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Research Article

Antimicrobial Activity of Ethanol Extract of *Senna Alata* Leaves against Some Selected Microorganism in Bayelsa State, Nigeria

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ABSTRACT

***Senna alata* is an underutilized shrub growing in many parts of the world and is known for its traditional use in the treatment of dermotophytes and other related ailment. The ethanol extract of *Senna alata* leaves was evaluated against some dermotophytes (*Malassezia pachydermatis, Malassezia furfure, and Malassezia restricta, Malassezia globosa*) and gastrointestinal bacterial pathogens (*Salmonella* Typhi, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas auriginosa*, *Klebsiella* spp). The extract was tested using well diffusion technique against pathogens and found effective against the selected pathogens. The highest zone of inhibition was observed against *Klebsiella* spp (27.4mm), followed by *S.* Typhi (26mm) and *P. auriginosa* (26mm), *P. mirabilis* (21.7mm) and *E. coli* (19.5mm). In case of fungi, it was most effective against *M. globosa* (19.7mm) and *M. pachydermatis* (17mm). It was not effective against *M. furfure* and *M. restricta*. Thus, *S. alata* is a good antimicrobial agent against bacterial and fungal pathogens of humans.**

Keywords: Antifungal, Antibacterial, Chemotherapy, dermotophyte, Ethanol Extract, *Senna alata.*

INTRODUCTION

Medicinal plants for centuries have been used as remedies for infectious diseases because of the presence of components with therapeutic value. Infectious disease accounts for one half of all deaths in the tropical countries irrespective of efforts made in controlling the incidence of epidemic (Iwu, 1993; Okigbo and Ajalie, 2005). It is therefore very necessary that the search for plants as antibiotic or therapeutics potential be a continued process. Plants are the cheapest and safer alternative sources of antimicrobials (Sharif and Banik, 2006; Doughari *et al*., 2007). Several findings has accumulated and proven the potentials of medicinal plants use in traditional medicine in the treatment of several human disease. Medicinal plants usage as therapeutics in the management of different ailment locally differs from society to society. Several studies have however identified compounds within herbal plants that are effective as antibiotics (Basile *et al*., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon *et al*., 2004), some traditional remedies have successfully been used against antibiotic-resistant strains of bacteria (Kone *et al*., 2004).

Phytomedical practice in Africa has been in existence for centuries even before the colonial administration and is still in use today with about 80% of the population depending on complementary medicine for primary health care delivery (Elujoba *et al*., 2005; Okigbo and Mmeka, 2006). However, in-spite of vast improved health care delivery in developed nations of the world, like the United States of America and Europe, are now turning back to traditional phytomedicine in order to prevent or treat many ailments (WHO, 2006). The demands for traditional herbal medicines is increasing globally, because it is cheap, safe and able to compete with most commercial antibiotics that bacterial pathogens are now even becoming resistant.

Senna alata Linn (Fabaceae) is an ornamental shrub that is widely distributed in the wild in all parts of Bayelsa state especially when dry season is approaching (Figure 1). This shrub is found in other southern part of the country including the south-east and south- west. It is traditionally used in Nigeria in the treatment of several infections which include ringworm, parasitic skin disease. The juice of fresh leaves of *Senna alata* is universally recognized by local healers as a remedy for parasitic skin disease and is used in the treatment of many skin condition by simply rubbing the crushed leaves either alone or mixed with oil on the skin (Oliver-Bever, 1986). The

leaf of this plant was reported to be useful in treating convulsion, onolthoea, heart failure, abnormal pain, oedema and as purgative, but it was especially useful in treating dermatophytosis. The plant is found in Nigeria, Malaysia, Australia, Thailand, tropical America and many other parts of the world. The effectiveness of *S. alata* against skin diseases was confirmed by recent studies (Makinde *et al*., 2007). In Nigeria, The phytochemical components such as alkaloids, anthraquinones, saponins, tannins, terpenes, steroids, flavonoids, carbohydrates present in *Senna alata* was investigated for fungal potency (Owoyale *et al*., 2005). The investigation of *Senna alata* on bacterial infection is limited. This study is therefore aimed at the confirmation of the antibacterial and antifungal activity of the leaves of *Senna alata* on some enteric organisms such as *Escherichia coli*, *Salmonella* Typhi, *Proteus mirabilis*, *Klebsiella spp* and *Pseudomonas auriginosa* and also on some opportunistic dermatophytic fungi such as *M. pachydermatis, M. furfure*, and *M. restricta, M.* globosa so as to justify the use of this plant traditionally by the populace *in vitro*.

MATERIALS AND METHOD

Collection of samples

The medicinal plants used was *Senna alata* leaves, collected in Agudama-Ekpetiama community in Yenagoa Local Government Area of Bayelsa state on the month of October 2013 and identified at the Department of Biological Sciences, Niger Delta University, Bayelsa state and sun-dried.

