

Screening of Chemical Constituents and Antifungal Potential of *Mitracarpus Scaber* (Button Grass)

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ABSTRACT

Plants have been used as valuable sources of natural products for maintaining animal and human health. The medicinal properties shown by different medicinal plants are due to the phytochemicals present in the plant. This study investigated the phytochemical and antifungal properties of the leaf extract of *Mitracarpus scaber*. Water and methanol were used as the solvents for the extraction to screen for their phytochemical properties and antifungal effects. The plant sample were extracted with water and methanol to yield light green and dark green aqueous extract respectively. Phytochemical analysis of the plant extracts in aqueous revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroid, terpenoids, phenols and absences of anthraquinones. In methanol extract alkaloids, tannins, cardiac glycosides, saponins, steroid, terpenoids, phenols are all present while flavonoid and anthraquinones are absent. The antifungal activities of the plant samples were tested against *Candida albicans* and *Taenia capitis*. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the extracts were determined. Antifungal activity of both extracts only showed inhibition on *Candida albicans* while no inhibition was observed on *Taenia capitis*, The MIC and MFC of the extracts recorded respective values of 12.5 mg/ml and 25 mg/ml only against *Candida albicans*.

Key Words: Antifungal, Phytochemical, *Mitracarpus Scaber*, Methanol, Aqueous

Introduction

Plants comparatively have a much greater biosynthetic ability than animals. Not only do plants make all the biomolecules that are universally required in living organisms, they also synthesize a vast array or complex chemicals which are unique to them (Hertog *et al.*, 1993). Phytochemicals are products of secondary metabolism defined variously as a peripheral metabolic pathways leading off from the universal (or primary) metabolism (Mojab *et al.*, 2003). Many phytochemicals have been shown to be bioactive that is they exhibit pronounced biological activity in other living organisms (Rashid *et al.*, 2008). Phytochemical analysis is a routine investigation in biochemical studies of plant extracts and in nutritional studies on foodstuff of plant origin. Some of the phytochemical substances that are usually tested for in plant extracts include alkaloids, glycosides, tannins, flavonoids and saponins among others (Onwuliri and Umezurumba, 2003).

Medicinal plants are the basis for the treatment of various diseases (Adams, 2007). Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants (Adewunmi *et al.*, 2004). However, the quality control of herbal

medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved (Agun *et al.*, 2003). Medicinal plants are the source of treatment for many diseases and ailments throughout the developing world (Rao *et al.*, 2004) because they contain various bioactive principles which have the potential to cause beneficial and/or detrimental effects (Adewunmi and Ojewole, 2004). A great number of Nigeria higher plants are traditionally noted for their medicinal and pesticidal properties (Wakeel *et al.*, 2004).

The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. More than 2000 Phytochemicals have been identified from plants (Taiz and Zeiger, 2006). The most important utilization of these plants is their application in medicines (Camciuc *et al.*, 1998 and Felter *et al.*, 2007).

Phytochemicals are naturally found in plants and are responsible for providing color, flavor and aroma to fruits and vegetables. They are biologically active and function to protect plants against invasion, disease and infection. Studies have shown that high intakes of fruits and vegetables are correlated with lower risks of chronic disease and obesity, partly because of these phytochemicals, also known as phytonutrients.

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova *et al.*, 2005). The history of plants being used for medicinal purpose is probably as old as the history of mankind. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar and Ravi, 2007).

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided as; primary or secondary constituents based on their role in plant metabolism. PRIMARY metabolites such as sugar, nucleic acids, amino acids, fats etc. which are found in all plants, and SECONDARY metabolites which are found in a smaller range of plants, serving a more specific function and are also the remaining plant constituents such as alkaloids, flavonoids, tannins, terpenoids, steroids, saponins etc. There are lots of medicinal plants which contain a number of phytochemicals and those phytochemicals are used for medicine purpose to treat various kinds of diseases (Zarai and Kadri 2011).

The secondary metabolites from plants, which are distinguished from primary metabolites such as nucleic acids, amino acids, carbohydrate, fat, etc. are extremely diverse; thousands of them have been identified in several classes (Thrane, 2001). A lot of scientific sources states that their role is not vital for living cells in normal growth, development, and reproduction but they play role in defense mechanism to protect a plant from any possible harm in the ecological environment. Therefore, they are usually synthesized in plants for particular needs. Secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolic, sterols, steroids, essential oils, lignin's and tannins etc). Their absence does not cause bad effects in the plants. These compounds or metabolites are accumulated by plant cells in smaller quantities than primary metabolites, and are synthesized in specialized cells at particular developmental stages making extraction and purification difficult (Stamp, 2003).

