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Phytochemical, Antifungal and Acute Toxicity Studies of *Mitracarpus scaber* Zucc. Whole Plant Extracts

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ABSTRACT

Mitracarpus scaber have been reported in the treatment of various ailments such as ulcer, cancer, skin diseases etc. It is therefore important to investigate these plant parts to ascertain their therapeutic potentials. The *Mitracarpus scaber* whole plant was extracted with water and methanol, screened for their phytochemical properties and antifungal effects. The plant samples were also investigated for alkaloid, flavonoids, saponins, tannins and phenolic contents using quantitative techniques. The antifungal activities of the plant samples were tested against *Candida albicans, Trichophyton mentagrophytes, Microsporum auduounii* and *Aspergillus flavus*. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the extracts were also determined. Flavonoid, steroid, triterpenes, tannins, carbohydrate, glycoside, phenols were detected in both extracts while anthraquinones was absent. Alkaloid was detected in the aqueous extract but absent in methanol extract. Quantitatively, the phenols (97.0 mg/g) was the highest phytochemical detected in the plant while the lowest was alkaloid (9.2 mg/g). Toxicity of the samples was expressed as LD_{50}, it was found above 5000 mg/kg and did not cause mortality in all the tested rats. Aqueous extract only showed inhibition on *Candida albicans* and *Trichophyton mentagrophytes* with no inhibition on *Microsporum auduounii* and *Aspergillus flavus* while ethanol extract only showed inhibition on *Candida albicans* with no inhibition on *Microsporum auduounii, Trichophyton mentagrophytes* and *Aspergillus flavus* at the lowest concentration 12.5 mg/ml. The aqueous extract has MIC and MFC of 12.5 mg/ml and 25 mg/ml respectively against *Candida albicans* and *Trichophyton mentagrophytes.* Thus, the traditional claims of the uses of the plants as antifungal agents were therefore supported.

Keywords: Antifungal, Minimum Fungicidal Concentration (MFC), Minimum Inhibitory Concentration (MIC), *Mitracarpus scaber*, Phytochemical

INTRODUCTION

The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Hammer *et al*., 1999). Antibiotic resistance has become a global concern (Westh *et al.*, 2004) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Bandow *et al.*, 2003). Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for the development of novel drugs because of the great diversity in their chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003). Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Benkeblia, 2004). Consequently, this has led to the screening of several medicinal plants for potential antimicrobial activity (Iwu *et al*., 1999).

Mitracarpus scaber (ZUCC) belongs to the family-Rubiaceae, genus-Mitracarpus and Species *M. scaber*. Common name is botton grasses, gogamasu (Hausa). The inflorescence is dense and clustered together with small white flowers at the leaf axils that are usually thick. *M. scaber* vary in its habitat and reproduces by seed. *Mitracarpus scaber* is widely employed in traditional medicine in West Africa for headaches, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases and leprosy (Bisignano *et al*., 2000). Among the folkloric uses, the juice of the plants is applied topically for the treatment of skin diseases (infectious dermatitis, eczema and

scabies) (Michael, 2016). Daiziel, (1937); Kerharo and Adam, (1974) observed that a lotion and a skin ointment made with the aerial part of *M. scaber* are used for skin infections or skin diseases and other infectious. Previous studies by Moulis *et al.* (1992) reported the isolation of pentalogin from fresh, aerial parts of *Mitracarpus scaber* which demonstrates a potent antifungal activity against *Candida albicans* and *Trichophyton soudanense*. Other investigations (Sanogo *et al.,* 1996) showed that different extracts of *M. scaber* exhibited broad antibacterial and antifungal activity against standard strains and clinical isolates of *Staphylococcus aureus* and *C. albicans* responsible for common skin infections. Germano *et al.* (1999) reported the hepato protective effects of *Mitracarpus scaber* decoction on tetrachloromethane (CCl4) induced hepatotoxicity *in vivo* as well as *in vitro* using isolated hepatocytes. The uses of *Mitracarpus scaber* like many other medicinal plants have been on folkloric among different communities in Africa without any scientific basis. (Michael, 2016).

