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Phytochemical and Antifungal Activity of Crude Extracts of *Mitracarpus scaber* Against Some Dermatophytes

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Abstract— *The aim of this study is to determine the antifungal activity of Mitracarpus scaber against some dermatophytes. The Mitracarpus scaber was extracted using ethanol and aqueous as solvents. The phytochemical constituents indicated that the plant contains saponins, tannins, terpenoids, flavonoids, alkaloids, steroids and phenol for ethanol extract while aqueous extract only contains saponins, terpenoids, flavonoids and alkaloids. The antifungal potential of M. scaber extracts were tested on T. mentagropytes, T. tonsurans and E. floccosum using poisoned food technique. Ethanol extract inhibited the growth of all the test organisms at concentration of 320mg/ml while the aqueous extract showed varied inhibition rate of 100%, 85% and 90% against T. mentagropytes, T. tonsurans and E. floccosum respectively. The MIC of the ethanolic extract was 20mg/ml against (T. mentagrophytes) to 80mg/ml against (E. floccosum) while aqueous extract ranged from 80mg/ml (T. mentagrophytes) to 160mg/ml against T. tonsurans and E. floccosum. The MFC of the ethanolic extract ranged from 20mg/ml (T. mentagropytes) to 160mg/ml (E. floccosum) and from 80mg/ml (T. mentagrophytes) to 320mg/ml against T. tonsurans and E. floccosum for aqueous extract. Hence M. scaber can be further developed to utilize its medicinal and pharmaceutical values.*

Keywords— *Antifungal, Susceptibility, Minimum inhibitory concentration (MIC), Minimum Fungicidal Concentration (MFC), Mitracarpus scaber, Phytochemicals.*

I. INTRODUCTION

Dermatophytes are parasitic fungus that invades the skin, hair and nails due to their ability to digest and utilize keratin as a substrate. They are referred to as keritonophilic and keritionolytic fungi. They secrete proteases that degrade skin and hair protein, which provide mechanism for fungal adherence and invasion of the skin (Summerbell, 2003). Dermatophytes are one of the most common cutaneous infections of man and pose public health problems to man which can be disfiguring, persistent and need to be treated with different antifungal agents (Nweze *et al*., 2007).

Most antifungal agent has broad spectrum activity although treatment of dermatophytosis has become difficult due to resistant to antifungal agents because of long term use and adverse effects associated with their administration (toxicity to humans). Often times these antifungal agents are not affordable by a large percentage of population (WHO, 2012).

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Avijgan *et al*., 2010).

Research have shown that these plants are rich in a wide variety of secondary metabolite such as tannins, alkaloids, cardiac glycoside, flavonoids, anthraquinones etc. which have been found in-vitro to have antimicrobial properties and different method are used with solvents to soften and break the plant's cell wall to release the soluble phytochemicals (Cowan, 1999).

The search for antimicrobial agents from plants has gathered interest in the last few decades and the geographical location of these plants make the phytochemicals differs greatly and varying in concentration depending on the environment where the leaves were harvested. Also plants parts/constituents have been use traditionally for the treatment of dermatophytosis but the minimum inhibitory concentration and minimum fungicidal concentration are not known (Inbathamizh *et al.,* 2013). However, results generated from many studies cannot be directly compared due to the lack of standardization in particular antimicrobial employed and results differ between authors (Ncube *et al*., 2008).

This research is aimed at determining the antifungal activity of *Mitracarpus scaber* against some dermatophytes.

II. MATERIALS AND METHODS

A. Collection and Extraction of plant material

Fresh leaves of *Mitracarpus scaber* was obtained in Modibbo Adama University of Technology, Yola premises and was identified at Plant Science Department, MAUTECH. *Mitracarpus scaber* leaves were air dried at room temperature. The dried leaves were crushed into powder with a mortar and pestle. The extraction was done using the cold maceration method. The plant material was soaked in a container with water and ethanol separately (solvent to sample 10:1 v/w). It was allowed to stand at room temperature for 3 days (Handa *et al*., 2008). The extract was filtered through Whatman No1 filter paper, the filtrate was allowed to dried and stored until needed.

B Determination of the presence of bioactive compounds in the crude extracts

Test for the presence of saponins

0.1g of the extract was added to 10ml of distilled water in a test tube, the test tube was corked and shaken. The

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production of frothing indicates the presence of saponins (Sofowora, 1993).

Test for the presence of tannins

A small portion of the extract was dissolved in 10ml of distilled water and then filtered, 2 drops of freshly prepared 5% ferric chloride solution was added to the filtrate. A bluish black color indicates the presence of tannins (Trease and Evans, 1989).

Test for the presence of cardiac glycoside

0.5g of the extract was diluted in 5ml of distilled water and 2ml of glacial acetic acid containing 1 drop of ferric chloride solution was added, followed by adding 1ml of concentrated sulphuric acid. A brown ring formation at the interface indicates the presence of glycoside. A violet ring may appear below the brown ring (Sofowora, 1993).