Antifungal

An antifungal medication known as antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), and serious systematic infections. There are two types of antifungals; local and systematic. Local antifungals are usually administered topically or vaginally, depending on the condition being treated. Systematic antifungals are administered orally or intravenously (Benitez *et al.*, 2019). Some classes of antifungal are: - Polyenes, Allylamines and Echinocandins.

Button Grass (*Mitracarpus Scaber Zucc*)

Mitracarpus scaber Zucc belongs to the family-Rubiaceae, genus-*Mitracarpus* and Species *M. scaber*. Common name is button grass, 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). It's 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 infections. Previous studies by (Moulis *et al.*, 1992) reported the 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. 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 plant is widely used traditionally in the treatment of skin diseases, particularly infectious dermatitis, eczema, ringworm and scabies (Shinkafi, 2013). *Mitracarpus scaber* has been reported to show antifungal efficacy against disease causing microorganisms such as *Salmonella enteritidis*, *Staphylococcus aureus*, *Campylobacter sp.*, *Escherichia coli*, *Candida albicans* and *Aspergillus* species. It has also been reported to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and contagious infections (Irodi and Daramola, 2009; Shinkafi, 2013).

Literature has revealed the wide-ranging medicinal applications of *Mitracarpus* species. Different *Mitracarpus* species have demonstrated antibacterial and antifungal activities. However, the leaves extract of *M. scaber* (Zucc). has been used to treat wounds, jaundice, inflammation, bacterial and fungal infections, toothache, headache, dyspepsia, amenorrhea, venereal diseases, liver diseases, sore throat, skin diseases, amenorrhea, skin infections and as an antidote for poisons (Ekpendu, P.A et al 1994). The plant is widely used traditionally in the treatment of skin diseases, particularly infectious dermatitis, eczema, ringworm and scabies (Shinkafi, 2013). *Mitracarpus scaber* has been reported to show antifungal efficacy against disease causing microorganisms such as *Salmonella*

enteritidis, *Staphylococcus aureus*, *Campylobacter* sp., *Escherichia coli*, *Candida albicans* and *Aspergillus* species (Irodi and Daramola, 2009; Shinkafi, 2013).

The methanol and chloroform extracts of the fresh leaves of *M. scaber* were found to have antioxidant activity using the phosphomolybdate method. Comparatively, the total antioxidant capacity of the methanol extract was higher than that of the chloroform extract with the values of 0.48 mg AAE/g and 0.21 mg AAE/g, respectively (B. Ouadja *et al.*, 2018). Also, the azaanthraquinone, 3,4,5-trimethoxybenzoic acid, and methoxy-4-acetophenone isolated from the aerial parts of *M. scaber* exhibited mild antioxidant activity, with IC₅₀ values of 12 µg/ml, and 45.74 µg/ml, respectively (L. Lagnika *et al.*, 2010).

(Cimanga *et al.*, 2004) evaluated the *in vitro* antibacterial activity of the n-hexane and 80 % MeOH extracts of the leaves of *Mitracarpus scaber* (Zucc). The maximum antibacterial activity was exerted against *Staphylococcus aureus*, *Staphylococcus faecalis*, and *Staphylococcus epidermidis*, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values < 65 µg/ml

The antifungal activity of *Mitracarpus Scaber*. ZUCC was widely reported. For instance, crude phenols, tannins, flavonoids, terpenes, and saponins present in the plant tested against clinical strains of *C. krusei*, *C. albicans*, *A. fumigatus*, *A. niger*, *T. verrucosum*, and *T. mentagrophytes* showed diverse activities. The crude tannin exhibited the most significant antifungal activity, with MIC values of 0.5-2.0 mg/ml and MFC values of 1.0-8.0 mg/ml. However, the ethanol extract of the plant exhibited antifungal activity, with MIC values of 25-50mg/ml (Lagnika *et al.*, 2010).

The ethanol extract from the leaf of *M. scaber* showed a remarkable anti-inflammatory effect in rats. The methanol extract of the *M. scaber* evaluated, displayed intense acute anti-inflammatory activity at dosages of 100 and 300 mg/kg. This activity was dose-dependent and showed selective inhibition of cyclooxygenase-2 (COX-2) expression (Aboh *et al.*, 2015).

MATERIALS AND METHODS

Sample Collection and Identification

The fresh *Mitracarpus scaber* whole plant was collected from a local farm located in Abukur, Katsina State, Nigeria and was taken to DR. Nasir Hassan Wagini of Department of Biology, UMYU Katsina for full identification and authentication. The collected plant was then washed and dried at room temperature for a week. Sample was grinded into powder using a mortar and pestle. The powdered plant sample was kept in polythene bag until extraction.

Collection of the Fungi

The fungi *Taenia capitis* and *Candida albicans* were obtained from the cultured microorganisms collected from Department of Microbiology Umar Musa katsina. The organisms were subcultured and maintained in Nutrient Agar at 40C.