Preparation of Extracts

Ethanol Extract Preparation

A slightly modified method by Hubert *et al.* (2012) was adopted for the extraction of *Senna alata* leaves. Precisely One hundred (100g) grams of the pounded leaves was weighed using a weighing balance. The weighed sample was soaked in 200mls of 99.9% ethanol contained in a conical flask. The mixture was swirled. After 24hours with interval stirring, the mixture was filtered using Whatman No.1 filter paper and concentrated to dryness at 70oC on a hot air oven. Extracts was stored in the refrigerator at 8oC until required for use.

Phytochemical analysis of crude and ethanol extract of *Senna alata* leaves

This was carried out according to the methods described by Owoyale *et al*. (2005), Ekpo and Etim (2009). Phytochemical analysis of the powdered crude extract of leaves of *S. alata* L. for the identification of phytochemicals such as tannins, alkaloid, steroid, phenols and terpenoid, flavonoid, carbohydrate and glycolsides.

Test Microorganism

The test organisms used in this study are stock cultures of *E. coli*, *S.* Typhi, *Proteus mirabilis, Pseudomona auroginosa, Klebsiella spp, M. pachydermatis, M. furfure, M. globosa and M. restricta* obtained from the Department of Medical Microbiology, Federal Medical Centre Yenagoa, Bayelsa State, Nigeria.

Tests for Potency of Bacteria and Fungi

The clinical Isolates of the microorganisms, *Escherichia coli, Salmonella* Typhi*, Proteus mirabilis, Klebsiella spp and Pseudomona auroginosa* were subcultured in Mcconkey Agar and *M. pachydermatis, M. globosa, M. furfure and M. restricta* was sub-cultured in sabouraud Dextrose Agar (SDA).

Antimicrobial assay

Antimicrobial activity of the ethanol extracts of *Senna alata* was evaluated by the agar well diffusion method as described by cheeseburough (2006). Prepared oxoid sensitest Agar and sabouraud dextrose agar (SDA) were inoculcated appropriately with the test organisms preadjusted to the 0.5 McFarland's turbidity standard. (*Salmonella* Typhi*, Escherichia coli, Proteus Mirabilis, Pseudomonas auriginosa, Klebsiella* spp and *M. pachydermatis, M. globosa, M. furfure and M. restricta*). By dipping the sterile swab sticks into the suspension and removing excess inoculum while pressing and rotating the swab firmly against the side of the tube. The inoculums were streaked all over the surface of the medium. About 1ml of the extract was thereafter, carefully pippeted on the agar holes that was bored with a sterile borer of 8mm in diameter. Ketoconazole drug was used as standard drug for fungi while that

of bacteria was ciprofloxacin. The plates were incubated at 37℃ for 24 hours in the case of bacteria while that of fungi were incubated at room temperature for 96 hours. At the end of the incubation period antimicrobial activity was determined by measuring the zone of inhibition around each well (including the diameter of the well), for each samples that was done in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Alikwe *et al.* (2013). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. Two fold serial dilutions were prepared to obtain a 0.1-50mg/ml concentration range after which about 1ml of the diluted extract were thereafter, carefully pippeted on the oxoid sensitest agar and sabouraud dextrose agar (SDA) that was already seeded with stock culture pre-adjusted to 0.5 McFarland's turbidity standard respectively. The plates were incubated at 37℃ for 24 hours in the case of bacteria while that of fungi were incubated at room temperature for 96hours. At the end of the incubation period antimicrobial activity was determined by measuring the zone of inhibition around each well (including the diameter of the well).

Statistical Analysis

SPSS version 16 was used for descriptive statistical analysis.

RESULT/DISCUSSION

All plant parts synthesize some chemicals in them which acts as protective materials against certain microorganism and are however valuable in the control of microorganism especially if their activities are tested invitro and invivo.

The present research work was focussed on the ethanol extracts of *Senna alata* leaves. The result in this study shows that the leaves of *Senna alata* shows both antibacterial and antifungal properties hence agrees with the traditionally use of this plant in Nigeria to treat bacterial and fungal infections (Idu *et al.*, 2006; Owoyale *et al.*, 2005). The extract of the plants showed varying degrees of antibacterial activities against the selected pathogens. All test bacterial isolates showed good susceptibility effect with extract. highest zone of inhibition observed was seen in *Klebsiella spp* (27.4mm), followed by *S.* Typhi (26mm) and *Pseudomonas auriginosa* (26mm), *Proteus mirabilis* (21.7mm) and *E. coli* (19.5mm) (Table 1). Antibacterial activity however is similar to the findings of Dounghari and Okafor (2007), on the other hand antifungal activity was seen for *M. globosa* (19.7mm) and *M. pachydermatis* (17mm) while *M. furfure* and *M. restricta* were completely resistant (Table 2).

