Biotechnol Res 2016; Vol 2(4):204-211
eISSN 2395-6763

ORIGINAL RESEARCH

Phytochemical Characteristics and *in vitro* Antibacterial Activities of *Senna alata* Leaves against Some Clinical Isolates

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• Received: 30 September 2016 • Revised: 28 November 2016 • Accepted: 05 December 2016 • Published: 17 December 2016 •

ABSTRACT
The antibacterial activities of ethanolic and methanolic extracts of *Senna alata* leaves against three infectious bacteria *(Escherichia coli, Salmonella spp* and *Staphylococcus aureus)* were examined using agar well diffusion method. The methanolic and ethanolic extracts were found effective against the tested clinical isolates. For the methanol extract, the highest zone of inhibition was observed against *Salmonella spp* with a diameter of 22.67 ± 0.67mm, followed by *E. coli* (20.33± 1.20mm) and *S. aureus* (17.00± 0.58mm). The highest zone of inhibition for ethanol extract was observed against *E. coli* (18.33± 1.20mm), followed by *S. aureus* (17.33±0.78mm) and *Salmonella spp* (14.67±2.40mm). The extracts demonstrated considerable activity against both Gram positive and Gram negative bacteria with the methanol extract showing higher activity than the ethanol extract. *E. coli* showed more susceptibility to the methanol extract of *S. alata* from the concentrations of 500mg/ml to 62.5mg/ml while *S. aureus* and *Salmonella spp* showed lesser susceptibility. The Minimum inhibitory concentration (MIC) result also showed that the MIC of *S. alata*. The phytochemical analysis carried out on the leaves of *S. alata* detected the presence of Alkaloids, Saponins, Tannins, Flavonoids and was fairly present for Phenol. The findings in this study with *Senna alata* leaves having antibacterial activities thus require more attention and evaluation needed against a wider range of microorganism.

KEY WORDS: *Antibacterial, Senna alata, solvent, Traditional medicine*

Introduction

Traditional medicinal plants are therapeutic resources used in many parts of the world specifically for healthcare and may also serve as starting materials for many available drugs today (Kigigha *et al*., 2015, 2016; Epidi *et al*., 2016a,b). Traditional medicines have been the focus for a wider coverage of primary healthcare delivery in Africa (Elujoba *et al*., 2005). Medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (Sofowora, 1993), which have beneficial properties. People of all continents have long applied poultice and imbibed infusions of indigenous plants dating back to pre-history for health purposes (Cowan, 1999). Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman *et al*., 2000; Karou *et al*., 2005; Okoro *et al*., 2010).

In Nigeria, various plant parts are used in treatment of different ailments with remarkable success. Among these, enormous numbers of medicinal plants are derived from members of the genus *Senna* (Ogunjobi and Abiala, 2013; Ehiowemwenguan *et al*., 2014). According to Odugbemi and Akinsulire (2006), the genus *Senna* has six species with medicinal value. Of these, three (*Senna tora, Senna*

orcidentalis and *Senna alata)* have been reported to have strong antifungal activities (Oliver-Bever, 1986). Furthermore, several reports have shown that *Senna alata* contain antimicrobial substances (Fuzellier *et al*., 1982; Palanichamy and Nagarajan, 1990; Crockett *et al.,* 1992; Caceres *et al.,* 1993; Ibrahim and Osman, 1995; Khan *et al.,* 2001; Somchit *et al.,* 2003; Timothy *et al.,* 2012; Selvi *et al*., 2012).

Senna is a native plant in Southeast Asia, Africa, Northern Australia and Latin America (Parsons and Cuthbertson, 1992; Timothy *et al*., 2012) and is grown as ornamental plants with diverse medicinal uses. It is commonly known as "Rai dore" in Hausa, "Asuwon oyinbo" in Yoruba,"Omirima" in Igbo and Efen-diri among the Ijo's of the coastal region of Nigeria.

Senna alata Linn (Fabaceae) is an ornamental shrub that is widely distributed in the wild in all parts of Bayelsa state and is usually abundant towards the onset of the dry season (November-March) (Zige *et al.,* 2014). *Senna alata* is recommended in some local communities in Nigeria for the treatment of ringworm and other skin diseases. Hence, there is great interest in characterizing its phytochemical components and evaluating the antimicrobial efficacy of different extracts of its leaves against a wide range of clinically important bacterial isolates. The phytochemistry and antibacterial activity of the plant is still largely unexplored despite the growing use of this plant. Therefore, this study seeks to evaluate the in vitro bacterial activity of ethanol and methanol leaf extracts of *Senna alata* against some clinical isolates and characterize the phytochemistry of its leaves.

