

## ***In vitro* Antifungal Activity of *Senna alata* Linn. Crude Leaf Extract**

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**Abstract:** This study reports on the *in vitro* antifungal activity of *Senna alata* Linn. crude leaf extract on clinical test Dermatophytes. The studies on the *in vitro* investigation of antifungal activities of ethanolic extracts of *Senna alata* L. leaf was carried out. The test was conducted on Dermatophytes which included Dermatophytes of the genera *Trichophyton*, *Microsporum* and *Epidermophyton*. These fungi are the causative agents of various types of dermatophytosis which 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 leaf exudates and the ethanol extract of the leaf of *Senna alata* L. exhil: had marked antifungal effects on *Microsporum canis*, *Trichophyton jirrucosum*, *Trichophyton mentagrophytes* and *Epidermophyton jlorrcozum*. The ethanolic extract showed the highest inhibition on *trinchophyton verrucosuf* and *Epidermophyton jloccosum* with 20.50 and 20.00 mm zone of inhibition, respectively. The Minimum Inhibitory Concentration (MIC) was also performed and the result showed that the MIC of *Senna alata* L. on all the tested Dermatophytes was 5.0 mg mL<sup>-1</sup> which is the standard. The results obtain from the biochemical analysis of the plant *Senna alata* L. revealed the presence of Alkaloids, Saponins, Tannins, anthracionones and carbohydrates.

**Key words:** Antifungal activity, biochemical components, crude extract, dermatophytes, Phytoconstituents, *Senna alata* L., MIC

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### **INTRODUCTION**

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases (Sher, 2009).

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. 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; Wang *et al.*, 2010). The acceptance of traditional medicine as an alternative form of health care hassled researchers to further investigate antimicrobial activity of medicinal plant. Countries in Africa, Asia and Latin America use traditional medicine to help meet some of their primary healthcare needs.

In Africa today, up to 80% of the population uses traditional medicine in primary health care (WHO, 2006). Many African plants are used in traditional medicine as antimicrobial agents but only few have documented. However, in spite of vast improved health and longevity in the United State and Europe, millions of their people are turning back to traditional herbal medicine in order to

prevent or treat many illness (WHO, 2006) and to circumvent resistance of many human pathogens to conventional (antibiotics), some of which have side effects like hypersensitivity and immunosuppression.

In Nigeria, traditional medical practitioners use a variety of herbal preparation to treat different microbial diseases. For example, the Ebira tribes of Kogi State, Nigeria use the fruits of *Solanum melongena* for weight loss allowed as dietary delicacy (Bello *et al.*, 2005). The use of herbal medicine predates the introduction of antibiotics predates social, economic and religious barriers (Akinyemi *et al.*, 2000).

The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists' worldwide (Sofowora, 1982). World Health Organization corroborated this in its quest to bring primary health care to the people. The plant kingdom has for long time served prolific source of helpful drugs, food, additives, flavouring agents, colorants binders and lubricants etc., as a matter of facts, it was estimated that about 25% of all prescribed medicines today are substances derived from plants (Bello *et al.*, 2005).

For control of microbial infections and diseases, various synthetic drugs and chemical formulations have been used. But due to their indiscriminate use, microbes have developed wide resistance against these synthetic drugs such as broad-spectrum antibiotics.

This resistance was developed after induction of new enzyme system in microbes which not only simplify drugs but also enhance drug threshold level in microbes. Therefore, to combat the problem of microbial infection and drug resistance new alternative of synthetic drugs have been explored, though antimicrobial activities of so many natural products have not been explored (Upadhyay *et al.*, 2010).

In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. It is on this basis that researchers keep on studying on medicinal plants in order to develop the best medicines for physiological uses (Usman and Otuji, 2007). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies (Okoro *et al.*, 2010).

*Senna alata* Linn (Fabaceae) is an ornamental shrub. It is locally used in Nigeria in the treatment of several infections which include ringworm, parasitic skin disease (Dalziel, 1965; Palanichamy and Nagorajon, 1990). *Senna alata* L. is also credited for treatment of haemorrhoids,

constipation, inguinal hernia, intestinal parasite, blennorrhagia, syphilis and diabetics (Abo *et al.*, 1998; Adjanahoun *et al.*, 1991). The leaf of this plant was reported to be useful in treating convulsion, onolthoea, heart failure, abnormal pain, oedema as and as purgative but it was espedally useful in treating dermatophytosis (Ogunti and Elujobi, 1993).