Often traditional healers use plants according to their analogy and morphological similarities to the ailment being treated. For example, plants containing red juice are used to treat ailments connected to menstruation problems and bleedings (Neuwinger, 2000). There is therefore the need to ascertain the basis for the claims of the efficacy of the plants used locally in ethno-medicine. The whole extracts of *Mitracarpus scaber* have been reported in the treatment of various ailments such as ulcer, cancer and skin diseases (Michael, 2016). It is therefore important to scientifically investigate these plant parts to ascertain their therapeutic potentials. Determination of their chemical composition as well as antimicrobial efficacy against specific pathogens is important in the recognition of this plant as a potent commercial medicinal plant (Michael, 2016). Tests can determine its efficacy against a pathogen and thus, establish the minimal dosage required for the treatment of ailments.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh *Mitracarpus scaber* whole plant was collected from local farms around Collage of Agriculture Dambatta, Kano State, Nigeria. Samples from the plant were taken to the Herbarium Section of the Department of Plant Biology, Bayero University, Kano, Nigeria for authentication. The voucher specimen was deposited at the Herbarium Section for future reference as BUKHAN649.

The collected whole plants of *M. scaber* were washed and dried at room temperature in the laboratory for one week. Samples were ground to powder using a mortar and pestle. The powdered plant samples were kept in polythene bags until extraction.

Preparation of Plant extracts

One hundred grams (100g) of the powdered whole plant was soaked in to 1000 ml each of methanol and distilled water. The mixture were allowed to stand for 3 days at room temperature (28 ±2ºC) with hourly agitations. Each extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50ºC until all the solvent evaporated.

Qualitative Phytochemical screening of *Mitracarpus scaber* Whole plant extracts

The plant extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

Tests for Carbohydrates

Molish's (General) Test for Carbohydrates

To 1 ml of the extract, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test

A portion of the extract was dissolved into 1-2ml of 50% methanol in the presence of heated metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 2009).

Test for Alkaloids

Wagner's Test

Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's test:

Equal volume of acetic acid anhydride were added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the

presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test:

Exactly 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for at least 5 mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

Quantitative Determination of Phytochemical Contents of the *Mitracarpus scaber* Whole plant

Alkaloid Determination using Haborne (1973) Method

About 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4hours. This was filtered and the extract is concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is completed. The whole solution was allowed to settle and the precipitates were collected and wash with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid Determination by the Method of Bohm and Kocipal – Abyazan (1994)

About 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later be transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Saponins Determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10g was weighed for each and put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with 200ml, 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was kept while the ether layer was discarded. The purification process was repeated and 60ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated as percentage yield.

Tannin Determination by Van-Burden and Robinson (1981) Method

About 500mg of each sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up of the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferro-cyanide. The absorbance was measure at 120mm within 10min.

Determination of Total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. About 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. About 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added also. The sample was made up to mark and allowed to react for about 30 minutes for colour development. This was measured at 505nm.

Collection of the Fungi

The fungi used were obtained from the cultured microorganisms collected from Department of Medical Microbiology Aminu Kano Teaching Hospital, Kano, Nigeria. This sample was subcultured and maintained in Sabouraud Dextrose Agar, SDA (slants) at 4^{0}C.

Identification of Isolated Fungi

The fungi species were identified by their cultural macroscopic and microscopic characters. The cultural macroscopic properties were studied. (i.e. the hyphae, presence of conidiophores, colour, shape of the conidia head etc. James and Natalie,

2001). The microscopic identification was aided by appropriate taxonomic keys and atlas of Dermatophyte (James and Natalie, 2001). Pure cultures were inoculated into slant bottles containing sterile SDA to serve as reservoir and store under low temperature inside the refrigerator (8-15^{0}C) to arrest their growth.

Lactophenol cotton blue stain was dropped on a clean slide using dropping pipette, while a small portion of mycelium was teased on the slide, using a needle. The slide was covered with pressure to eliminate air bubbles. The slide was then mounted and observed under the microscope at x10 and x40 objective lenses respectively. The species encountered were identified in accordance with Cheesbrough (2000).