Materials and Methods

Collection of Plant Samples

Senna alata leaves used for this study was collected from bushes between Otuoke and Onuebum communities in Ogbia Local Government Area of Bayelsa state, Nigeria in the month of October 2015 and identified at the Department of Biology, Federal University Otuoke, Bayelsa state, the plant material was air dried with the aid of circulating air flow at room temperature for seven days. It was weighed daily for the until it attained a steady weight. The dried leaves were homogenized into fine powdered form and stored in an air tight container.

Extractions of *Senna Alata* Leaves

100g of dried pulverized leaves of *Senna alata* was weighed out differently for methanol and ethanol using the analytical weighing balance. The sample was wrapped with fifteen pieces of Whatman filter paper, each filter paper containing about 3.3g of the powdered *Senna alata.* The soxlet jacket was loaded with the wrapped sample. 500ml of absolute ethanol and methanol was measured using the measuring cylinder and was transferred into the soxlet flat bottom flask distinctively. The soxlet apparatus was set, the water inlet and outlet of the soxlet condenser was connected, the heating mantel was put on and set to heat at 70^0C. After about four (4) hours of extraction, the resulting solution of the ethanolic and methanolic extract was evaporated to dryness in a water bath at 40^0c. The resultant crude extract was transferred into airtight sample bottles and kept at 4^0c inside the refrigerator until when needed.

Test Organisms

The test isolates *(Escherichia coli, Salmonella spp* and *Staphylococcus aureus)* used in this study were obtained from stock cultures from the Microbiological unit of Gloryland Medical centre Yenagoa, Bayelsa state. The bacterial isolates were confirmed for viability in nutrient agar grown overnight and bacterial isolates were re-identified using Gram reaction, Biochemical tests namely; Kigler Iron Agar, Motility, Indole, Urea, Catalase and Coagulase test following the scheme of Cheesbrough (2006). The test organisms were sub-cultured on a nutrient agar plate and incubated at 37^0C for 24 hours. A colony from the pure culture of *Eschericia coli*, *Staphylococcus aureus* and *Salmonella* sp was picked and transferred into test tubes containing 5ml of normal saline, it was mixed and the absorbance and transmittance was measured on a spectrophotometer and was adjusted to the Mcfaland's turbidity standard by adding more inoculum or normal saline. The resulting suspension now contain 1×10^8 colony forming units per ml (cfu/ml).

Phytochemical Analysis

Preparation and analysis

Extraction method previously described by Awomukwu et al. (2014, 2015) was adopted in this study. The plant materials were air-dried and milled into uniform powder using Thomas-Willey milling machine. The aqueous extract of each sample was determined by soaking 50g of dried powdered sample in 10ml of distilled water for 12hours. The mixtures were filtered

using Whatman filter paper and excess water removed by concentration by boiling the extract to 100^0C for 10 minutes.

Qualitative Determination of the Chemical Constituents

Freshly prepared ground samples are chemically tested for the presence of chemical constituents using standard procedures (Trease and Evans, 1989).

Test for alkaloids

One mL of extract of the sample was shaken with 5.0ml of 2% HCl on a steam bath and filtered. To 1.0mL of the filtrate was treated with Wagner's Reagent (Iodine in Potassium-Iodine solution) and observed for reddish brown precipitate.

Test for tannins

To 1.0mL of extract was added an equal volume of bromine water. The formation of a greenish to red precipitate was taken as evidence for presence of condensed tannins.

Test for flavonoids

To 1.0mL of extract 1.0ml of 10% lead acetate was added. The formation of yellow precipitate is taken precipitate for flavonoids.

Test for saponins

One mL of extract was boiled with 5.0ml of distilled water for 5 minutes and decanted while still hot. The filtrate is used for the test. 1.0ml of the filtrate was diluted with 4.0ml of distilled water, shaken vigorously and observed on standing for stable froth.

Test for phenols

To 1.0mL of extract was added 1.0ml of 10% ferric chloride. The formation of a greenish-brown or black precipitate or colour is taken as positive for phenolic nucleus.