A similar study in Malaysia by Ibrahim and Osman (1995) reported that ethanolic extracts of *Senna alata* L. plant show high antifungal activity against dermatophytic fungal such as *Trichophyton mentagrophyte* var. *interaligitale* and var. *Metagrophytes*, *Trichophyton rubrum* and *Microsporium gypseum*. Several studies (Akinsinde and Olukoya, 1995; Akinyemi *et al.*, 2000) have documented the basis of the leaf of *Senna alata* in herbal medicine. Adebayo *et al.* (1999) documented that Minimum Inhibitory Concentration (MIC) of the plant extract was low on all fungal agents except *Aspergillus niger*.

It has been observed that the antimicrobial activity of *Senna alata* is associated with the presence of component such phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates. The ethanolic and metanolic extract *Senna alata* L. exhibited very long antimicrobial activity against fungi like *Candida albicans*, *Aspergillus flavus* *Trichophyton mentagrophytes*, *Microsporium canis* and *Blastomyces dermatitidis* and some bacteria with maximum activity in the fractions containing alkaloid salts and base (Makinde *et al.*, 2007).

The plant can be found in Nigeria, Malaysia, Australia, Thailand, tropical America and many other parts of the world (Palanichamy and Nagorajon, 1990). Several medicinal plants have also been evaluated for their activities on different species of microorganisms. It has been reported that crude hot water extract of light edible leafy vegetable of *Lasianthera africana* and *Heinsia pulchella* exhibited bacteriostatic effects on six canned food borne pathogenic bacteria while *Ocimum gratiticimum* and *Vernonia amygdalina* produced bacteriocidal effect on *Bacillus cereus* and *S. aureus*, respectively (Itah and Opara, 1990; Itah, 1999; Ekpo and Etim, 2009).

The aqueous and ethanolic extracts of *Azadirachta indica* leaves have been shown to have anti-dermatophytic activity when tested *in vitro* against 88 clinical isolates of dermatophytes using the agar dilution technique (Sher, 2009). The activity was more prominent in ethanolic extract as compared to that in aqueous extract (Venugopal and Venugopal, 1994). Rai (1996) observed antimycotic activity against the test pathogen *Pestalotiopsis mangiferae* in 14 medicinal plants.

Essential oil obtained from the herb of *Santolina chamaecyparissus* showed significant antifungal activity both *in vitro* (against 13 strains of *Candida albicans*) and *in vivo* (experimentally induced vaginal and systemic candidiasis in mice) (Suresh *et al.*, 1997).

It also showed activity against experimentally induced superficial cutaneous mycosis in guinea pigs by the hair root invasion test (Suresh *et al.*, 1995). The natural xanthenes isolated from the fruit hulls of *Garcinia mangostana* showed good inhibitory activity against the three phytopathogenic fungi, *Fusarium vasinfectum*, *Alternaria tenuis* and *Dreschlera oryzae* (Gopalakrishnan *et al.*, 1997). The root of *Withania somnifera* was found to be effective in prolonging the survival of Bulb/C mice infected intravenously with *Aspergillus fumigatus*.

This protective activity is probably due to the observed increase in phagocytosis and intracellular killing capacity of peritoneal macrophages induced by treatment with *Withania somnifera*, thus suggesting that the plant has potential to activate macrophage function in infectious states (Dhuley, 1998). *Moringa olifera* (commonly known as drum stick plant or Sahijan herb), *Ficus bengalensis* (Banyan tree), *E. officinalis* (Indian Goose Berry plant) and *T. dioica* (pointed gourd), have been extensively explored in the laboratory for their antidiabetic and *in vivo* antioxidant activities (Rai *et al.*, 2008; Jaiswal *et al.*, 2009; Singh *et al.*, 2009; Sharma *et al.*, 2009).

As a result of the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics (Cowan, 1999). There is need to develop alternative antimicrobial drugs. One approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents.

The petroleum ether, chloroform, acetone and ethanol (95%) extracts of the leaves of the *Cassia alata* also showed significant *in vitro* antifungal activity against various fungi viz. *Aspergillus niger*, *R. Japonicum*, *candida albicans*, *C. tropiathis* and *R. glutinis* (Sakharkar and Patil, 1998a, b). In a study by Owoyale *et al.* (2005) in Kwara State, Nigeria, *Senna alata* Linn was found to have antifungal activities.