Table 1: Antibacterial activity of *Senna alata* against test bacterial isolates

	E coli	***S* Typhi**	***Proteus mirabilis***	***P auriginosa***	***Klebsiella mirabilis spp***
Plant extract	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)
Senna alata	19.5 ± 1.5	26± 2.1	21.7±1.2	26± 1.0	27.4±0.98
Negative Control	0±00	0±00	0±00	0±00	0±00
Positive control (5µg),	15.8±0.2	22.7±1.9	21.3±1.5	18.0±2.1	19.0±1.5

Generally, the observed antimicrobial effectiveness of *Senna alata* extracts were favourable against all test bacteria, and some fungi particularly *M. pachydermatis and M. globosa*. This then suggest that, it is a promising antimicrobial agent which agreed with suggestion made by Takazawa and Miyashita (1982). It is not common to have commercial antibiotics with antifungal and antibacterial effectiveness, this report reveals the need to concentrate on the possibility of searching for active ingredients in these plants that can be deployed into world's chemotherapeutic usage. A similar finding by Ogujobi and Abiala (2013) and Oliver-bever (1986) also observed the efficacy of *Senna alata* on fungi and support the use in treating fungal skin infections and diseases.

Table 2: Antifungal activity of *Senna alata* against test fungal isolates

Plant extract	*M pachydermatis*	*M furfure*	*M globosa*	*M restricta*
	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)
Senna alata	17.0±1.7	0±00	19.7±1.5	0±00
Negative control	0±00	0±00	0±00	0±00
Positive control (8ug/ml)	21.8±1.01	20.8±1.3	17.3±1.2	21±1.0

MIC determination of the extracts ranged from 0.1-50 mg/ml for the bacteria and from 0.1-50 mg/ml for fungi isolates as well. Results shows that even at 50mg/ml dilution, extracts were still active against all bacteria isolate, while that of fungi *M. globosa* and *M. restricta* still shows complete resistance. However, there was increase in diameter with increase in concentration (Table 3). High efficacy of the plant extract on selected microorganism is determined with the least concentration still showing efficacy while in high concentration, a low efficacy is indicated due to possible development of resistance by the microorganisms to the antimicrobial (Majore, 1999). The plant can therefore be used in the treatment of gastrointestinal, urinary tract, as well as some mycotic infections because it competes favourably with the commercial antibiotics used as control.

Table 3: Minimum Inhibitory Concentration (MIC) of ethanol extract of *Senna alata* leaves against test bacterial and fungal isolates

Microorganism	Extract/Concentration *Senna alata*		
	0.1mg/ml	**25mg/ml**	**50mg/ml**
Bacteria	18.6mm	18mm	10mm
E coli	29.2mm	23mm	19mm
S Typhi	23mm	19mm	12mm
Proteus mirabilis	22mm	19mm	12mm
P Auriginosa	23mm	16mm	16mm
Klebsiella mirabilis spp			
Fungi	15.3mm	15mm	12.4mm
M pachydermatis	R	R	R
M furfure	17mm	16.3mm	15mm
M globosa	R	R	R
M restricta	R	R	R
Key: R- Resistance, mm-milimeter			

The result of phytochemical analysis showed that *S. alata* contained tannins, steroids, phenols and saponins (Table 4). 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 (Sule *et al*., 2010, Anosike *et al.,* 2012). The biochemical screening of *S. alata* L. leaf revealed the following important phytoconstituents: saponins, steroids, phenols, tannins, and absence of glycosides, alkaloids, carbohydrate and flavonoid. Thus indicating the therapeutic potentials of *S. alata* L. leaf. Several reports showed that the activities of these plants were due to the presence of tannins, saponin, flavonoids and anthraquiones in the plant extract. Similar phytochemical constituents (flavonoids and tannins) were also revealed in the plant (*Stachyterpheta indica*) against pathogenic bacteria as reported by Kumar *et al*. (2012). The tannins present in the leaf extracts of *S. alata* in this study make it useful in bathing or cleansing of skin surfaces. Documented literatures has it that tannins can be toxic to filamentous fungi, yeasts and bacteria (Treese and Evan, 2004). And also inhibitory to viral reverse transcriptase (Onwuliri and Wonang, 2004, 2005). Saponins were reported as a major components acting as antifungal secondary metabolite (Onwuliri and Wonang, 2004, 2005). A wide range of physiological activity of saponins, steroids, phenols and tannins are found to be more predominant and therefore may be responsible for the antimicrobial action (Sule *et al*., 2010).

Table 4: Results of Phytochemical screening of *Senna alata*

Phytochemicals	Presence of colour formation
Tannins	+
Alkaloid	_
Steroid	+
Phenols	+
Flavonoid	_
Carbohydrate	_
Glycolsides	_
Saponins	+

+ Present, - absent

CONCLUSION

The ethanolic extract of leaves of *Senna alata* demonstrated antimicrobial activity implying, it can be useful in antiseptic and disinfectant formulation as well as in chemotherapy. So there is need for more research on the activity of the extracts against a wider range of bacteria and fungi and even viruses. The anti-diarrhoeal, anti-typhoidal and antidermatomycotic activities of the plant extracts can be further explored invivo.

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