Preparation of Fungal Media

Sabouraud Dextrose Agar (SDA)

The medium was prepared according to manufacturer's instruction (Oxoids Limited Basingstoke, Hampshire England). This was prepared by dissolving 42g of its powder in distilled water to produce a litre and then heated gently to dissolve the agar completely. The SDA was distributed into l0mls and 20mls bottles, capped and autoclaved at 121^{0}C for 15 minutes. After sterilization, 10 ml SDA bottles were slanted and allowed to set firmly to give agar slope (slants).

Sabouraud Dextrose Liquid Medium (SDLM)

The medium was prepared by dissolving 30g of SDLM-powder (Oxiods) in one liter of distilled water and distributed into the final containers and sterilized at 121°C for 15minutes.The media were allowed to cool to 45^{o}C and 20ml of the sterilized medium was poured into sterile petri dishes and allowed to cool and solidify (Olowosulu *et al*., 2005).

Preparation of test organisms

The fungal culture was prepared on sabouraud dextrose agar incorporated with 0.5% cycloheximide and 0.45% chloramphenicol and incubated at 30°C for 7days, thereafter, kept in triplicates as stock fungal spores cultures at 4°C (Aberkane *et al*., 2002).

Maintenance of Test Fungal Stock Culture

To do this, six freshly prepared sabouraud dextrose agar slants were inoculated each and harvested, washed isolated and incubated at 30°C for 5-7 days. (Aberkane *et al*., 2002).

Preparation of spore suspension

This was prepared by harvesting SDA slants containing stock fungal culture. The harvested fungal spores were washed using harvesting medium. The supernatants were discarded and spore-pellets re- suspended in harvesting medium, standardized and stored at 4°C until required for use within two weeks (Olowosulu *et al*., 2005).

Determination of Zone of Inhibition

The antifungal activity of *Mitracarpus scaber* crude extracts (Methanol) against the test fungi was evaluated using agar well diffusion method for susceptibility testing (Srinivasan *et. al.,* 2009*)*. Sabouraud Dextrose agar were inoculated with 0.1ml of standardized inoculum of each bacterium and fungus (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Wells of 6 mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml each of the crude extracts were dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed in millimeters after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, fluconazole was used as positive control parallel with the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution technique was employed (Shanmugapriya *et al.,* 2012) equal volume (10 ml) of double-strength SDA was mixed with crude extract, poured aseptically in plates and allowed to set. Sterile paper disc in duplicates were aseptically placed on the agar. The standardized fungal spores were inoculated with sterile paper disc aseptically. The minimum concentration that showed no growth was the MIC of the crude extract.

Determination of Minimum Fungicidal Concentration (MFC)

The well from the plates in MIC determination that showed no growth were removed and the fungal spores were harvested after 7 days. SDA Slant culture was washed with 10ml normal saline inoculated into SDLM with 0.05% Tween 80 with glass beads aseptically. These broths were incubated for 48hrs at 37°C and then examined for the presence or absence of visible growth. Positive and negative controls were set up to confirm the viability of the organism (positive control), the tube contained the media inoculated with sterile water and test organism while in the negative control, and the tubes contained only media inoculated with sterile distilled water. Re-inoculation was done into recovery SDLM and incubation was done for 5days at 30°C. Visual observation of visible growth was made and the fungicidal concentration of the crude extract for that organism was examined (Hafidh *et al.,* 2011).

Acute toxicity studies of methanol extract of *Mitracarpus hirtus* Whole plant

Lethal Dose (LD_{50}) Determination

The method of Lorke (1983) was employed for this test. Thus, the phase I involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adult wister albino rats. In the fourth group, three adult male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered the extract using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extract and hourly for the next 6 hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the phase II was employed, only one animal was required in each group. Groups 1–4, animals were orally given 1,500, 2,200, 3250 and 5,000mg/kg dose levels of the crude extract. All the animals were left for observation as in stage one.

