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Antimicrobial assessment of extracts of the stem piths of *Alchornea cordifolia* and *Senna alata* against *Trichophyton mentagrophyte* and *Trichophyton verrucosum*

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Abstract

Fungal infections are often chronic, hence requiring long-term treatment for them to be effectively treated. The antifungal activity of the ethylacetate and hot water extracts of the stem piths of *Alchonea cordifolia* and *Senna alata* were tested against *Trichophyton mentagrophyte* and *Trichophyton verrucosum*. Extracts of both crude drugs were obtained by maceration using ethyl acetate and hot water respectively. Identification of dermatophytes, microscopic and macroscopic examination of cultures was carried out using standard procedures. Phytoconstituents of both plants were evaluated qualitatively using standard methods. The antifungal activity of extracts was tested using the agar well diffusion method. Minimum inhibitory concentration (MIC) of both extracts was carried out using the agar dilution method. The minimum fungicidal concentration (MFC) was also done using standard procedures. Phytochemical analysis showed the presence of saponins and tannins in both crude extracts. *Senna alata* showed the highest antifungal activity against both dermatophyteswith the zone of inhibition ranging from 12.85±2.44mm to 20.85±2.44mm. *Trichophyton verrucosum* was most susceptible with the zone of inhibition of 20.85±2.44mm at a concentration of 2.50mg/ml. The hot water extract of the stem pith of *Senna alata* and *Alchonea cordifolia* did not inhibit any of the dermatophytes. Conclusively, the results of this study support the traditional use of *Senna alata* for the treatment and management of ringworm and athlete's foot caused by *Trichophyton mentagrophyte* and *Trichophyton verrucosum*.

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Introduction

Traditional medicine is the oldest method of managing diseases. Over the years, plants have been used in different parts of the world, including Nigeria to treat human diseases and infections (Nweke *et al*., 2004). For several years, humans have been faced with enormous health challenges that have made them depend on medicinal plants for their survival. According to WHO (1996), medicinal plants are finished, labeled, medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations, therefore, whether in crude state or as plant preparations. Historically, plants have been used as a good source for novel drug compounds, as plant-derived medicines have made large contributions to human health and well-being (Igbinosa *et al*., 2009). The role of plants in the development of new drugs is in two-folds. Firstly, they may become the base for the development of novel drugs or; secondly, they could serve as a phytomedicine directly used in the treatment of diseases (Iwu, 1993). Different plant parts have also been used for the treatment of dermatophytes (Wokoma *et al*., 2007), respiratory tract infection and diabetes (Adeleke *et al*., 2006) and skin infections (Ekpo, 2009). In Nigeria, many plants are used against infectious diseases which today are frequent due to very poor hygienic conditions, high cost, and antimicrobial resistance to conventional antibiotics[8]. The incessant incidence of fungal infections together with the gradual rise in resistance of bacterial and fungal pathogens for antibiotics and antifungals highlights the need to establish an alternative source from medicinal plants.

Dermatophytes are a unique group of fungi that affect the keratinous tissues of lower animals and humans (Weitzman and Summerbell, 1995). They are known for their ability to invade the superficial layers of the epidermis, particularly, the stratum corneum and high keratin-concentration-containing appendages, the hair as well as nails of a living host (Santos *et al*., 1997). The antifungal activities of some plants have been reported by various researchers throughout the world. The use of antimycotic drugs in controlling superficial mycoses has been a long-time practice, but may not be used as a routine treatment for every case because of the cost, duration of treatment, and their notable impermeable nature. Some of the newer drugs such as clotrimazole, miconazole, fluconazole, itraconazole, and ketoconazole are also very effective but they can elicit undesirable side effects, which could be harmful to human health (Shahi *et al*., 1999).

The objective of this study revolves around determining the antimicrobial efficacy of the stem piths of both the hot water and ethylacetate extracts of *Alchornea cordifolia* and *Senna alata* against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* in comparison to griseofulvin which is the control drug, investigating the phytochemical constituents present in the plant extracts, ascertain the minimum inhibitory concentration of the plant extracts against the test dermatophytes as well as deducing the minimum fungicidal concentrations of the plant extracts against the test dermatophytes.

Materials and methods

Materials

The materials used for this study include:Ethylacetate (Guanghua chemical factory co, ltd) Shartau, ghuandghua, china, Sabouraud dextrose, and Mueller Hinton agar (Titan Biotech), Petri-dishes, methylated spirit, cotton wool, aluminium foil, scapel, spatula, conical flask, beaker, measuring cylinder, mortar, and pestle, Griseofulvin (Green field Pharmaceutical, China).

