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Phytochemical properties and *in-vitro* antifungal activity of *Senna alata* Linn. crude stem bark extract

Sule W. F.¹, Okonko I. O.^{2*}, Omo-Ogun S.³, Nwanze J. C.⁴, Ojezele M. O.⁵, Ojezele O. J.⁶,
Alli J. A.⁷, Soyemi E. T.⁷ and Olaonipekun T. O.⁷

¹Department of Biological Sciences, College of Science, Engineering and Technology, Osun State University, P. M. B. 4494, Osogbo, Osun State, Nigeria.

²Department of Biochemistry and Microbiology, Lead City University, Ibadan, Nigeria;

³Department of Microbiology, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria.

⁴Department of Pharmacology and Therapeutics, Igbinedion University, Okada, Edo State, Nigeria.

⁵Department of Nursing Science, Administration and Public Health, Lead City University, Ibadan, Oyo State, Nigeria.

⁶Analytical Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria

⁷Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria.

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This study reports on the phytochemical properties and *in-vitro* antifungal activity of *Senna alata* Linn. crude stem bark extract on clinical test dermatophytes. The studies on the antifungal activities of crude extracts of *Senna alata* L. stem bark were carried out. The test was conducted on dermatophytes, which included clinical dermatophytes of the genera *Trichophyton*, *Microsporum* and *Epidemophyton*. These fungi are the causative agents of various types of dermatophytosis that attack various parts of the body and tend to the following conditions, Tinea capitis, Tinea cruris, Tinea corporis and Tinea pedis. The results obtained showed that the crude extract of the stem bark of *Senna alata* L. exhibited marked antifungal effects on *Microsporum canis*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*. The crude extract showed the highest inhibition on *T. verrucosum* and *E. floccosum* with 21.00 and 20.05 mm zones of inhibition respectively. The Minimum Inhibitory Concentration (MIC) was also performed and the result showed that the MIC of *Senna alata* L. crude stem bark extract on all the tested dermatophytes was 5.0 mg/mL. The result showed that the crude extract of *S. alata* L stem bark was fungicidal for all tested dermatophytes at concentration of 10.00 and 5.00 mg/mL except *E. floccosum* which was only fungicidal at concentration of 10.00 mg/mL. The crude extract of *S. alata* L. stem bark screening showed vary degree of activities against all the tested dermatophytes. Phytochemical analysis revealed the presence of important secondary metabolites (tannins, steroids, alkaloids, anthraquinones, terpenes, carbohydrates and saponins) in the plant thus indicating the therapeutic potentials of the plant. It is concluded that *S. alata* L. stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is therefore advocated.

Key words: Antifungal activity, crude extract, dermatophytes, phytochemicals, *Senna alata* L., stem bark, MIC.

INTRODUCTION

Medicinal plants are various plants which can have

medicinal properties. Plants in most developing countries, as a basis for promoting and maintenance of good health, has been widely recognized (UNESCO, 1996). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well

*Corresponding author. E-mail: mac2finney@yahoo.com. Tel: +234-080-3538-0891.

being (Igbinosa et al., 2009). Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases (Iwu, 1993). Different plant parts have also been used for various forms of diseases and infections. The following plants have been reported for the treatment of the following ailments: Garlic (*Allium sativum*) for dermatophytes (Wokoma et al., 2007), *Garcinia kola* for respiratory tract infection and diabetes (Adeleke et al., 2006), *Sida acuta* for skin infections (Ekpo and Etim, 2009). Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections (Nweze et al., 2004; Vineela and Elizabeth, 2005; Ekpo and Etim, 2009). According to the World Health Organization (WHO, 2002), traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world and, in African countries; this rate is much higher. In recent years, however, medicinal plants have represented a primary health source for the pharmaceutical industry (Ajose, 2007). No less than 400 compounds derived from plants are currently used in the preparation of drugs, such as vincristine and vinblastine used in the treatment of cancer (Ajose, 2007).