Quantitative determination of the chemical constituents

Alkaloids Determination

Alkaloids in the test sample were determined by the gravimetric method described by Harborne (1973), Mshelia *et al*. (2016), Belonwu *et al*. (2014) and Obadoni and Ochuko (2001). A measured weight of the processed sample (5g) was soaked in 100ml of 10% acetic acid solution in ethanol. The mixture was allowed to stand for 4 hours of room temperature before being filtered. The filtrate was concentrated to about a grater of its original volume. The alkaloid in the extract was precipitated by drop wise addition of concentration. Aqueous ammonia with full precipitate was obtained. The mixture was filtered through a weighed filter paper to collect a precipitate. The precipitate was dried in the oven at 60^0C, cooled in desiccators and reweighed by difference; the weight of alkaloid (precipitate) was determined and expressed as a percentage of the weight of the sample analyzed. The alkaloid content was given by the formula.

% Alkaloids = $(W_2 – W_1)$/Weight of sample

Where; W_1 = Weight of filter paper

W_2 = Weight of paper with alkaloids precipitate

Tannins Determination

Tannins in this test sample as determined by Van-Burden and Robinson (1981) method. 5.0g of each of the sample was weighed into a 100 mL plastic bottle. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M Potassium Ferocyanide ($K_3Fe(CN)_3$). The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured

Tannin (mg/100mL) is expressed as: X-Blank/Standard-Blank

Where; X = Absorbance

Flavonoids Determination

Flavonoids in the test sample were determined by the acid hydrolysis gravimetric method (Harborne, 1973; Boham and Kocipai, 1994; Eleazu *et al*., 2012). A measured weight of 5.0g of each processed plant sample was mixed with dilute 1m HCl solution to form a ratio of 1:10w/v. The mixture was boiled for 30 minutes. The boiled extract was allowed to cool and filtered through Whitman No.42 filter paper. A portion of the extract (20mL) was measured with a beaker and treated with Ethyl Acetate to precipitate the flavonoids. The precipitate was measured by filtering with a weighed filter paper and determined by weight difference. It was given by:

% Flavonoids = $W_2 – W_1$ x 100/Weight of sample

Where; W_1 = Weight of filter paper

W_2 = Weight of paper with flavonoid precipitate

Saponins Determination

The saponins content was determined by the combined solvent extraction method (Harborne, 1973; Belonwu *et al*.,

2014). A measured weight of 5.0g of each processed sample was mixed with 20% of ethanol to form a ratio 1:10w.v (10g-100ml), the mixture was incubated with agitation in a water bath at 55^0C for 12 hours. It was filtered through Whatman No.42 filter paper. The residue was re-extracted with 200mL of the same extract (20% of ethanol) the combined extract was reduced by evaporation to about 40mL over a water bath. The concentration extract was transferred to a separation funnel and re-extracted by partitioning and shaking vigorously with 20ml of diethyl ether. The aqueous layer was discarded. The re-extractant was contained until a colourless water layer was obtained. The pH of the colourless aqueous layer was adjusted to 4.5 by the use of NaCl. Partitioning successively with 60 and 80mL portion of normal butanol showed the resulting mixture. The combined extract was washed twice with 50% of NaCl solution and transferred to a tarred evaporation dish. It was evaporated to dryness 80^0C cooled in desiccators and re-weighed by difference; the weight of crude saponins extract was determined and expressed accordingly. Saponin content in mg/100g sample was given by the expression.

Saponins mg/100g = 100 x 100 (W_2 x W_1)/Weight of sample

Where W_1 = Weight of evaporation dish empty

W_2 = Weight of dish of saponins extract

Phenols Determination

The Folin-Ciocalteau colorimetric method described by Harborne (1973) and Obadoni and Ochuko (2001) was used to determine the phenol contents. 5.0g of the dried powdered sample was dissolved into a 10 mL of methanol in a test tube and was shaken thoroughly. The mixture was allowed to settle for 15 minutes, before using Whatman filter paper 42.1mL of the extract was measured into a test tube and 1mL Follin-Ciocaltean reagent was added to it with 5.0mL of distilled water. The colour was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760nm. The experiment was repeated two more times for confirmation. The phenol content was calculated to get average.

