, red or crimson color which indicate the absence of flavonoids.

### 3.1.3 Test for Tannins

The extract solution (2ml) was mixed with 2ml of 15% $Fecl_3$ solution. The appearance of blue-black precipitate was taken as the presence of tannins in the sample.

### 3.1.4 Test for Anthraquinones

One ml of the extract solution was boiled with 4ml of alcoholic KOH for 2min and was diluted with 4ml of water and filtered. The filtrate was acidified with diluted Hcl, filtered, cooled and shook with benzene. The layer was separated and transferred to a clean test tube and shaken with 2ml of the dilute ammonia solution. The appearance of orange-red color in the aqueous layer was taken as the presence of anthraquinone.

### 3.1.5 Test for Carotenoids

Exactly 2ml of the extract solution was mixed with 2ml of chloroform, and centrifuged. The clear solution was mixed with concentrated sulfuric acid. The appearance of blue color was taken as the absence of carotenoids in the sample.

## 3.2. Determination of Extraction Yield (% Yield of the Extract)

The yield (%) from the dried extracts was calculated as:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of powder extracted}} \times 100$$

## 3.3 Antimicrobial Screening Test

### 3.3.1 Preparation of Mueller-Hinton Agar

38g of Mueller-Hinton Agar weighed using weighing balance machine. It was dissolved in 1 liter of distilled water, which was then heated to dissolve the media completely. The media was sterilized by autoclaving at 15 IBS pressure at 21$^{o}$C for 15 minutes. It was mixed and poured into a sterile petridish.



### 3.3.2 Preparation of Chocolate Agar

Chocolate agar is a heated blood agar. Required percentage of blood was added to the prepared Mueller-Hinton Agar while the temperature was still at 75$^{o}$C.

### 3.3.3 Preparation of Working Extract

The working extract was prepared using Ditch method. Three test tubes were arranged, labeled concentrated, diluted and double diluted. 5.0 g of the plant extract was dissolved in 5ml of normal saline.

### 3.3.4 Inoculation

### 3.3.5 Preparation of Organism for Inoculation

The organisms were diluted in four folds. It was inoculated on a Mueller-Hinton Agar and blood agar, the excess were drained. Four wells were made using cock borer labeled concentrated, diluted and double diluted, with the control at the center (Fluconazole and Amoxicillin was used as a positive control), and each well has a diameter of 8mm and a depth of 6milliliter. About 0.25 (250µl) of the respective extract were then placed in the well. The plates of the bacteria were incubated at 37$^{0}$C for 24 hours while those of fungus were incubated at 37$^{0}$C for seven days. The zones of inhibition were determined by measuring with a ruler for each organism

### 3.3.5 Determination of Minimum Inhibitory Concentration (MIC)

The determination of the Minimum Inhibitory Concentration (MIC) was carried out on the Ethanol extract of the leaves of *Mitracarpus scaber*. Using peptone water, about 9 test tubes were arranged and 2ml of peptone water were placed in all the test tubes. 2ml of the extract are added to the first test tube. It was mixed and 2ml was transferred to the third test tube and it was repeatedly up to the 9th test tube respectively. The last 2ml was discarded. The respective organisms were added in equal volume to each test tube. The result was examined where there was no turbidity is the MIC.

### 3.3.6 Determination of Minimum Fungicidal Concentration (MFC)

This was carried out to know if the organisms could be killed completely or their growths could be inhibited. The MFC was determined by sub-culturing the tubes that does not show turbidity. It was inoculated at 37$^{o}$C for 24 hours. The result shows antifungal action of the extract to the respective organisms.

### 3.3.7 Statistical Analysis

The results were expressed as mean ± SEM of the studied organisms using the analysis of variance test(one way ANOVA), followed by statistical package for the social science software(SPSS). Values of P <0.05 considered significant.

## 4. Results and Discussion

Percentage Yield = 5.48%



**Table 4.1 Phytochemical screening of the Ethanolic leaves Extract of *Mitracarpus scaber***

| Phyto constituents | Observation |
|---|---|
| Alkaloids | + |
| Flavonoids | - |
| Tannins | + |
| Anthraquinones | + |
| Carotenoids | - |

**Key: + = presence - = Absence**

**Table 4.2: Zones of inhibition (mm) produced by Ethanolic leaves extract of *M.scaber* at different concentrations on *C.* albicans**

| Concentrations | Test Organism *Candida albicans* |
|---|---|
| Concentrated | 29.33±0.66$^{a}$ |
| Diluted | 20.50±0.23$^{c}$ |
| Double diluted | 0.00±0.00$^{d}$ |
| Fluconazole | 27.50±0.28$^{b}$ |

Values are expressed as Mean ± SEM (mm) of triplicate determinations.
$^{abc}$ Means on the same column with different superscript are significantly different($p< 0.05$).

**Table 4.3: Zones of inhibition (mm) produced by Ethanolic leaves extract of *M.scaber* at different concentrations on *Escherichia coli***

| Concentrations | Test Organism *Escherichia coli* |
|---|---|
| Concentrated | 28.83±0.44[a] |
| Diluted | 19.50±0.28[b] |
| Double diluted | 0.00±0.00[c] |
| Amoxicillin | 28.83±0.44[a] |

Values are expressed as Mean ± SEM (mm) of triplicate determinations.
[abc] Means on the same column with different superscript are significantly different($p < 0.05$).