Recently, some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as antimicrobials, such plant products would be biodegradable and safe to human health (Kumar et al., 2008; Sugar et al., 2008; Krishnamurthy et al., 2008; Wang et al., 2010). Furthermore, the increasing awareness on the use of medicinal plants in industrialized countries has been traced to the development of several drugs and chemotherapeutics from the traditionally used herbs (UNESCO, 1998). Nigerians still depend largely on crude herbal remedies or traditional medicine. They also use wild plants for cosmetics and perfumery. Some of these herbal remedies have been observed to be effective in certain skin diseases (Ajose, 2007). Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoids, bioflavonoids, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax-quinones (Ekpo and Etim, 2009).

The numbers of suitable antifungal drugs are limited. Selective toxicity is much more difficult to achieve in the eukaryotic fungal cells than in prokaryotic bacteria, and although the available antifungal agents have greater activity against fungal cells than they do against human cells, the difference is not as marked as it is for most antibacterial agents. Treatment of fungal infections is rather

hampered by problems of solubility, stability and absorption of the existing drugs (Cedric, 2004). Azole antifungal agents inhibit cell membrane synthesis and also act by inhibiting lanosterol (14-demethylase), an important enzyme in sterol biosynthesis. Ketoconazole has become the agent of choice for many serious fungal infections like ringworm (dermatophytes) while Ketoconazole and Miconazole are full preparations, which are also used for fungal infections (Cedric, 2004). However, the more current and most effective antibiotics (antifungal) are very expensive and out of reach of many Africans, majority of whom reside in the rural areas. These antibiotics are also associated with some serious side effects. A medicinal plant, such as *S. alata* L., is readily available and affordable. *S. alata* Linn (fabaceae) is an ornamental shrub, which grows well in forest areas of West Africa (Owoyale et al., 2005). *S. alata* L. plants contain a group of phytochemicals like saponin, alkaloid, steroid, flavonoid, tannin, phenol and carbohydrate (Akinyemi et al., 2000). These chemical compounds are well known for their laxative and pharmacological effects on humans and animals. Stem bark of *S. alata* L. is used to treat fungal infections such as ringworm. It is a common ingredient in soaps, shampoos and lotions because of its antifungal properties. The ethanolic extract of the stem bark of *S. alata* L. could be fungistatic in which it inhibits fungi multiplication but not necessarily kill them. The ethanolic extract could also be fungicidal causing cell death and lysis. The action may also be against protein synthesizing machinery or against an enzyme involved in nucleic acid synthesis (Dawang, 2005). *S. alata* L. is locally used in Nigeria in the treatment of several infections, which include ringworm, parasitic skin diseases. The leaves are reported to be useful in treating convulsion, venereal diseases (syphilis and gonorrhoea), heart failure, abdominal pains, oedema, stomach problems, fever, asthma, snake bite and is also used as a purgative (Owoyale et al., 2005). The effectiveness of *S. alata* L. against skin diseases was confirmed by modern scientific studies (Makinde et al., 2007). In Kwara State, Nigeria, *Senna alata* was investigated for fungi activity (Owoyale et al., 2005). The phytochemical components such as alkaloids, anthraquinones, saponins, tannins, terpenes, steroids, flavonoids, carbohydrates have been investigated for their therapeutic potency (Owoyale et al., 2005).

This study was carried out with the aim of contributing to previous work on the list of plants used for treatment of infections. This study therefore reports on the phytochemical properties and *in-vitro* antifungal activity of *S. alata* Linn. crude stem bark extract on dermatophytes.

MATERIALS AND METHODS

Identification of *Senna alata* Linn plant

S. alata Linn Plant was identified at the plant taxonomy laboratory,

Department of Botany, University of Jos, Jos, Plateau State, Nigeria.

Test organisms

Four pure cultures of Dermatophytes isolates of *Microsporium canslaslomyces*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, used in this study were obtained and identified from Dermatophytosis Research Center, National Veterinary Research Institute, VOM, Jos, Plateau State, Nigeria.