Isolation of Test Organisms

The method of Shinkafi and Manga (2011), was adopted with few modifications. Samples of infected hairs and toes were collected from infected individuals with clinical manifestations of dermatophytosis within Abraka, Delta State. The sites of infections were first cleaned with methylated spirit; specimens from the scalp and foot were collected by scraping outwards with a blunt scalpel, on a clean piece of paper and were folded to enclose the specimen. They were labeled and transferred to the Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy, Delta State University, Abraka. In accordance with the methods of Hartman and Rhode (1980), the specimen collected were identified

by subjecting to wet preparation of 10% potassium hydroxide (KOH) and viewed under the microscope at both low and high power magnification. The culture was done in triplicates. The specimen was inoculated into petri-dish containing sabouraud dextrose agar with 0.05% chloramphenicol. Plates were incubated at room temperature for 5 days and observed for growth. A series of sub-culturing was carried out until a pure culture that was free from contamination was observed. Macroscopic examination of the culture was carried out to colonial morphology and colonial characters. Also, with little modification, the microscopic examination of the culture was carried out using standard method of Mackie and McCartney (1999). Dermatophyte identification chart by Hardly diagnostic (2013) was used to identify the dermatophytes. The organisms identified were Trichophyton mentagrophyte and Trichophyton verrucosum.

Collection of Plant Samples and Preparation of Plant Extracts

The plants were collected from the botanical garden of Department of Pharmacognosy, Faculty of Pharmacy, Delta State University, Abraka. The stems were broken and the stem pith was aseptically removed. The stem piths were allowed to dry under room temperature, pulverized, weighed, and thereafter subjected to maceration using the standard method (Eqwakhide and Gimba, 2007). Both crude extracts were concentrated using a water bath to remove the solvents of extraction (ethyl-acetate and water). The concentrated extracts were stored in a refrigerator until they are needed for the research work.

Phytochemical Analysis

Extracts from the stem piths of *Alchornea cordifolia* and *Senna alata* were subjected to phytochemical tests to detect the presence of secondary metabolites such as alkaloids, tannins, saponins, cardiac glycosides, anthraquinones, phlobatannins, flavonoids and terpenes using procedures of Ekpo and Etim (2009) with little modification.

Antifungal Activity

Antifungal activity of extracts was tested using the agar well diffusion method described by Hassan *et al.* (2007), with little modification. An antifungal drug (Griseofulvin) was used as a standard drug. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) at ambient temperature until they produced spores. The fungal spores were harvested after sporulation by pouring a mixture of sterile distilled water on the surface of the plate, thereafter the spores were scraped with a sterile glass rod. The fungal spore suspension was evenly spread on the sabouraud dextrose agar (SDA) using a glass spreader. Wells were then bored into the agar plate using a sterile 6mm cork-borer and the wells were carefully filled with the solution of the extract to avoid spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1hr to allow for proper diffusion of the extracts into the media. 0.03mg/ml of griseofulvin solution was dispensed into the wells using sterile pipettes. Plates were incubated at room temperature for 96 hours and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with a griseofulvin concentration of 0.03mg/ml.

Minimum Inhibitory Concentration (MIC)

The agar dilution method of Ali-shtayeh and Abu-Ghdeib (1999) was adopted with minor adjustments. The test isolates were inoculated into sabouraud dextrose agar (SDA) plates and incubated at ambient temperature for seven (7) days to obtain young, actively growing cultures consisting of mycelia and conidia. Mueller Hinton agar was prepared, and the plant extracts were diluted serially in order to obtain different concentrations.

The required amount of plant extracts and griseofulvin was dissolved in 2ml sterile distilled water and then mixed with the amount of pre-sterilized Mueller Hinton agar required in order to give a final concentration of 15mg/ml. A mycelia disc, 5mm in diameter, cut from the periphery of the seven (7) day old cultures was aseptically inoculated onto the medium. In controls, sterile distilled water inoculated plates were then incubated at ambient temperatures, and the colony diameter was measured and recorded after seven (7) days.

Minimum Fungicidal Concentration (MFC)

It was carried out as described by Wokoma *et al* (2007), with little modification. The MFC was determined by culturing the content of the plate cultures that showed no visible growth in the MIC determination test described above. A loopful of the mixture contained in the plate was sub-cultured on freshly prepared sabouraud dextrose agar plates and incubated at ambient laboratory conditions for 7 days. The minimum fungicidal concentration was regarded as the lowest concentration of the extracts that did not permit any visible colony growth on agar medium after the period of re-incubation. The MFC test was set up in triplicates.

