PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITIES OF *Mitracarpus scaber* CRUDE EXTRACTS ON SOME PATHOGENIC FUNGI.

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Abstract

Preliminary phytochemicals screening of acetone, aqueous and methanol extracts of Mitracarpus scaber was carried out using Harbone and Hassan's method. The phytochemical screening of acetone extract revealed the presence of anthroquinone, flavonoids, saponin and tannin, while methanol extract possessed anthroquinone, saponin, steroid, flavonoids and tannins. However, aqueous extract revealed the presence of tannins and flavonoids, while alkaloid, terpenes, resin, reducing sugar were absent in all extracts. The antifungal activities of acetone, aqueous and methanol extracts of Mitracarpus scaber were analysed using discs diffusion method. After 72 hours of incubation, both extracts at 10mg/disc inhibited the growth of Aspergillus fumigatus, Aspergillus flavus, *Aspergillus niger*, Mucor mucedo, Microsporium canis, Fusarium solani, Candida albican, Epidermophyton floccosum and Trichopyton verrucosum. The methanol extract of Mitracarpu sscaber was more active than acetone and aqueous extracts. The activities of the extracts may be responsible for their utilization in the traditional treatment of various ailments associated with the test organisms.

Keywords: Antifungal Activities, Phytochemical, Screening, Isolates, disc and *Mitracarpus scaber*.

Introduction

Traditional medicinal plants are rapidly growing and are used globally in various treatments for example; African traditional medicine based immune booster and infectious diseases (Yuan *et al*., 2016). Plant derived drugs remain an important resource, especially in developing countries to combat serious diseases. In recent years, infections have increased to great extent and resistance against antibiotics becomes an even increasing therapeutic problem (Austin *et al*., 1999). For many years, interested in new safer and more effective antifungal agents has grown with the increasing incidence of fungal infections. In the science of natural products, the antifungal activities of small and higher plants remain largely unexplored compared with that of other micro-organism (Sammy *et al*., 1998).

Fungal disease may not be as common as other microbial infections but when present,

they could be difficult to eradicate especially in immune suppressed situations (Bryce, 1992). Increasing prices of drugs especially in development countries need to search for alternative drugs becomes imperative. In Nigeria, the crude extracts of some local plants are used by the natives to cure fungal diseases in man and these include *Mitracarpus scaber*, *Richandia braciliensis*, *Crossopteryos febrifuga*, *Melochia corehorifolia* and *Triumfetta pentanda* (Mann *et al*., 2003).

Mitracarpus scaber is herb widely distributed in the Northern part of Nigeria especially in Niger, Kwara state and Federal Capital territory (F.C.T), Abuja. During the rainy season, the plant leaves are used in traditional medicine, for the treatment of sores and skin diseases among children. This is more popular among the Nupe tribe as "Eka'a "(Ringworm). Several studies on antifungal substances from plants have been conducted by a number of Authors (Ali, 2006 and Jabiru, 2010). Bioactive constituents of plant include the alkaloids saponins, steroid, tannins, terpenes, phenols, quinines, flavonoids and resins. Research on bioactive substances from plant sources has scope and could lead to the provision of value-added (Fadayi *et al*., 1987; Odebiyi, and Sofowora, 1978).

In this study, the antifungal activity of the crude extracts of *Mitracarpus scaber* was investigated. The bioactive constituents of plant were further screened to ascertain therapeutic potency of the plant.

Materials and methods

Collection and identification of the plant material.

The plant material used was collected from Alhaji Bagudu Waziri farm, popularly Known as (BCCC) near Wuya-Suman village along Kutigi–Mokwa road, Niger state, Nigeria. The plant material was authenticated using text vernacular names of medicinal plant or literature from Mann *et al.* (2003). This was done by Dr Adebola of Department of Biological sciences, Federal University of Technology, Minna, Niger state. A voucher specimen of the plant was deposited at Department of Biological Science, Federal University of Technology, Minna. The whole plants were air dry for seven days. The dried materials were ground using mortar and pestle and sieve with a mesh of size (0.50mm). The powdered samples obtained were stored in clean bottle at ambient temperature until when needed for use (Hassan *et al.*, 2005).

Test fungal isolates

The fungal isolates used were obtained from Microbiology Laboratory of Federal University of Technology, Minna, Niger state. The isolates include *Aspergillus flavus*, *Aspergillus solani*, *Aspergillus fumigates*, *Aspergillus niger*, *Epidermatophyton* sp., *Mucor mucedo*, *Microsporium canis* and *Trichophyton verrucosum*. The fungi were stored on Sabourand dextrose agar (SDA) slants in the refrigerator at 4^0C prior to use (Chessbrough, 2000).

Extraction of plant material

A quantity (40g) grams of the powdered material was weighed using automated balance and placed or dispensed into 200ml of methanol, acetone and aqueous in conical flask 500ml capacity respectively. The

mixture was allowed to stand overnight. The mixture was passed through sterile cotton wool placed in sterile glass funnels to separate the extracts from the residue (Hassan *et al*., 2005). The extracts was concentrated using a rotary evaporator. The dried crude aqueous, acetone and methanol extracts were reconstituted with 10ml of glycerol in order to impregnate the improvised paper discs.