Selection and preparation of crude extracts *S. alata* Linn. stem bark

Stems of *Senna alata* Linn were collected at the Kogi State University, Anyigba, Kogi State, Nigeria in July, 2008. The stem barks were carefully peeled from the stem using a sharp knife. The stem barks were air-dried at room temperature for seven days. It was weighed daily for the seven days until it attained a constant weight. It was then pulverized with clean mortar and pestle to fine powder. It was then stored in a sterilized glass container at room temperature (25 - 30°C) until used. For the preparation of ethanolic extract, ethanol Soxhlet extraction method as described by Akinyemi et al. (2000) and modified by Abdulrahman et al. (2004) was used. Eighty (80) grams of the powdered stem bark of *S. alata* L. was extracted using Soxhlet extractor with 300 mL of 96% ethanol at 80°C for 8 h for complete extraction in the Chemistry laboratory Kogi State University, Anyigba. Eighty (80) g of the powdered plant was weighed with weighing balance and packed in a serviette paper and tied well with white rope, this was placed inside the thimble of the extractor and the condenser was placed on the thimble containing the packed powdered stem bark. Afterwards, it was placed inside the round bottom flask containing the ethanolic solvent and placed on the Barnstead/electrothermal heater for 8 h. The ethanolic extract of the stem bark was concentrated to dryness using Rotary-evaporator at 78°C for 1 h. The resulting organic extract was stored in sterile bottles at refrigeration temperature (4 - 8°C) before assay.

The weight of the chaff after extraction was weighed using a weighing balance to determine the percentage yield of the crude extract using the formula:

$$\text{Percentage yield} = X_1 - X_2 / X_1 \times 100.0\%.$$

[Where, X_1 = weight of the dry powdered leaves before extraction (80.0g);

X_2 = weight of the dry chaff after extraction].

The weight or mass of crude ethanolic extract can be calculated using the formula:

$$\text{Percentage yield} \times X_1 / 100$$

Phytochemical analysis of the crude extract of stem of *S. alata* Linn

The powdered crude extract of stem of *S. alata* L. were subjected to phytochemical analysis for the presence of plant biomolecular and secondary metabolites, anthraquinone, tannins, saponins, flavonoid, steroid, terpenes, alkaloids carbohydrate, and glycosides using the standard qualitative procedures in accordance with Trease and Evans (1989), Harborne (1998) and Ekpo and Etim (2009) with little modification. For alkaloids, 0.5 g of each extract was stirred with 5 mL of 1% aqueous hydrochloric acid on a steam bath. 1 mL of the filtrate was treated with a few drops of the

Dragendorff's reagent. The formation of orange colour indicated the presence of alkaloids. For saponins, 0.5 g of the extract was added and mixed with Fehling's solution and then 5% of sodium trioxocarbonate solution was later added. The mixture was then boiled. The pink precipitate indicated the presence of saponins. For flavonoids, 0.5 g of the extract and few pieces of magnesium strips was mixed with concentrated HCl. An orange faint colour of effervescence solution indicated the presence of flavonoids. For tannins, 0.5 g of the plant extract was stirred with 1 mL of distilled water, filtered and ferric chloride solution or reagent was added to the filtrate. A blue black or blue green precipitate was taken as evidence for the presence of tannins. For anthraquinones, 0.5 g of the plant extract was boiled with 1 mL of 10% sulphuric acid and filtered. 2.5 mL of benzene was added to the filtrate and shaken. The benzene layer was separated and half its own volume, 10% ammonia solution was added. The presence of a pink or red-violet colour in the lower ammonia phase indicated the presence of anthraquinones.

Determination of pH of crude extract of *S. alata* stem bark

The pH of the crude ethanolic extract of *S. alata* stem bark was determined using calibrated pH meter, model 3510 (Jenway). One gram of the crude ethanolic extract of *S. alata* Leaf was dissolved in 100 mL of distilled water to give a concentration of 10 mg/mL. The electrodes of the calibrated pH meter, model 3510 (Jenway) was immersed in the homogenized extract to obtain the pH of the crude extract.