The phytochemical component such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates were also investigated as a scientific assessment of the claim of therapeutic potency. This research was undertaken with the aim of contributing to previous study or the list of plants used for the treatment of fungal infection. This study therefore reports on the

assay of the *Senna alata* L. leaf for pertinent phytoconstituents. It also aimed to investigate the *in vitro* antifungal activities of leaf extracts of *Senna alata* L. on isolates of dermatophytes. This screening is of significant importance because of the urgent need for compounds that would be added to or replace the current antimicrobial agents to which microbes have become largely resistant (Chopra *et al.*, 1997; Okoro *et al.*, 2010).

## MATERIALS AND METHODS

**Identification of *Senna alata* L. plant:** *Senna alata* L. plant was identified at the plant taxonomy laboratory, Department of Botany, University of Jos, Plateau State.

**Pure cultures:** 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 *Senna alata* L. leaf:** Leaves of *Senna alata* L. were collected at the student walk way of Kogi State University, Anyigba Kogi State, Nigeria in July, 2008. The leaves were shade dried and at room temperature for 7 days to attained a constant weight. It was not dried under sun so as not to lose some of the biochemical constituents of the plant in it. 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. Ethanol soxhlet extraction method as described by Akinyemi *et al.* (2000) and Abdulrahman *et al.* (2004) was adopted for study and the complete extraction of the plant was carried out at the Chemistry Laboratory, State University, Anyigba. The stock extract was stored in a sterile bottle and kept in refrigerator at 7°C.

**Phytochemical analysis of the crude extract of leaf of *Senna alata* L.:** The phytochemical analysis of the leaf extract was carried out for presence of biomolecular such as: anthraquinones, carbohydrates, flavonoids, saponins, tannins, alkaloids, terpenes and steroids using the standard qualitative procedures as described by Trease and Evans (1989) and Owoyale *et al.* (2005). The ethanolic leaf extracts were screened for their phytochemical bases using the standard method of Trease and Evans (1989) and Harborne (1998). The Phytochemical component analysed were alkaloids, saponins, flavonoids, tannins,

anthraquinones and cardiac glycosides. For alkaloids, 0.5 g of each extract was stirred with 5 mL of 1% aqueous hydrochloric acid on a steam bath. About 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 *Senna alata* leaf:**

The pH of the crude ethanolic extract of *Senna alata* leaf was determined using calibrated pH meter, model 3510 (Jenway). About 1 g of the crude ethanolic extract of *Senna alata* leaf was dissolved in 100mL of distilled water to give a concentration of 10 mg mL<sup>-1</sup>. 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**

**tract of *Senna alata* leaf:** Nutrient agar and Nutrient Broth was used for the microbiological analysis and antifungal activity of crude extract of *Senna alata* leaf on the four pure cultures of Dermatophytes. All media was prepared and sterilized according to the manufacturer's specifications. The crude extract of *Senna alata* leaves were tested *in vitro* for purity by plating them out on four Petri-dishes containing prepared nutrient agar aseptically and incubated at 37°C for 24 h. Antifungal activities of the ethanolic extract were tested using screwed diffusion method described by Cheesbrough (2006). The four prepared Sabouraud Dextrose Agar (SDA) plates were aseptically inoculated with test dermatophytes in duplicate, after which wells (5mm in diameter) were

aseptically used to make four wells on the medium using a sterilized cork borer 0.1 m (equivalent to mg). The liquid extract was introduced in the wells using a sterilized pipette. An antifungal drug (Clotrimazole) was used as standard drug, 100 mg of which was dissolved in 100 mL of distilled water according to the manufacturer's specifications.

About 10 g of the solution was introduced in the well using a sterilized pipette and it was on repeated on the different plates containing SDA in which one of the wells were bore in duplicates. The plates were left on the bench undisturbed for few minutes, after which the plates inoculated with fungi isolates were incubated at room temperature (28±2°C) for 5 days. The external diameters of visible zones of growth inhibition were measured after incubation.

#### **Determination of Minimum Inhibitory Concentration (MIC) of the leaf crude extract:**

The Minimum Inhibitory Concentrations (MIC) were determined using the broth dilution method as described by Atlas (1995). About 1 g of the extract was weighed and dissolved in 100 mL of the solvent (ethanol) to give a concentration of 10 mg mL<sup>-1</sup>.

The 10 mg mL<sup>-1</sup> was serially diluted by 2-fold dilution with pipette to concentrations of 5, 2.5, 0.625 and 0.325 mg mL<sup>-1</sup>. Four groups of test tubes were used for the four different dermatophytes. Each group consisted of seven test tubes. A group was used for particular dermatophytes. Each group was labeled 1-7. A set of seven test tubes covered with clean sterile cotton wool were used against each dermatophytes.