Preparation of paper discs.

Improvised discs measuring 8 mm in diameter were cut from absorbent Whatman No1 filter paper using paper puncher. The discs were transferred into clean bottle and sterilized in hot air oven at 160^{o}C for 90 minutes. The reconstituted extract of 10ml was used for the preparation of paper disc. Thirty (30) sterile paper discs were put into two different bottle that contain 10ml of aqueous and methanol extracts respectively in order to absorb the 10ml volumes of the concentrated extract thereby become impregnated with the extracts. The impregnated discs were dried in an oven operated at 70^{o}C over 2 hours and kept ready for used (Chessbrough, 2000).

Phytochemical screening of acetone, aqueous and methanolic extract of *Mitracarpus scaber*

Phytochemical screening for major chemical constituent was carried out using standard qualitative methods. The phytochemical ingredients tested include flavonoids, tannins, saponins, alkaloids, steroid, phenol, terpenoids and glycosides.

Test for anthroquinone

To 4mL of each extract in a test tube, 4mL of 100% ammonium solution was added. A pink violet or red coloration in the ammoniac layer in each test tube indicated the presence of anthroquinone (Odebiyi and Sofowora, 1978).

Test for alkaloids

To 3mL of extract introduced into different test tube, 1mL of 1% of hydrochloric acid (HCL) was added. The mixture was later treated with few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids (0gukwe *et al*., 2004).

Test for flavonoids

To 1mL of each extract, 3 drops of ammonia solution was added. Half (0.5) mL of concentrated HCL was further added to the mixture. A pale brown coloration indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for glycosides

To 1 mL of each extract, 2 mL of acetic acid was added and then cool in an ice bath at 4^{0}C. To the mixture, 1 ml of conc. H_2SO4 was added drop wise. Oil layer formed on top of the solution indicated the presence of glycosides (Ogukwe et al., 2004; Elmahmood and Amey, 2007; Odebiyi and Sofowora, 1978).

Test for resins

To 5mL of each extract, 5mL of copper acetate solution was added and shaken vigorously and then allowed to separate. A reddish brown precipitate indicated the presence of resins (Elmahmood and Doughari, 2008).

Test for saponins

Half milliliter (0.5mL) of each extract in a test tube, 5.0mL of distilled water was added. The mixture was then shaken vigorously for 2 minutes; the froth formation indicated the presence of saponins (Hassan *et al*., 2005).

Test for steroid

To 1mL of each extract, 1mL of concentrated tetraoxosulphate (IV) acid (H_2SO_4) was added. A reddish coloration indicated the presence of steroid (Hassan *et al*., 2005).

Test for tannins

To 5mL of each extract, 2 drops of 5% $FeCL_3$ was added. A dirty green precipitate indicated the presence of tannins (Ogukwe *et al*., 2004).

Determination of antifungal activity of *Mitracarpus scab*er extracts

The Paper disc technique described by Kirby–Baurer *et al*. (1968) was used for antifungal assay. Freshly prepared plates of Sabourand dextrose agar were allowed to solidify. A micropipette was used to introduce 0.lml of spore or conidia suspension on the agar plate before spreading with a glass spread rod under sterile conditions. Paper discs impregnated with extracts were placed aseptically and pressed firmly on the surface of the agar plate and later incubated at 28^{o}C for 72 hours. The sterile paper discs (not impregnated) serve as control. The agar plates were examined for evidence of inhibition which is usually indicated by an area for the discs completely devoid of fungal growth observed. The diameter of zones of inhibition was determined using transparent plastic ruler, recorded and expressed in millimetre.

Results

The result of phytochemicals screening showed in Table 1 indicated that aqueous extract possessed flavonoids and tannin, while acetone extract have anthraquinone, flavonoids, saponin, tannin. Alkaloids, steroid, resin, terpenes and reducing sugar were found to be absent in both aqueous and acetone extracts of *Mitracarpus scaber*. However, anthraquinone, flavonoids, saponin, steroid and tannin were found to be present in methanol extract. Table 2 showed the result of antifungal activity of aqueous and methanol extract of *Mitracarpu sscaber* at concentration of 10mg/disc against the test isolates. *Candida albican* was more susceptible to both methanol, aqueous and acetone extract with diameter of zone of inhibition of 19mm, 17 and 15mm respectively. However, *Fusarium solani* was less susceptible to aqueous and acetone extract with diameter of zone of inhibition of 8mm, while *Epidermaphyton floccosum* was also less susceptible to acetone extract with diameter of zone of inhibition of 11mm. Methanol extract was more active against all the test fungi isolates.