Microbiological assay and antifungal activity of crude extract of *Senna alata* stem bark

Nutrient agar, Nutrient Broth and Sabouraud dextrose agar (SDA) (Oxoid) were used for the microbiological analysis and antifungal activity of crude extract of stem bark on the four pure cultures of Dermatophytes. All media was prepared and sterilized according to the manufacturer's specifications. The crude extract of *S. alata* stem bark were tested in vitro for purity by plating them out on four Petri-dishes containing prepared nutrient agar and SDA aseptically and incubated at 37°C for 24 h and at 25°C until they sporulated.

Antifungal activity

Antifungal activity of the crude extract was tested using agar diffusion method described by Cheesbrough (2006). An antifungal drug (Ketoconazole) was used as standard drug. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD_{600nm} of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Two hundred milligram (200 g) of the Ketoconazole drug was dissolve in 100 mL of distilled water according to the manufacturers' instructions. 1 mg of the solution was dispensed into the wells using sterile pipettes. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with Ketoconazole at a

Table 1. Phytochemical characteristic of the crude stem bark extract of *Senna alata* L.

Biochemical components	Presence of colour formation
Alkaloid	Reddish-brown colouration
Anthraquinone	Grey-green
Carbohydrate	Brick-red precipitate
Flavonoid	Creamy or light-yellow
Saponin	Formation of froth
Tannin	Blue-green precipitate
Terpenes and steroids	Reddish colour interface

concentration of 1 mg/mL.

Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the crude extract was prepared and 2 mL aliquots of different concentrations of the solution were added to 18 mL of pre-sterilized molten nutrient agar and SDA for bacteria and fungi respectively at 40°C to give final concentration regimes of 0.050 and 10 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial and fungal cultures. The plates were later incubated at 37°C for 24 h and at 25°C for up to 72 h for bacteria and fungi respectively, after which they were examined for the presence or absence of growth (Adesokan et al., 2007). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Minimum fungicidal concentration (MFC)

This was carried out to assess the crude extract for fungicidal or fungistatic effect. It was carried out as described by Cheesbrough (2006). Emphasis was mostly placed on the tubes with MIC and the preceding tubes. A loopful from each of these tubes were sub-cultured into appropriately labeled quadrants of sterilized nutrient agar plates using sterilized wire loop and streaked uniformly on the labeled quadrants. This was incubated for 7 days at 25°C after which they were observed for growth (Adesokan et al., 2007). The MFC was the quadrant with the lowest concentration of the extract without growth.

RESULT

The pH determination showed that the crude extract of *Senna alata* stem bark had a pH value of 7.3. The weight of the chaff after extraction was 39.72 g.

$$\text{Percentage yield} = \frac{X1 - X2}{X1} \times 100\%$$

Where X1 = 80g and X2 = 39.72g;

$$\text{Percentage yield} = \frac{(80 - 39.72) \text{g}}{80 \text{g}} \times 100\% = 50.35\%$$

Therefore the percentage yield of the extract was 50.35%.

The weight of the crude extract =

$$\text{Percentage Yield} \times X1 / 100 = 50.35 \times 80 / 100 = 40.28 \text{g.}$$

Therefore the weight of the crude extract was 40.28g.

Table 1 shows the phytochemical analysis of the crude stem bark extract of *S. alata* L. The result indicated the presence of alkaloid which was confirmed by reddish-brown colouration, grey-green colour confirmed the presence of anthraquinone, black-red precipitate which confirmed the presence of carbohydrate, creamy or light-yellow coloration confirmed the presence of flavonoid. Formation of froth confirmed the presence of saponin, formation of blue-green precipitate confirmed the presence of tannin and reddish colour interface confirmed the presence of terpenes and steroids in the crude stem bark extract of the *S. alata* L. (Table 1).

The effects of different concentrations of the crude stem bark extract of *Senna alata* L. and standard drug are presented in Table 2. The result showed that the crude stem bark extract had antifungal activity on the tested Dermatophytes. It showed the highest antifungal activity on *T. verrucosum* and *E. floccosum* with 21.00 and 20.05 mm zone of inhibition respectively (Table 2). This also showed that at higher concentration of the crude extract, all the tested clinical dermatophytes were not inhibited. However, only *T. mentagrophytes* was inhibited at concentration of 2.5 mg/mL (Table 2).