About 9 mL of prepared sterile Nutrient broth was transferred into each test tube; 1 mL of the liquid extract with highest concentration (10 mg mL<sup>-1</sup>) was transferred into the test tubes and was robbed on the palm and diluted serially by 2 fold dilution. A 10<sup>9</sup> full from the culture of dermatophytes was introduced into each of the 6 test tubes.

This experiment was carried out on the three other dermatophytes. The positive controls were equally set up by using 0.1 mL (1 mg) of the liquid extract without the test dermatophytes. All the test tubes were covered with clean sterile cotton wool and incubated at 25°C for 48 h. At the expiration of the time, the plates were examined for zones of inhibition which were measured and recorded.

#### **Determination of Minimum Fungicidal Concentration (MFC) of the crude extract:**

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. The MFC was the quadrant with the lowest concentration of the extract without growth.

## RESULTS AND DISCUSSION

*Senna alata* plant was identified as belonging to the family Fabaceae or Leguminosae, genus *Senna* and species *Senna alata* Linn. The pH determination showed that the crude ethanolic extract of *Senna alata* L. leaf had pH value of 7.2. The result for the purity of the crude extract of the *Senna alata* L. leaf after 24 h of culture showed no growth which confirmed no contamination and that indicate the purity of the crude extract.

Table 1 shows the phytochemical analysis of the crude extract of *Senna alata* L. leaves. 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 was 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 of the acetic anhydride with concentrated sulphuric acid confirmed the presence of terpenes and steroids in the

Table 1: Phytochemical characteristic of the crude extract of *Senna alata* L. leaf

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

crude extract of the *Senna alata* L. leaf (Table 1). The effects of the crude leaf extract of *Senna alata* L. and standard drug at various concentrations are shown in Table 2. The result of the antifungal activity showed that the plant extract had antifungal activity on some dermatophytes. It showed that the highest activity on *Trichophyton verrucosum* and *Epidermophyton floccosum* with 20.05 and 20.00 mm zone of inhibition, respectively (Table 2). The result of the antifungal activity also showed that at higher concentration of the crude extract, all the tested clinical dermatophytes were not inhibited. However, only *Trichophyton mentagrophytes* was inhibited at concentration of 1.25 and 2.5 mg mL<sup>-1</sup> (Table 2).

Table 3 shows the Minimum Inhibitory Concentration (MIC) of the crude leaf extract of *Senna alata* L. leaf. The MIC values of the crude leaf extract for all the tested dermatophytes were all at 5.0 mg mL<sup>-1</sup> (Table 3). The Minimum Fungicidal Concentration (MFC) of the crude leaf extract for all the tested clinical dermatophytes were shown in Table 4. The results obtained in this study shows that MFC of *Microsporum Canis* and *Trichophyton mentagrophytes* were the same at 10.00 and 5.00 mg mL<sup>-1</sup> but 2.5 mg mL<sup>-1</sup> concentration if it is fungistatic but all were fungicidal at 10 and 5 mg mL<sup>-1</sup> with the exception of *Epidermophyton floccosum* that was fungistatic at 5.00 mg mL<sup>-1</sup> (Table 4). The effects of the crude ethanolic extract of the *Senna alata* L. leaf have been documented in this study. Firstly, the pH of the leaf crude extract *Senna alata* L. in this study showed that the ethanolic extract had pH of 7.2 which is alkaline.

The pH of the extract falls within the range of physiological pH of 7.2-7.4 which is an important factor in determining its suitability in formulations. This is because the stability and physiological activity of most preparations depend on the pH (WHO, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc., which have been found *in vitro* to have antimicrobial properties

Table 2: The effects of the antifungal activity of the crude leaf extract of *Senna alata* L. at various concentrations on the test dermatophytes

Test dermatophytes	10.00 mg mL <sup>-1</sup>	5.00 mg mL <sup>-1</sup>	2.5 mg mL <sup>-1</sup>	1.25 mg mL <sup>-1</sup>	Clotrimazole (Control drug)
<i>Microsporum canis</i>	13.00	12.05	00.00	00.00	25.50
<i>Trichophyton verrucosum</i>	20.50	16.50	00.00	00.00	22.50
<i>Trichophyton mentagrophytes</i>	19.50	17.00	13.50	11.00	23.00
<i>Epidermophyton floccosum</i>	20.00	16.50	00.00	00.00	21.00