Table1: Phytochemical properties of acetone, aqueous and methanol extracts of *Mitracrpus scarber*

Phytochemical constituents	Acetone extract	Aqueous extract	Methanol extract
Alkaloids	–	–	–
Anthroquinone	+	–	+
Flavonoids	+	++	++
Saponin	+	–	++
Steroid	–	–	+
Resin	–	–	–
Reducing sugar	–	–	–
Tannin	+	+	++
Terpenes	–	–	–

Key: - - = Absent += Present ++=Moderately present

Table 2: Antifungal activities of crude acetone, aqueous and methanolic extract of *Mitracarpus scaber* on test fungal isolates

Test isolates	Diameter of zone of inhibition (mm) Concentration of extract (mg/disc)			
	AQE10mg/disc	ACE 10mg/disc	MTE10mg/ml	DST Water (Control)
Aspergillus flavus	14.0	11.0	15.0	0.0
Aspergillus fumigatus	13.0	11.0	14.0	0.0
Aspergillus niger	09.0	08.0	13.0	0.0
Candida albican	17.0	15.0	19.0	0.0
Epidermatophyton floccosusum	11.0	09.0	11.0	0.0
Fusarium solani	08.0	08.0	11.0	0.0
Microsporium canis	11.0	10.0	12.0	0.0
Mucor mucedo	11.0	09.0	12.5	0.0
Trichophyton verrucosum	13.0	11.0	13.5	0.0

Key: 0.0=No zone of inhibition, AQE=Aqueous extract, ACE=Acetone extract, MTE=Methanol extract, DST Water=Distilled water.

Discussion

The pronounced presence of flavonoids, tannin, anthroquinone, saponin, and fairly presence of steroid from the whole plant (*Mitracarpus scaber*) as presented in table 1 clearly indicated the phytotherapeutic potentials of the plant. However, aqueous extract of *Mitracarpus scaber* showed in table1 revealed the presence of flavonoids at high concentration, tannin at low concentration and other compounds tested were absent. Also methanol extract revealed the presence of flavonoids, saponin, steroid and tannin, while acetone indicated the presence of anthroquinone, flavonoids, saponin and tannins. The phytochemicals evaluation of bioactive compounds of *Mitracarpus scaber* extracts is in line with findings of Suleiman and Ali (2004) who reported in their studies of antifungal activity of *Mitracarpus scaber* crude extracts. The result of this study is a bit contrary to the findings of Cimaga *et al*. (2004), who reported in their studies of Antibacterial and antifungal activities of extracts and fractions of *MItracarpus scaber*.

The result of in-vitro antifungal activity of aqueous extract of the *Mitracarpus scaber* exhibited an average antifungal effect on the test fungi with respective zone of their inhibition namely *Aspergillus flavus* (14mm), *Aspergillus fumigatus* (13mm), *Aspergillus niger* (9mm), *Candida albican* (17mm), *Epidermatophyton floccosum* (11mm), *Fusarium solani* (8mm), *Microsporium canis* (11mm), *Mucor mucedo* (12.5mm) and *Tricophyton verrucosum* (13mm). The high sensitivity showed by the extract on the fungal isolates may be either due to the presence of anthroquinone, flavonoids and tannins.

The implication of phytochemicals: Flavonoids was one of the major constitute detected in this study and have been reported to have anti-oxidant activity, protecting cells against oxidative damage

and reduce the risk of developing certain types of cancer (William *et al.,* 1996). Saponins are glycoside in nature and have been reported to have expectorant and cardio-tonic activity (Clarke, 1975; Finar, 1989). Also saponin has hypoglycaemic and anti-diabetic effects which are very useful in the management of diabetes mellitus (Anila *et al*., 2000). In medicine, saponin is used in hypercholesterolemia, hyperglycemias, antioxidant, anticancer, anti-inflammatory, weight loss and also has antifungal properties (Anila *et al*., 2000; Sui *et al*., 1994). Plant tannin have been recognized for pharmacological properties and also known to make trees and shrubs a difficult meal for many caterpillars (Heslen, 1989). Tannins are reported to exhibit antiviral, antibacterial, antitumor activities and capable of inhibiting HIV replication selectively (Heslen, 1989).

The presence of phytochemical ingredients (compounds) may be attributed to their use by traditional medicine practitioners and biomedicine health care system in the treatment of fungal infections. The result of the antifungal activity shows that the plant could be used to treat sores, rashes, boils, scabies and other skin diseases caused by *Microsporium canis, Trychophyton verrucosum* and *Epidermatophyton floccusum*. The inhibitory activities exhibited by the extracts tend to agree with the report of Irobi *et al*. (1993). The methanol extract of *Mitracarpus scaber*

exihibited greater inhibitory effect on the test organism than the aqueous and acetone extracts. This is due to the ability of methanol to extract essential oil and other compounds inhibitory to the test organism.

Conclusion

The study has confirmed that the crude extracts of *Mitracarpus scaber* contain anthroquinone, flavonoids, saponin, steroid and tannin. This study has provided the basis for the uses of *Mitracarpus scaber* in the treatment of fungal infections.

Recommendation

The potential antifungal effect of the *Mitracarpus scaber* could be enhanced if extraction is done through thin layer and column chromatography in order to have a pure bio-active compound which could be further test against fungal isolates.

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