Table 3 shows the minimum inhibitory concentration (MIC) of the crude stem bark extract of *S. alata* L. The MIC of the crude extract for all the tested dermatophytes was at 5.0 mg/mL (Table 3).

The minimum fungicidal concentration (MFC) of the crude stem bark extract on the tested dermatophytes is presented in Table 4. The results obtained in this study shows that the crude stem bark extract was fungicidal for all the tested dermatophytes. MFC of *M. Canaslalomyces*, and *T. mentagrophytes* were the same at 10.00 and 5.00 mg/mL but 2.5 mg/mL concentration if it is fungistatic, but all were fungicidal at 10.00 and 5.00 mg/mL with the exception of *E. floccosum* that was fungistatic at 5.00 mg/mL (Table 4).

DISCUSSION

Higher plants have been shown to be a potential source for the new antimicrobial agents. The screening of plant

Table 2. The effects of different concentrations of the crude stem bark extract of *Senna alata* L. on the tested dermatophytes.

Test dermatophytes	10.00 mg/mL	5.00 mg/mL	2.5 mg/mL	1.25 mg/mL	Ketoconazole (Control drug)
<i>Microsporum canslaslomyces</i>	13.50	12.00	00.00	00.00	25.50
<i>Trichophyton verrucosum</i>	21.00	15.00	00.00	00.00	22.50
<i>Trichophyton mentagrophytes</i>	19.00	17.00	10.00	00.00	23.00
<i>Epidermophyton floccosum</i>	20.05	15.50	00.00	00.00	21.00

Note: The values are the average of two measurements across each zone of inhibition and in duplicates, measured in millimeter (mm). The zero values indicate no inhibition.

Table 3. The minimum inhibitory concentration (MIC) of the crude stem bark extract of *Senna alata* L. on the tested dermatophytes.

Test dermatophytes	10.00 mg/mL	5.00 mg/mL	2.5 mg/mL	1.25 mg/mL	0.625 mg/mL	0.325 mg/mL	Ketoconazole (Control drug)
<i>Microsporum canslaslomyces</i>	NG	NG	G	G	G	G	NG
<i>Trichophyton verrucosum</i>	NG	NG	G	G	G	G	NG
<i>Trichophyton mentagrophytes</i>	NG	NG	G	G	G	G	NG
<i>Epidermophyton floccosum</i>	NG	NG	G	G	G	G	NG

Key: NG = No growth (Clear); G = Growth (Turbid).

Table 4. The minimum fungicidal concentration (MFC) of the crude stem bark extract on the tested dermatophytes.

Test dermatophytes	10.00 mg/mL	5.00 mg/mL	2.5 mg/mL	1.25 mg/mL	0.625 mg/mL	0.325 mg/mL
<i>Microsporum canslaslomyces</i> ,	NG	NG	G	G	G	G
<i>Trichophyton verrucosum</i>	NG	NG	G	G	G	G
<i>Trichophyton mentagrophytes</i>	NG	NG	G	G	G	G
<i>Epidermophyton floccosum</i>	NG	G	G	G	G	G

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). A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literature, but the vast majority has not been adequately evaluated (Sokmen et al., 1999). This is also particularly valid for the Turkish flora which has one of the most extensive floras in continental Europe with more than 9000 flowering plant species (Davis, 1965 - 1984). Owing to its strategic position, the accumulation of the knowledge of traditional medicine from the west and the east enabled this region to have a rich tradition in terms of the uses of medicinal plants (Alim et al., 2009).

Many scientists have worked on the anti-microbial properties of several plants in Nigeria (Danalap and Akueshi, 2005; Ekpo et al., 2007; Okungbowa and Edema, 2007; Ayodele et al., 2009; Ekpo and Etim, 2009). It is on this note that we investigated the phytochemical and antifungal properties of *S. alata* L.