Test for the presence of flavonoids

Small portion of the extract was boiled with 5ml of water and filtered. To 2 ml of the filtrate, 2 drops of freshly prepared ferric chloride solution was added. A dark green coloration indicated the presence of flavonoids (Trease and Evans, 1989).

Test for the presence of Terpenoids

To small portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a layer. Formation of pink colour indicated the presence of terpenoids (Trease and Evans, 1996).

Test for the presence of alkaloids

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of mayer's reagent was added. Presence of green colour or white indicates the presence of alkaloids (Trease and Evans, 1996).

Test for the presences of Phenols

To 1ml of the extract mixed with 2ml of distilled, 5 drops of 10% ferric chloride was added. The formation of blue or green coloration n indicates the presence of phenols (Sofowora, 1993).

Test for the presences of steroids

4 ml of extract was treated with 0.5ml of acetic anhydride and 0.5ml of acetic acid. Then concentrated sulphuric acid was added slowly and reddish brown ring color indicates the presence of steroids (Trease and Evans, 1996).

C. Isolation of Dermatophytes

Clinical isolates of dermatophytes were collected from the Department of dermatophytosis, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Test organisms was sub-cultured and grown on Sabouraud dextrose agar

D. Determination of antifungal activity of crude extract

The antifungal activity was determined using poisoned food technique (Adjou, 2012). To every 15 ml of molten sabouraud dextrose agar (SDA), 5ml of the extracts was incorporated into it and mixed for thorough diffusion, it was poured into petri dishes and allowed to solidify at room temperature. A hole (6mm in diameter) was bored at the center of the agar using a sterile cork borer. Agar disc with mycelia (6mm in diameter) was cut from the periphery of actively growing regions of the pure culture using a sterile cork borer and was aseptically inoculated at the center of the petri dishes.

Ketoconazole was used as positive control, while water was used as negative control and the plates was incubated at 28°C for 7days.

The percentage inhibition of mycelia growth of the test fungi was calculated using the formula by Philippe *et al*. (2012).

Inhibition of mycelial growth (%) = dc-dt/dc X 100

Where: Dc= growth of mycelial colony in the control set subtracting the diameter of inoculum disc.

dt= growth of mycelial colony in the treatment set subtracting the diameter of inoculum disc.

E. Determination of minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC)

The crude extract was prepared by reconstituting the dried extract in water at different concentration: 10, 20, 40, 80, 160 and 320 mg/ml respectively as described by Hafidh *et al*. (2011). The minimum inhibitory concentration was taken as the lowest concentration of crude extract at which no growth occurs. The minimum fungicidal concentration was determined by re-inoculating the plte with the lowest concentration of extract that did not show visible growth as described by Hafidh *et al*. (2011).

III. Result and Discussion

The phytochemical screening of the crude ethanolic and aqueous extracts of *M. scaber* carried out showed that the ethanolic extract contains seven (7) bioactive components: saponins, tannins, terpenoids, flavonoids, alkaloids, steroids and phenol while the aqueous extract contains Four (4) bioactive components namely saponins, terpenoids, flavonoids and alkaloids as presented in Table 1.

TABLE 1. Phytochemical bioactive compounds in the crude extracts of *Mitracarpus scaber*

Bioactive components	**Crude Extracts of *M. scaber***	
	Ethanol extract	**Aqueous extract**
Saponins	+	+
Tannins	+	-
Terpenoids	+	+
Flavonoids	+	+
Alkaloids	+	+
Steroids	+	-
Phenols	+	-
Cardiac glycoside	+	-

Key: - = Absent, + = Present

The result of the determination of the antifungal activity of the crude ethanol and aqueous extracts of *M. scaber* against three dermatophytes is presented in Table 2. At concentration of 320 mg/ml of the ethanol extract, growth of all tested organisms was inhibited. The aqueous extract at a concentration of 320 mg/ml showed varied inhibition rate of 100%, 85% and 90% against *T. mentagropytes*, *T. tonsurans* and *E. floccosum* respectively.

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TABLE 2. Antifungal Activity of the crude Extracts of *M. scaber*

Organisms	Crude Extracts of *M. scaber* (%)		
	Ethanol extract	Aqueous extract	Control
T. mentagrophytes	100	100	100
T. tonsurans	100	85	100
E. floccosum	100	90	100

The minimum inhibitory concentration of the crude ethanol and aqueous extracts of *M. scaber* is shown in Table 3. The MIC of the ethanolic extract ranged from 20mg/ml against (*T. mentagrophytes*), 40 mg/ml against (*T. tonsurans)* to 80mg/ml against (*E. floccosum)*. The MIC of the aqueous extract ranged from 80mg/ml against (*T. mentagrophytes*) to 160mg/ml against *T. tonsurans* and *E. floccosum.*

TABLE 3. The minimum inhibitory concentration of crude ethanol and aqueous extracts of *M. scaber*

Organisms	Crude Extracts of *M. scaber* (%)	
	Ethanol extract	Aqueous extract
T. mentagrophytes	20	80
T. tonsurans	40	160
E. floccosum	80	160

The determination of the minimum fungicidal concentration of crude ethanolic and aqueous extract of *M. scaber* is presented in Table 4. The results showed that the MFC of the ethanolic extract ranged from 20mg/ml against (*T. mentagropytes)* to 160mg/ml (*E. floccosum)* while that of the aqueous extract ranged from 80mg/ml against (*T. mentagrophytes)* to 320mg/ml against *T. tonsurans* and *E. floccosum*.