Results

Table 1. Phytochemical Screening Results.

Phytoconstituents	*A. cordifolia* Hot water extract	*A. cordifolia* Ethyl acetate extract	*S. alata* Hot water extract	*S. alata* Ethyl acetate extract
Alkaloid	-	-	-	+
Saponin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	-	+
Anthraquinone	+	+	-	+
Terpenes	-	-	-	+
Steroids	-	-	-	+
Tannin	+	+	+	+
Cardiac glycoside	+	+	-	+

+ Present -Absent

Table 1 shows the qualitative phytochemical analysis of the different extracts of both plants. For *Senna alata* ethyl-acetate extract, the analysis revealed the presence of alkaloids, tannins, anthraquinones, saponins, flavonoids, terpenes and steroids but phlobatannin was absent.

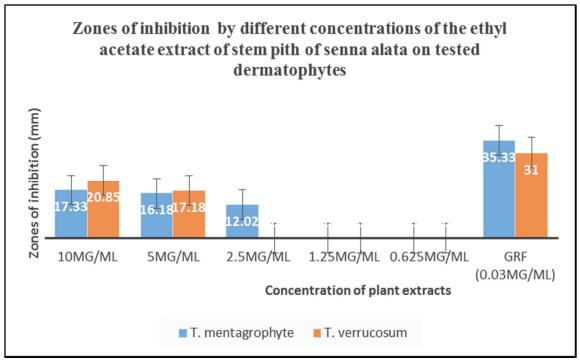

Fig. 1. Zones of inhibition of different concentration of ethyl-acetate extract of *Senna alata* stem pith against test dermatophytes.

The hot water extract indicated presence of only saponin and tannin. For *Alchornea cordifolia*, both the ethyl-acetate and the hot water extract contain similar phytoconstituents. The result showed presence of saponin, anthraquinones, flavonoids, tannins and cardiac glycosides. Alkaloids, terpenes, phlobatannin and steroids were absent.

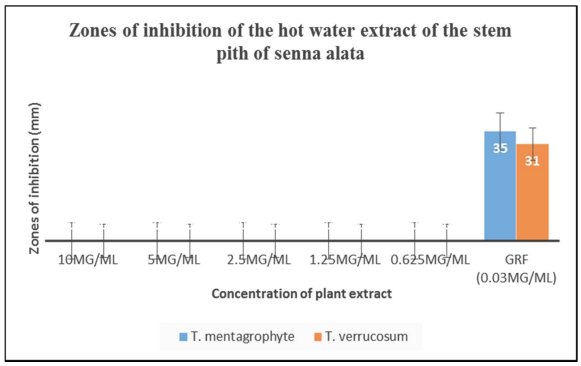

Fig. 2. Zones of inhibition of different concentration of the hot water extract of senna alata stem pith against test dermatophytes.

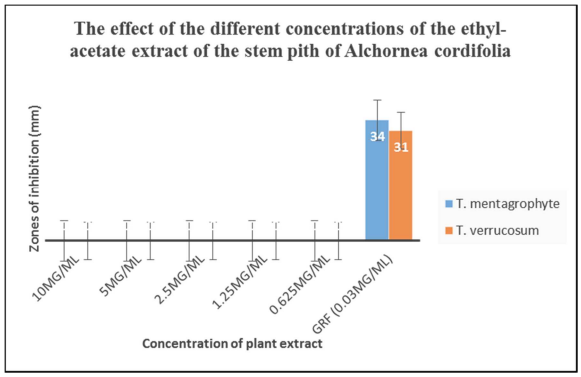

Fig. 3. The effect of the different concentrations of the ethyl-acetate extract of the stem pith of *Alchornea cordifolia*.

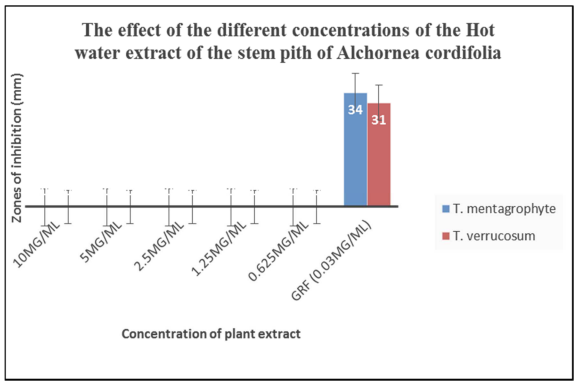

Fig. 4. The effect of the Hot water extract of the stem pith of *Alchornea cordifolia*.