The results obtained may contribute to the alternative use of plant materials for medicinal purposes. In this present study, the effects of the crude extract of *Senna alata* L. stem bark were documented. The investigations on the phytochemical and biochemical screening of the crude extract of *S. alata* L. stem bark revealed the presence of alkaloid, anthraquinone, carbohydrate, flavonoid, saponin, tannin, terpene and steroid. These compounds have been known to be biologically active and therefore aid the antimicrobial activities of *S. alata* L. stem bark. It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates. These secondary metabolites exert antimicrobial activity through different mechanisms and have been previously reported by Owoyale et al. (2005) to have inhibitory effect or antifungal activity on fungal genus.

The observation in this study supported the reports of Onwuliri and Wonang (2005) and Ayodele et al. (2009),

who attributed the antifungal properties of *A. sativum* to its phytochemical components. The anti-fungal properties of this *S. alata* may also be due to their phytochemical contents which caused inhibitory effects on the mycelia growth of the fungi. Similarly, *Cassia alata* has been reported to contain anthraquinones, the principal laxative constituent of many plants used as purgatives (Owoyale et al., 2005). Thus, from literature search, there is no evidence that flavonoid glycoside; a main constituent of the leaf extract of *S. alata* is responsible for antifungal activity (Owoyale et al., 2005). Meanwhile, the methanolic extracts of leaves, flowers, stem and root of *Cassia alata* had been shown to have a broad spectrum of antibacterial activity after fractionating with petroleum spirit, dichloromethane and ethyl acetate (Owoyale et al., 2005). The dichloromethane fraction of the flower extract was found to be the most effective (Khan et al., 2001).

Tannins act by coagulating the cell wall proteins (Onwuliri and Wonang, 2004, 2005). Tannins have been found to form irreversible complexes with prolinerich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *S. alata* L. in herbal cure remedies. The presence of tannins in *S. alata* L. supports the traditional medicinal use of this plant in the treatment of different ailments.

Saponins were reported as a major components acting as antifungal secondary metabolite (Onwuliri and Wonang, 2004, 2005). Saponins were found to be present in *S. alata* extracts and have supported the usefulness of this plant in managing inflammation. Saponins were also reported as surface active agents who interfere with or alter the permeability of the cell wall, and this facilitates the entry of toxic materials or leakages of vital constituents from the cell (Onwuliri and Wonang, 2004, 2005). Just et al. (1998) revealed the inhibitory effect of saponins on inflamed cells.

Flavonoids, another constituent of *S. alata* stem bark extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Flavonoids were phenolic in nature and acts as cytoplasmic, poisons, which also have been reported to inhibit the activity of enzymes (Dathak and Iwu, 1991).

Steroidal compounds present in *S. alata* L. extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Quinlan et al. (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al. (2004) also confirmed the antiviral

property of steroids. Generally, however these active components are responsible for the diverse pharmacological actions of the crude extract used. Hence, the presence of these compounds in *S. alata* L. corroborates the antimicrobial activities observed (Igbinosa et al., 2009).

The result of the crude extract of *Senna alata* L. showed that the crude extract had a pH of 7.3, which is alkaline. The pH of the extract falls in the range of physiologic pH of 7.2 to 7.4 because pH is an important factor in determining stability and physiological activity of most preparations (WHO, 2006). The inhibition of tested dermatophytes by the crude extract of *Senna alata* L. is most likely due to the action of different phytoconstituents of the plant stem bark. The crude extract has a wide range of physiological activity of saponin, alkaloid, carbohydrate, favonoid, anthraquinone, steroid, and tannin. Of all these, favonoid was found to be the biochemical constituents of *S. alata* L. responsible for the antifungal action on dermatophytes (Trease and Evans, 1989). A literature search on 38 of the plants used by herbal prescribers revealed the presence of established antimicrobial agents, immune modulating agents, antioxidants, other vitamins and minerals, volatile oils and emollients, and anti-inflammatory agents. Some of the plants may be contaminated by mycotoxins because of poor storage (Ajose, 2007).