TABLE 4. The minimum Fungicidal Concentration of crude ethanol and aqueous extract of *M. scaber*

Organisms	Crude Extracts of *M. scaber* (%)	
	Ethanol extract	Aqueous extract
T. mentagrophytes	80	80
T. tonsurans	160	320
E. floccosum	160	320

IV. Discussion

This study was carried out to determine the antifungal activity of crude ethanolic and aqueous extract of *M. scaber* against clinical isolates of *T. mentagropytes*, *T. tonsurans* and *E. floccosum.* In our search, both aqueous and ethanolic extracts of *M. scaber* have bioactive components. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins which are responsible for the antimicrobial properties of such plants (Ubani *et al.,* 2012). The results thus indicate that both ethanolic and aqueous extracts of *M. scaber* may exhibit antimicrobial activity. Seven phytochemicals were detected in the ethanol extracts while four were in aqueous extract. This could be due to the difference in the polarity of the extracting solvents (Ncube *et al.,* 2008). The results obtained agree with the work of Abere *et al,* (2007) and Onawunmi *et al.,* (2012). There was however a difference in the type and numbers of bioactive components present in crude ethanol extracts. Onawunmi *et al.* (2012) however reported the presence of 4 phytochemicals (tannins, flavonoids, saponins and phenols) and Namadina *et al*., (2020) reported Flavonoid, steroid, triterpenes, tannins, carbohydrate, glycoside, phenols from methanolic extracts of *M. scaber*, while Barile *et al*. (2007) reported the presence of six phytochemicals (saponins, terpenoids, flavonoids, alkaloids, tannins, and steroids) from ethanolic extracts. This difference in number and type of phytochemicals could be as a result of difference in extracting solvent used (ethanol and methanol) and geographical location where the plants were obtained (Inbathamizh *et al.,* 2013).

The antifungal potential of the plant extracts indicate that the two extracts exhibit antifungal activity against the entire test organism although the effectiveness varied between organisms. The ethanolic extract had a 100% inhibition rate at a concentration of 320mg/ml against the entire test organisms, the aqueous extract showed percentage inhibition rates at 100%, 85% and 90% against *T. mentagropytes*, *T. tonsurans* and *E. floccosum*. In agreement with our findings, Shinkafi *et al*. (2011) reported that the methanolic extract of *M. scaber* exhibited antifungal activity against *T. mentagrophytes, T. tonsuran and M. gyseum*. Crude aqueous and ethanol extracts of *M. scaber* contain bioactive components and can thus exhibit antimicrobial activity. The antifungal activities of the crude ethanol extract seemed to be more effective than that of aqueous extract. The reason for this differences may be attributed to the solubility level of the phyto-constituents in the extracting solvents and the ability of ethanol to bring out the main bioactive component such as tannins, saponins which are responsible for its antifungal activities (George *et al.,* 2002).

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the crude ethanolic and aqueous extract of *M. scaber* showed that the crude ethanol extract showed better activity at lower concentrations than the aqueous extract. The MIC of the crude ethanolic extract against *T. mentagropytes, T. tonsurans* and *E. floccosum* was 20mg/ml, 40mg/ml and 80mg/ml respectively while the aqueous extract had MIC of 80mg/ml against *T. mentagropytes* and 160mg/ml against *T. tonsurans* and *E. floccosum.* The MFC results had similar trend. The MFC of the ethanolic extract was 20mg/ml, 80mg/ml and 160mg/ml against *T. mentagropytes*, *T. tonsurans* and *E. floccosum* respectively while the MFC of the aqueous extract was 80mg/ml against *T. mentagropytes* and 320mg/ml against both *T. tosurans* and *E. floccosum*. Ubani (2012) reported similar observation that methanolic extract of *M. scaber* exert inhibitory effect on some fungi (*T. mentagropytes* and *T. rubrum*) at a concentration of 100mg/ml to 200mg/ml.

V. Conclusion

In this study, crude ethanol and aqueous extracts of *M. scaber* contain bioactive components possess antifungal activity against the test organisms. The crude ethanol extract however contained more bioactive components, was more effective at lower concentration and showed better antifungal activity than the aqueous extract on the clinical isolate of *T. mentagropytes, T. tonsurans* and *E. floccosum.* Therefore,

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ethanol is a more effective solvent for obtaining the bioactive components in M. scaber.

VI. RECOMMENDATION

In this study, the extract was found to possess considerable antifungal properties. This partly justifies the claim for the traditional use of the plant in the treatment of skin infections. It is therefore recommended that M. scaber should use at the tested concentration for effective treatment.

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