%Phenol content = 100/w x C/100 x VF/VA x D

Where:-

W = Weight of sample analyzed

Au = Absorbance of the test sample

As = Absorbance of standard solution

C = Concentration of standard in mg/ml

VF = Total filtrate volume

VA = Volume of filtrate analysed

D = Dilution factor where applicable

Disc Diffusion Method

Antimicrobial activity of the ethanol and methanol extracts of *Senna alata leaves* was evaluated by the agar well diffusion method as described by Cheesbrough (2006). Prepared oxoid sensitest Agar were inoculcated appropriately with the test organisms (*Salmonella* spp*, Escherichia coli and S. aureus*) pre-adjusted to the 0.5 McFarland's turbidity standard in different tubes. By streaking the inoculum all over the surface of the medium. Next, holes of 6mm diameter was bored onto the agar plate making use of a cork-borer. Using a calibrated micropipette, 20ul of the extract was introduced into the wells. Gentamycin drug was used as standard positive control. The plates were incubated at 37°C for 24 hours. At the end of the incubation period antimicrobial activity was determined by measuring the zone of inhibition around each well for each samples that was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of MIC of both ethanol and methanol extract was carried out using the following concentration 500mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml respectively. Test bacteria cells from overnight culture was inoculated on pre-sterilized nutrient broth and adjusted to 0.5 mcfaland's turbidity standard. 1ml of the extract from the different concentration was pipetted using a Pasteur pipette and introduced into the wells and readings taking after 18-24hrs incubation for the minimum inhibitory concentration measured in millimetres using a meter rule.

Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis. The data were expressed as Mean ± standard error. A one-way analysis of variance was carried out at P = 0.05, and Duncan Statistic was used to determine the source of the detected differences.

Results and Discussion

Results of qualitative phytochemical analysis of leaves of *Senna alata* reveals the quality of the alkaloids, tannins, flavonoids, saponins and phenols, the presence of the

phytochemicals, shows varying degree, with alkaloid and saponin been highly present, followed by tannin and flavonoid which is moderately present while phenol is fairly present (Table 1).

Table 1: Qualitative analysis of the Phytochemicals in *Senna alata*

Phytochemicals	*Senna alata* (Leaf)
Alkaloids	+++
Tannins	++
Flavonoids	++
Saponins	+++
Phenols	+

+ = Fairly present; ++ = Moderately present; +++ = Highly present

The quantitative determination of phytochemical compounds reveals that saponins have the highest percentage whereas phenol the least. These data are presented in Table 2.

Table 2: Quantitative Analysis of the Phytochemicals in *Senna alata*

Phytochemicals	*Senna alata* (Leaf)
Alkaloids (%)	4.06
Tannins (%)	1.54
Flavonoids (%)	1.48
Saponins (%)	4.28
Phenols (%)	0.36

The phytochemical screening of *Senna alata* showed various bioactive agents present which demonstrated antibacterial activity against all tested bacteria species, this result is in agreement with the traditionally use of this plant in Nigeria to treat bacterial infections (Idu *et al.*, 2006; Owoyale *et al.*, 2005; Zige *et al*., 2014) .Phytochemical compounds present includes tannins which was in all the extracts as well as saponins, alkaloids, flavonoid in varying degree, with the highest recorded level being saponins and alkaloids. The antibacterial activity of *S. alata* leaves could be due to the phytochemicals present which have been reported by Dweck (1994) to act as plant protectants against pathogens in the wild. The protection is equally conferred on humans when plant parts are drunk as concoctions, decoctions in ethnomedicine.

Table 3 presents the results of antibacterial efficacy of methanol and ethanol extracts of leaves of *Senna alata* against *E. coli, S. aureus and Salmonella* spp. zone of inhibition produced by methanol extracts on *E.coli* showed 20.33±1.20, *S.aureus* (17.00±0.58) and *Salmonella spp* (22.67±0.67). Typically, the zone of inhibition of *S.aureus* were significantly (P<0.05) lower compare to *E.coli* and *Salmonella spp*). On the other hand ethanol extract produced zones of inhibitions for *E. coli (*18.33±1.20), *S.aureus (*17.33±0.76) and *Salmonella spp (*14.67±2.40) measured in millimetre. There was significant variation (P>0.05) among the various test bacterial cell.

Table 4 Presents the MIC for leaves of *Senna alata* with their extracting solvent against test bacteria cell. Results showed that at concentration of 500mg/ml for methanol extract, there was no significant difference (p>0.05) between all test bacteria (15.67±0.88b, 15.00±1.00b and 7.33±0.67b) while at the same concentration for methanol there was significant difference.