**Table 4.4: Zones of inhibition (mm) produced by Ethanolic leaves extract of *M.scaber* at different concentrations on *Penicillium***

| Concentrations | Test Organism *Penicillium* |
|---|---|
| Concentrated | 25.50±0.28[a] |
| Diluted | 18.00±0.57[b] |
| Double diluted | 0.00±0.00[c] |
| Fluconazole | 24.50±0.28[a] |

Values are expressed as Mean ± SEM (mm) of triplicate determinations.
[abc] Means on the same column with different superscript are significantly different($p < 0.05$).



**Table 4.5: Zones of inhibition (mm) produced by Ethanolic leaves extract of *M.scaber* at different concentrations on *Aspergillus spp***

| Concentrations | Test Organism *Aspergillus spp* |
|---|---|
| Concentrated | 27.50±0.28[a] |
| Diluted | 18.66±0.88[b] |
| Double diluted | 0.00±0.00[c] |
| Fluconazole | 28.83±0.88[a] |

Values are expressed as Mean ± SEM (mm) of triplicate determinations.
[abc] Means on the same column with different superscript are significantly different($p < 0.05$).

**Table 4.6: MIC of Ethanolic leaves extract of *Mitracarpus scaber***

| Test Organisms | Different Concentration of Extract (Leaves of *M.scaber*). | | |
|---|---|---|---|
| | Concentrated | Diluted | double diluted |
| *C.albicans* | - - | *- - | ++ |
| *E-coli* | *- - | ++ | ++ |
| *Penicillium* | *- - | ++ | ++ |
| *Aspergillus spp* | - - | *- - | ++ |

**Key: * = MIC; - - = No Growth; ++ = Growth.**

**Table 4.7: MFC of the Ethanolic leaves extract of *Mitracarpus scaber***

| Test Organisms | Different Concentration of Extract (Leaves of *M.scaber*). | | |
|---|---|---|---|
| | Concentrated | Diluted | double diluted |
| *C.albicans* | - - | *- - | ++ |
| *Penicillium* | *- - | ++ | ++ |
| *Aspergillus spp* | - - | *- - | ++ |

**Key: * = MFC; - - = No Growth; ++ = Growth.**



The phytochemical screening revealed the presence of alkaloids, tannins, anthraquinones, and absence of flavonoids and carotenoids as reported by Oghenejobo *et al.,* (2013). The presence of some of these secondary metabolites in the leaf extract of *Mitracarpus scaber* could be responsible for both the anti-fungal and anti-bacterial activities. This result agrees with the findings of Bisignano *et al.*,(2000). All bioactive compounds obtained in the crude extracts of the leaves showed antimycotic activity against the test organisms in this study.

Based on the zones of inhibition produced by the plant (*M.scaber*) the Ethanolic crude extracts of *Mitracarpus scaber* showed significant activity at Diluted concentration and concentrated, after 24 hours on both *C. albicans, Aspergillus spp, Penicillium* and *E.coli.* Such observation has also been reported by Karaye *et al.,* (2017) and Bisignano *et al.*, (2000).

The antimicrobial studies reveal that highest zone of inhibition of growth was observed as 29.33±0.66 mm in the Ethanolic leaves extracts against *C.albicans* which shows a significant difference when compared with the standard drug (27.50±0.28 mm) and the lowest zone of inhibition of growth was observed as 0.00±0.00 in the Ethanolic leaves extracts against all the tested organisms which also shows a significant difference when compared with the standard drugs. The result of this study revealed that the minimum inhibitory

concentration (MIC) and the minimum fungicidal concentration (MFC) of the Ethanolic leaves extract of *Mitracarpus scaber* indicated higher concentrations. This observation is based on the fact that the concentration of the crude extract required to completely eliminate an organism must be higher than the concentration required to inhibit the growth as reported by Andrews (2001). The lowest MIC of this plant part (leaves) was observed at diluted concentration of the extracts against *C. albicans* and *Aspergillus spp* with corresponding MFC also at diluted concentration of the extract against *C.albicans* and *Asprgillus spp.* The highest MIC and MFC was observed at concentrated solution of the leaves extract against almost all the tested organisms. The findings in this work have justified the use of this plant in the treatment of skin infections caused by some bacteria and fungi used in this study.

## 5.0 Conclusion

The results of this study showed that the antimicrobial activity of the Mitracarpus ethanol extract against Candida albicans, Penicillium, Aspergillus and E-coli may be related to the presence of alkaloids, tannins, and anthraquinones, indicating that the plant is potent and contains therapeutic potentials. It therefore means that the compounds identified in this plant showed that they could be responsible for the antimicrobial activity observed and hence it can be used to formulate herbal mixtures for the treatment of infections caused by some bacteria and fungi .

## 6.0 Recommendations

The findings in this work have justified the use of this plant in the treatment of infections caused by some bacteria and fungi used in this study, it is therefore recommended that; Further studies should be carried out to identify more secondary metabolites that may contribute to the antifungal activity of *M.scaber* either by using the roots bark or the stem. Further research should also be done on quantitative analysis on the phytochemical constituents of *M.scaber* plant that have already been quantitatively determined. Public awareness should be created on the uses of plants for medicinal purposes and discouraging cutting down (deforestation) of most of these medicinal trees in the developed countries.

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