Table 2. Minimum Inhibitory Concentration (MIC) of the ethyl-acetate extract of the stem pith of *Senna alata*.

Test dermatophyte	Ethylacetate pith extract (mg/ml)
T. mentagrophyte	2.50
T. verrucosum	5.00

Table 3. Minimum Fungicidal Concentration (MFC) of the ethyl-acetate extract of the stem pith of *Senna alata*.

Test dermatophyte	Ethyl acetate pith extract (mg/ml)
T. mentagrophyte	5.00
T. verrucosum	5.00

Discussion

The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases (Alim *et al*., 2009). The antifungal assay revealed that only the ethyl-acetate extract of *Senna alata* exhibited a fungal inhibitory effect against the test dermatophytes. The Minimum Inhibitory Concentration (MIC) of the aforementioned extract is 2.50mg/ml for *T. mentagrophyte* and 5.00mg/ml for *T. verrucosum*, hence the result of this study is in tandem with the report of Sule *et al* (2011). However, the ability of this extract to inhibit the growth of these test dermatophytes is a clear indication of the broad-spectrum antimicrobial potential of *S. alata*.

Furthermore, only Trichophyton mentagrophytes were inhibited at a lower concentration of 2.50mg/ml of the ethyl-acetate extract. The plant extracts exhibited a lower antimicrobial effect in comparison with the control drug (Griseofulvin). This contradicted the findings of the previous study in Malaysia (Ibrahim and Osman, 1995) that reported a higher antifungal activity of the ethanolic extracts of the senna plant against dermatophytic fungi. The investigations on the phytochemical properties of the ethyl-acetate extract of *Senna alata* revealed the presence of alkaloids, carbohydrates, anthraquinones, tannins, saponin, flavonoid, terpenes, and steroids which agrees with the report of Doughari and Okafor (2007). These bioactive compounds have been reported to be used for protection against bacterial, fungal, and pesticidal infections and are responsible for antimicrobial activity (Srinivasan *et al*., 2001). These secondary metabolites exert antimicrobial activity through different mechanisms and have been reported previously (Owolaye *et al*., 2005) to have an inhibitory effect on the fungal genus.

The ethyl-acetate and hot water extracts of the stem piths of *Alchornea cordifolia* had no activity on the tested dermatophytes but Okoye *et al.* (2012) reported that the methanol and chloroform extracts of *A. cordifolia* were both active against *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. In a similar report by Akpo and Owhe-ureghe (2016) *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Clostridium tetani* have been shown to be susceptible to the stem pith of *A. cordifolia*. These results are also in consonance with reports of Akpo and Owhe-ureghe (2013) that the stem pith of *A. cordifolia* was active against oral bacteria. The investigation of Adeshina *et al.* (2010) also showed that the ethylacetate extract of *Alchornea cordifolia* leaves possesses antimicrobial activity against clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans*. Due to these reports, it may be possible that *A. cordifolia* is more active against only bacteria and some fungi but not dermatophytes.

Conclusion

Based on this study, the ethyl-acetate extract of *Senna alata*, showed inhibitory activity against *T. mentagrophyte* and *T. verrucosum* but the hot water extract exhibited zero activity on the test dermatophytes. This therefore indicates that the ethyl-acetate extract of the stem pith of *Senna alata* is useful for treating Tinea capitis and Tinea pedis caused by Trichophyton mentagrophyte and Trichophyton verrucosum. For *Alchornea cordifolia*, the ethylacetate and hot water extracts were both not inhibitory against *T.mentagrophyte* and *T. verrucosum*, hence may not be useful for treating Tinea capitis and Tinea pedis.

Recommendation

Though the hot water extract of *Senna alata*, the ethyl-acetate and the hot water extracts of *Alchornea cordifolia* were not active against the test dermatophytes, there may be possibilities of them

having activity on other dermatophytes. The inactivity of these extracts against the tested dermatophytes may be due to incomplete exhaustion of the active ingredients. Different solvents have different solubility capacities for different phytoconstituents, hence the differences in the activities of the various extracts. It is recommended that more work should be done on these plants using solvents with better solubility capacity.

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Declaration of Interest

The authors declare no conflict of interest.

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