Based on Table 3 and Table 4, both the ethanolic and methanolic extract of *Senna alata* leaves had antimicrobial activity of on *S. aureus, E. coli*, and *Salmonella* spp. The zone diameter of inhibitions for methanolic and ethanolic extract respectively reveals a dose-dependent antimicrobial activity. The methanoic extracts gave an MIC of 62.5mg/ml for *E.* coli which is the lowest recorded MIC and this points towards the efficacy of the plant extract to cure ailments caused by *E. coli.* On a similar note, the ethanoic extract gave an MIC of 250mg/ml for *Salmonella* showing that it may be effective in treating salmonellosis. However, the MIC obtained for *S. aureus* is high (500mg/ml). This concentration may not be administrable in vivo as it may cause systemic toxicity.

The result obtained for *S. aureus* from previous studies (Doughari and Okafor, 2007; Zige *et al*., 2014) contrasts to those obtained in this present study. While the present study showed similar finding for the other test isolates, it should be noted that the bioactive components of any medicinal plant may differ in their solubility depending on the solvents used for extraction. This may account for the differences observed in the effective ethanol and methanolic extracts. The results obtained from the present study provide evidence that ethanolic extracts and methanolic extract of the *Senna alata* exhibit useful antibacterial activity against test bacteria cells. Effective exploitation of a plant derived bacterio-potential ability is dependent on its bioavalibility, in this case *Senna alata*, which is abundant in many parts of the world and grows effectively as the dry season approaches (Zige *et al.,*

Table 3: Results of efficacy test of *Senna alata* against *E.coli, S. aureus* and *Salmonella* spp.

Test bacteria cell	Extracting Solvent (Diameter mm)		
	Methanol	Ethanol	Control
E.coli	20.33±1.20b	18.33±1.20a	23.00±0.58a
S.aureus	17.00±0.58a	17.33±0.76a	24.67±1.76a
Salmonella spp	22.67±0.67b	14.67±2.40a	20.33±1.20a

Table 4: Results of Minimal inhibitory concentration (MIC) of *Senna alata* against test bacteria cells

Extracting Solvent	Assayed Species	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)
		(Inhibitory diameter, mm)			
Methanol	*E. coli*	17.33±0.67b	12.33±0.88c	9.33±0.67b	7.33±0.33b
	S. aureus	15.00±1.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Salmonella spp	15.67±0.88b	0.00±0.00a	0.00±0.00a	0.00±0.00a
Ethanol					
	E. coli	15.33±0.88b	0.00±0.00a	0.00±0.00a	0.00±0.00a
	S.aureus	9.33±0.67a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Salmonella spp	11.00±0.58a	8.33±0.33b	0.00±0.00a	0.00±0.00a

2014). Previous study also proves the entire plant parts like the flower, leaf, root, stem and bark have potential medicinal property, therefore exploiting other parts of this plant involves harming the plant and possibly decline their bioavailability. However, the antimicrobial potential may cause its competitive demand for the formulation of bioactive decoction, and alternative source of management of bacterial infections as it is active against both gram positive and negative bacteria.

Conclusion/Recommendations

The control and management of several ailments is a global challenge as many synthetic drugs are becoming resistant to many antibiotics and are also in many cases expensive and therefore unaffordable, especially in socio-economically poor society where most infectious diseases such as diarrhoea, typhoid and many waterborne diseases are endemic. Plants and plant products have been used extensively throughout history to treat ailments. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. *Senna alata* plant has found potential application as an eco-friendly potential antimicrobial due to its activity against *E. coli, S. aureus* and *Salmonella spp*. The fact that the pathogenic strain of *S. aureus* can cause localized abscesses and septicaemia in humans implies that the ethanolic and methanolic extract of *Senna alata* could be employed for the treatment of such infections and also in the management of diarrhoea and salmonellosis. The agar *in vitro* assay might not be conclusive in determining the antibacterial activity of plant extract. Thus, further *in vivo* activity of the plant should be evaluated to

ascertain its viability for inclusion in formulation of drugs by pharmaceutical industries. The phytochemical evaluation of the leaves of *Senna alata* through this study was found to have Flavonoids, Tannins, Alkaloids, and Saponins. The presence of this phytochemical constituent can be isolated, identified and utilized by the Pharmaceutical industries in the production of drugs. Further research needs to be carried out on the leaves and other parts of *Senna alata* to isolate and identify the active compound (s) responsible for its medicinal properties.

Acknowledgements

This publication is based on undergraduate project work of third author (Evelyn C. EZIUKWU) supervised by Douye V. ZIGE at Federal University Otuoke, Bayelsa state, Nigeria.

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