RESULTS AND DISCUSSION

The plant material was extracted with water and methanol to yield light green and dark green aqueous extract (18.8 g) and methanol extract (16.4 g), respectively (Table 1). Flavonoid, steroid, triterpenes, tannins, carbohydrate, glycoside, phenols were detected in both extracts while anthraquinones was absent. Alkaloid was detected in the aqueous extract but absent in methanol extract (Table 2). Table 3 showed the phytochemical contents of *Mitracarpus scaber* in the methanol extract. The phenols (97.0 mg/g) was found to be the highest phytochemical detected in the extract while the lowest content was observed in alkaloid (9.2 mg/g). Toxicity study indicate no death recorded in the first phase of the investigated rats. In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol whole plant-extract of *Mitracarpus scaber* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed (Table 4).

Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will helps the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle (Mishra *et al.,* 2010). Phytochemical analysis of the whole plant extracts had revealed the presence of some secondary metabolites namely carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroid/triterpenes and absences of anthraquinones; this result is in agreement with the finding of Mann *et al*. (2010). The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008). Some of the observed phytochemical constituents present in this plant have been reported to possess antifungal properties (George *et al*., 2002; Ubani *et al*., 2012). Tannins have been described to contain a large number of complex substances that are widely distributed in almost all parts of the plant. They are usually localized in the various parts of the plant such as the leaves, stem, roots and barks (Oyi *et al.,* 2001). Phytochemicals are the natural bioactive compounds found in plants. The phytochemicals work nutrients and fibres to form an integrated part of defense system against various diseases and stress condition (Koche *et al*., 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids terpenoid, carbohydrates and phenolic compound (Pascaline *et al*., 2011). Tannins and flavonoids was also seen in moderate quantity. These phytochemicals are known to exhibit medicinal activity as well as pharmacological activity. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) (Kittakoop *et al.,* 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al.,* 2014), and antihyperglycemic activities (e.g., piperine) (Qiu *et al.,* 2014). Tannin is one of the major active ingredients found in plant based medicines (Haslam, 1996); they are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulant in rubber production as well as possessing antiviral, antibacterial, and antitumor activity (Haslam, 1996; Khanbabaee and Van Ree, 2001). Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al*., 1992). Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumors (Kim *et al.,* 1994); reduction of coronary heart disease has been reported to be associated with intake of flavonoid (Hertog *et al.,* 1993).

Table 1: Mass and Percentage Yield for the Extracts of *Mitracarpus scaber*

Extract	Mass (g)	Percentage Yield (%)	Extract Appearance
Methanol	16.4	16.4	Dark green
Aqueous	18.8	18.8	Light green

Table 2: Phytochemical screening of the whole plant of *Mitracarpus scaber*

Metabolite	Inference	
	Aqueous extract	Methanolic extract
Alkaloid	+	-
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	+	+
Anthraquinones	-	-
Carbohydrate	+	+

Table 3: Quantitative Phytochemical screening of methanolic whole plant extract of *Mitracarpus scaber*

Metabolite	Quantity (mg/g)
Alkaloids	9.20±0.50
Flavonoids	21.40±0.60
Saponins	51.00±0.33
Tannins	80.0±0.20
Phenols	97.0 ±0.60

Table 4: Acute toxicity studies of methanolic whole plant extract of *Mitracarpus scaber*

Treatment	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

Antifungal activity of aqueous extract only showed inhibition on *Candida albicans* (11-20 mm) and *Trichophyton mentagrophytes* (09-16 mm) while no inhibition was observed on *Microsporum audouinii* and *Aspergillus flavus* (Table 5). The MIC and MFC of the extract recorded respective values of 12.5 mg/ml and 25 mg/ml against *Candida albicans* and *Trichophyton mentagrophytes.*

Antifungal activity of methanol extract only showed inhibition on *Candida albicans* (08-14 mm) while no inhibition was observed on *Trichophyton mentagrophytes, Microsporum audouinii* and *Aspergillus flavus* (Table 6). The MIC and MFC of the extract recorded respective values of 12.5 mg/ml and 25 mg/ml against *Candida albicans.*

The higher yield of the aqueous extract over the methanol extract could be due to their difference in polarity. During extraction solvent diffused into the solid plant material and solubilized compounds with similar polarity (Prashant *et al.* 2011). In a study, Cimanga *et al.* (2004) reported that the percentage yield of the methanol extract of *Mitracarpus villosus* was 14.80%. This was less than the percentage yield of aqueous and methanol extracts recorded in this study. This could be due to the difference in the method of extraction used. Batch cold maceration method with various solvents of increasing polarity was employed in this study, while successive cold maceration only was employed in the study by Cimanga *et al.* (2004).