Table 3: The minimum inhibitory concentration (MIC) of the crude extract of *Senna alata* L. leaf at various concentrations on the test dermatophytes

Test dermatophytes	10.00 mg mL <sup>-1</sup>	5.00 mg mL <sup>-1</sup>	2.5 mg mL <sup>-1</sup>	1.25 mg mL <sup>-1</sup>	0.625 mg mL <sup>-1</sup>	0.325 mg mL <sup>-1</sup>	Clotrimazole (Control drug)
<i>Microsporum canis</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

NG: No Growth (Clear); G: Growth (turbid)

Table 4: The Minimum Fungicidal Concentration (MFC) of the crude plant extract for all the tested clinical dermatophytes

Test dermatophytes	10.00 mg mL <sup>-1</sup>	5.00 mg mL <sup>-1</sup>	2.5 mg mL <sup>-1</sup>	1.25 mg mL <sup>-1</sup>	0.625 mg mL <sup>-1</sup>	0.325 mg mL <sup>-1</sup>
<i>Microsporum canis</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

NG: No Growth (Clear); G: Growth (turbid)

(Sher, 2009). The biochemical screening of *Senna alata* L. leaf revealed the following phytoconstituents: alkaloids, carbohydrates, saponins, anthraquinones, steroids, tannins and absence of digitalis glycosides and lipids. Alkaloids are heterocyclic nitrogen compounds (Sher, 2009). Solamargine, a glycoalkaloid from the berries of *Solanum khasianum* and other alkaloids may be useful against HIV infections (McMahon *et al.*, 1995) as well as intestinal infections associated with AIDS (Phillipson and O'Neill, 1987).

Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes (Freiburghaus *et al.*, 1996) and plasmodia (Omulokoli *et al.*, 1997). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmine is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987).

Anthraquinones, a quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. The switch between diphenol (or hydroquinone) and diketone (quinone) occurs easily through redox reactions (Sher, 2009). In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in protein often leading to inactivation of the protein and loss of function.

For that reason the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides and membrane bound enzymes. Quinones may also render substrates unavailable to the microorganism (Stern *et al.*, 1996). Anthraquinones act on the gastro-intestinal tract to increase the peristalsis action (Egunyomi *et al.*, 2009). Anthranols, anthrones, oxanthronea and dianthrones are all derivatives of anthraquinones (Evans, 1989).

The presence of anthraquinones in *S. alata* used in this study is an indication that it may be useful as a mild laxative especially in cases where patients complain of constipation (Egunyomi *et al.*, 2009). Kazmi *et al.* (1994) also described an anthroquinone from *Cassia italica* which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphtherium* and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*. Tannins were another phytochemical

documented in this study. Tannins are general descriptive name for a group of polymeric phenolic derivatives and are non-nitrogenous plant constituents with astringent properties on mucous membranes (Egunyomi *et al.*, 2009). They are also substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency.

They are found in almost every plant part: bark, wood, leaves, fruits and roots (Scalbert, 1991). The tannins present in the leaf extracts of *S. alata* in this study make it useful in bathing or cleansing the surface of the skin as a result of skin infection/disease. Tannins in this extract were believed to acts by coagulating the cell wall proteins (Onwuliri and Wonang, 2004, 2005). Many human physiological activities such as stimulation of phagocytic cell, host mediated tumor activity and a wide range of anti infective actions, have been assigned to tannins (Haslam, 1996).

Their mode of antimicrobial action as described for quinone, may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope, transport-proteins etc.

They also complex with polysaccharide (Ya *et al.*, 1988). According to a number of studies, tannins can be toxic to filamentous fungi, yeasts and bacteria (Scalbert, 1991). At least two studies have shown tannin to be inhibitory to viral reverse transcriptase (Kaul *et al.*, 1985; Norton and Addy, 1989).

The presence of cardiac glycosides indicates that they may be potent in curing cardiac insufficiency, coughs and circulatory problems. Also they may act as good sedatives and have antispasmodic properties (Kenner and Requena, 1996; Egunyomi *et al.*, 2009). Saponins are surface active agents which interfere with or alter the permeability of the cell wall.

This therefore, facilitates the entry of toxic materials or leakages of vital constituents from the cell (Onwuliri and Wonang, 2004, 2005). Saponins were reported as a major components acting as antifungal secondary metabolite.

Flavonoids are phenolic in nature and acts as cytoplasmic poisons. They also have been reported to inhibit the activity of enzymes (Dathak and Iwu, 1991). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Their

activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996). Flavonoid compounds exhibit inhibitory effects against multiple viruses. Generally, these active components were responsible for the diverse pharmacological actions of the extract used.