Preparation of Plant Extract

One hundred grams (100g) of the powdered leaves sample was extracted by maceration in 500 ml each of methanol and distilled water in which 50g each was used. The mixtures were allowed to stand for 72 hours at room temperature with occasional agitations.

Phytochemical Qualitative Analysis

The plant extracts of methanol and aqueous solutions were assessed for the existence of the phytochemical analysis which are: - Alkaloid, Flavonoid, Saponins, Glycoside, Tannins, Steroid, Terpenoids, Phenols, Anthraquinones

TEST FOR ANTIFUNGAL ACTIVITY.

Preparation of Stock Solution

0.5g of the extracts was dissolved in 100ml of their respective solvents (distilled water and methanol to produce a concentration of 100mg/ml, the solution was then diluted with water to produce a series of concentrations 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Preparation of Agar Plate

40g of agar powder was weighed. The agar was dissolved in 1L of distilled water. Magnetic stirrer was used to stir the mixture to ensure proper mixing. After stirring, the agar solution was autoclaved for sterilization at temperature of 121°C for 15 minutes. After autoclave, the hot sterilized nutrient agar solution was poured into the petri-plates and cool to solidify.

Preparation of the Disk and Inoculum

From the four different concentrations of complexes which were 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. Sterile disk punched using Whatman No. 2 filter paper were impregnated into each of the respective tubes allowed to soak for 30 minutes and dispensed in each of the already seeded plates of *Candida albicans*. Ciprofloxacin was used as a fungal positive control while fresh disc was used as a negative control and incubated at 37⁰ C for 24 hours.

Determination of Zone of Inhibition

The antifungal activity of *Mitracarpus scaber* extracts against the test fungi was evaluated using agar well diffusion method for susceptibility testing. Sabouraud Dextrose agar were inoculated with 0.1ml of standardized inoculum of fungus 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 of the extract was dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for 10 minutes, allowing the diffusion of the extract into the incubation at 37 °C for 24 hours in an incubator.

Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution technique was employed equal volume (10 ml) of double-strength SDA was mixed with the extracts, 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.

Determination of Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentrations (MFCs) were determined by subculturing of 2 µl from each of the wells showing no growth into micro titer plates containing 100 µl of broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Positive and negative controls were set up to confirm the viability of the organism (positive control).

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of the leaves of *Mitracarpus scaber*

Metabolite	Aqueous extract	Methanol extract
Alkaloid	+	+
Flavonoid	+	-
Saponins	+	+
Glycoside	+	+
Tannins	++	+
Steroid	+	+
Terpenoids	+	++
Phenol	++	++
Anthraquinones	-	-

+ = present, ++ = present in higher quantity, - = absent

Table 2: Result of Antifungal activity of the Methanol Extract of *M. Scaber*.

Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MIC	MFC
<i>Candida albicans</i>	20	18	14	11	12.5	25
<i>Taenia capititis</i>	ND	ND	ND	ND		

Key: ND = Not detected.

Table 3: Result of Antifungal activity of the aqueous Extract of *M. Scaber*.

Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MIC	MFC
<i>Candida albicans</i>	14	12	10	08	12.5	25
<i>Taenia capititis</i>	ND	ND	ND	ND		

Key: ND = Not detected

DISCUSSION

The plant sample was extracted with water and methanol to yield light green and dark green aqueous extract and methanol extract, respectively. Alkaloid, steroid, terpenoids, tannins, glycoside, phenols were detected in both extracts while anthraquinones was absent. Flavonoid was detected in the aqueous extract but absent in methanol extract as in (Table 1).

Phytochemical analysis of the plant extracts had revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroid/terpenoids 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 (Ubani *et al.*, 2012).

Antifungal activity of both extracts only showed inhibition on *Candida albicans* while no inhibition was observed on *Taenia capitis*, The MIC and MFC of the extracts recorded respective values of 12.5 mg/ml and 25 mg/ml only against *Candida albicans* as in (Table 2 and 3).

CONCLUSION

The methanol and aqueous 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 and as the MFC of the extracts is higher than that of the MIC then the compounds are fungistatic. This justifies the claim for the traditional use of the plant in the treatment of skin infections.

RECOMMENDATION

Based on the result obtained above, it is clear that *Mitracarpus scaber* plant extract in both aqueous and methanol solution provide a great phytochemicals with a very good pharmacological activities therefore this plant is highly recommended. If further research were to be carried out, it is recommended that extensive clinical trials should be carried out on antifungal activity of *Mitracarpus Scaber* to synthesize fungal drugs. As claimed in traditional medicine, *Mitracarpus Scaber* may be useful in many cases, further work should be carried out on it to determine other pharmacological activities.

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