Antifungal activity of aqueous extract only showed inhibition on *Candida albicans* (11-20 mm) and *Trichophyton mentagrophytes* (09-16 mm) while no inhibition was observed on *Microsporum audouinii* and *Aspergillus flavus* (Table 5). The MIC and MFC of the extract recorded respective values of 12.5 mg/ml and 25 mg/ml against *Candida albicans* and *Trichophyton mentagrophytes* while methanol extract showed inhibition on *Candida albicans* (Table 6*).* The antifungal activity revealed that there was no zone of inhibition for the methanol and aqueous extracts on *Microsporum audouinii* and *Aspergillus flavus*. This agreed with the finding that biological activities of the plant extracts are solvent dependent (Zohra and Fawzia, 2011). Furthermore, the results of this study, signifies that degree of antifungal activities of the plant extract varied from one test microorganism to another. It was observed that

there was an increase in antifungal activity with increase in the concentration of extract used. In the susceptibility test of the fungi to the different extracts, it was observed that the aqueous extract of *Mitracarpus scaber* gave the highest antifungal activity against the two tested fungi. This was indicated by the diameter of zone of inhibition which was shown to increase with the increase in concentration of the extracts. The increased antifungal activity of the aqueous extract over the methanol extracts of *Mitracarpus scaber* is an indication that distilled water was able to extract out most of the active components of the plant. The zone of inhibition of the aqueous extracts against the *Candida albicans* at 100mg/ml was comparable to the standard drugs (Terbinafine) used. The result of minimum inhibitory concentration of extracts suggests that the extracts may act as fungicidal agents to *Candida albicans* and *Trichophyton mentagrophytes*. This finding is similar to the work of Uduak *et al.* (2010), who carried out a study on antifungal activities of some *Euphorbiaceae* plants used in traditional medicine in Nigeria. They observed that the inhibitory effect validated the use of the plants to treat infections caused by these microorganisms. Aqueous extract had MIC of 12.5mg/ml and MFC of 50mg/ml on *C. albicans.*

The oral median lethal dose value for the methanol whole plant extract of *Mitracarpus scaber* obtained in rats was found to be above 5000mg/kg. This suggests that the plant extract is non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.*, 2002). The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for rats found to be above 5000 mg/kg, is relatively safe orally.

Table 5: Antifungal activity of Aqueous extract of *Mitracarpus scaber* whole plant

Clinical isolates	Concentration/Diameter zone of inhibition						MIC	MFC
	100	50	25	12.5	Terbinafin	DMSO		
C. albicans	20	18	14	11	30	06	12.5	25
T. mentagrophytes	16	14	12	09	28	06	12.5	25
M. audouinii	06	06	06	06	30	06	-	-
A. flavus	08	06	06	06	31	06	-	-

Table 6: Antifungal activity of Methanol whole plant extract of *Mitracarpus scaber*

Clinical isolates	Concentration/Diameter zone of inhibition						MIC	MBC
	100	50	25	12.5	Terbinafin	DMSO		
C. albicans	14	12	10	08	30	06	12.5	25
T. mentagrophytes	06	06	06	06	28	06	-	-
M. audouinii	06	06	06	06	30	06	-	-
A. flavus	06	06	06	06	31	06	-	-

CONCLUSION

The methanol and aqueous whole plant extracts of *Mitracarpus scaber* were found to possess several bioactive constituents including flavonoids, saponins, tannins, cardiac glycosides among others, associated with potent pharmacological activities. The extract was found to possess considerable antifungal properties at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of skin infections.

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