The inhibition of tested clinical dermatophytes by *Senna alata* L. leaf confirmed their antifungal activity and this is most likely due to the action of different phytoconstituent present in the leaf extract. A wide range of physiological activity of saponins, alkaloids, carbohydrates, flavonoids, anthraquinones, steroids and tannins.

These were found to be more predominant and therefore may be responsible for the antifungal action on the clinical dermatophytes (Trease and Evans, 1989).

The findings of this study indicated that *Senna alata* extract showed antifungal activity to the dermatophytes tested in this study. This is in agreement with Okoro *et al.* (2010) who documented that the acetone extract of *S. alata* showed the highest antioxidant activity compared to other ethnomedicinal plants (*Momordica charanta* and *Nauclea lafifolia*). In their study, the medicinal plants displayed different polyphenols contents and antioxidant activities. In addition, varying antimicrobial susceptibility patterns were exhibited.

The highest amount of total phenolic compounds was shown by *S. alata* and the lowest one was *M. charanta* (Okoro *et al.*, 2010). However, the dermatophytes used in this study were associated with skin infections such as ringworm and eczema. The ethanolic extract showed that the highest inhibition on some of the tested clinical dermatophytes at higher concentrations. It showed the highest activity on *Trichophyton verrucosum* and *Epidermophyton floccosum* with 20.50 and 20.00 mm zone of inhibition, respectively which competes favorably with clotrimazole. The clotrimazole showed activity on *Trichophyton verrucosum* and *Epidermophyton floccosum* with 22.00 and 21.00 mm zone of inhibition. However, only *Trichophyton mentagrophytes* was inhibited at lower concentration of 1.25 and 2.5 mg mL<sup>-1</sup> of the ethanolic extract, respectively. However, crude plants preparation generally exhibit lower antifungal activity than pure antibiotic substance as cotrimazole.

The Minimum Inhibitory Concentrations (MIC) of the ethanolic extract are reported in this study. The study showed that for the ethanolic extract of the tested dermatophytes, all had Minimum Inhibitory Concentration (MIC) of 5.00 mg mL<sup>-1</sup>. The Minimum Fungicidal

Concentrations (MFC) of the leaf extract at 10.00 and 5.00 mg mL<sup>-1</sup> was fungicidal for *Microsporium canis*, *Trichophyton mentagrophytes* and *T. verrucosum* while for *Epidermophyton floccosum*, it was fungicidal at 10.00 and at 5.00 mg mL<sup>-1</sup> it was fungistatic. The MFC varies with concentration.

This is in consonance with Okoro *et al.* (2010). Okoro documented that some micro organisms (*S. aureus* and *C. albicans*) were susceptible to the *S. alata* polyphenol extracts with MIC values between 1.25-5.00 mg mL<sup>-1</sup> while other microorganisms (*S. pyogenes* and *E. coli*) appeared to be resistant to the extracts. In line with Sher (2009), clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited so new sources especially plant sources are also being investigated.

Second, the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plants compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and national food stores and the self medication with these substances is a common practice to certain extent (Sher, 2009).

Development of resistance to chemotherapeutic agents shown by the microorganisms appears to be a continuous process since the time antibiotics were discovered. So every antibiotic has certain life span regarding its efficacy. Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Jazet *et al.* (2007, 2008) documented antifungal activity of *C. latifolia* oil against *P. angolensis*.

This is interesting and indicates that *C. latifolia* oil could be used as easily accessible source of natural antifungal agent against this fungus which is responsible for heavy losses on Citrus fruits (Jazet *et al.*, 2007, 2008). Further study on isolation and characterization of active principles from medicinal plants and their pharmacodynamic study using latest techniques would be highly beneficial to human beings.

## CONCLUSION

In this study, the extracts of *Senna alata* L. leaf crude extract have high potential as antimicrobial agent. It

showed varying degrees of activities against all the tested dermatophytes with better antifungal activity against *Microsporum canis*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. The phytochemical analysis revealed the presence of important secondary metabolite (alkaloids, saponins, tannins, steroid and anthraquinones), thus indicating the therapeutic potentials of *Senna alata* L. leaf.

It showed the presence of bioactive compounds as well as the antifungal properties of ethanolic crude extract. However, this finding provides an insight into the usage of this plant in traditional treatment of foot infections, subcutaneous parasitic infection, intestinal parasitism, venereal diseases and other diseases associated with bacterial and fungal infections.

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