The results of this study showed that the crude extract of *S. alata* L. stem bark showed antifungal activity on all the tested dermatophytes. At higher concentration of 10.00 and 5.00 mg/mL, the crude extract has the highest inhibition. It showed the highest antifungal activity on *Trichophyton verrucosum* and *Epidermophyton floccosum* with 21.00 and 20.05 mm zones of inhibition respectively, which compete favorably with the control drug (Ketoconazole) by showing antifungal activity on *Trichophyton verrucosum* and *Epidermophyton floccosum* with 22.50 and 21 mm zones of inhibition, respectively. In a study by Ajose (2007), partial relief was achieved in dermatophytoses, ichthyosis, leprosy, and systemic lupus erythematosus (SLE) using remedies prepared mostly by heating and boiling, infusion, and maceration. In most cases in Ajose (2007) study, mixtures of plants (*Adansonia digitata*, *Aframomum melegueta*, *Aloe species*, *Azadirachta indica*, *Cassia alata*, *Alstonia boonei*, *Ficus asperifolia*, *Cocos nucifera*, *Jatropha gossypifolia*, *Ocimum gratissimum*, *Ricinus communis*) or other substances were used.

In this study however, only *Trichophyton mentagrophytes* was inhibited at lower concentration of 2.50 mg/mL of the crude extract. The plant extract generally exhibited lower antimicrobial activity than the pure antibiotic substance (*Ketoconazole*). This is in consonance with the findings of previous studies. A study in Malaysia (Ibrahim et al., 1995) reported that ethanolic extract of the *Senna* plant showed high activity against

dermatophytic fungi: *T. mentagrophytes var interdigitale*, *T. Mentagrophytes var. mentagrophytes*, *T. rubrum* and *Microsporium gypseum* (MIC: 125 mg/mL) and *M. canslaslomyces*, (MIC: 25 mg/mL). In a recent review, the methanolic fraction of the leaves has been shown to be active against *Trichophyton mentagrophytes* at a concentration of 50 mg/mL but has no activity against moulds and *Candida albicans* (Villasenor et al., 2002). Much earlier, the antifungal activity of *C. alata* leaf extract has been reported (Owoyale et al., 2005).

The MICs of the extract in this study also showed that the crude extract of the *Senna alata* L stem bark had MIC of 5.00 mg/mL and the MFC showed that the crude extract of *Senna alata* L stem bark was fungicidal for all tested dermatophytes at concentration of 10.00 mg/mL and 5.00 mg/mL except *E. floccosum* which was only fungicidal at concentration of 10.00 mg/mL. The ability of the extracts to inhibit the growth of several fungal species is an indication of the broad spectrum anti-microbial potential of *S. alata* L, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs. Several other studies (Akinoyemi et al., 2000; Owoyale et al., 2005) have been conducted to provide scientific basis for the efficacy of plants used in herbal medicine. However, Ajose (2007) claimed that some forms of alopecia, onychomycosis, and vitiligo, as well as allergic dermatoses, were not improved by herbal medicines.

This study in line with other studies showed that many infectious diseases are known to be treated with herbal remedies throughout the history of man kind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Alim et al., 2009). Plants still continue to be almost the exclusive source of drugs for the majority of the world's population. In western medicine substances derived from higher plants constitute ca. 25% of prescribed medicines and 74% of the 121 bioactive plant-derived compounds currently in worldwide use were identified via research based on leads from ethno medicine (Alim et al., 2009).

The crude extract of *S. alata* L. stem bark screening showed vary degree of activities against all the tested dermatophytes (*M. canslaslomyces*, *T. verrucosum*, *T. mentagrophytes* and *E. floccosum*). Phytochemical analysis revealed the presence of important secondary metabolites (tannins, steroids, alkaloids, anthraquinones, terpenes, carbohydrates and saponins) in the plant thus indicating the therapeutic potentials of the plant. The phytochemical tests have revealed the presence of bioactive compounds as well as the antifungal properties of the crude stem bark extract. It is concluded that *S. alata* L. stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is therefore advocated.

Also, in line with Ajose (2007), there appears to be clinical, scientific, and pharmacologic basis for the use of herbal preparations. Nigeria needs to provide effective

coordination of the practice of herbal medicine to ensure safety, standardization, and preservation of the flora. As a result of these, a further study on isolation and characterization of the active compounds from the crude stem bark extract is necessary. The same is necessary for the chemical structures and mode of action of the isolated crude stem bark extract.

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