

The Antibacterial Activity of Ethanolic Leaf Extracts of Six Senna Species

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| Received: 19.03.2019 | Accepted: 27.03.2019 | Published: 31.03.2019

DOI:10.21276/haya.2019.4.2.5

Abstract

Leaf ethanolic extracts of six Senna species namely *S. occidentalis*, *S. hirsuta*, *S. siamea*, *S. obtusifolia*, *S. polyphylla* and *S. alata* were obtained using the cold extraction method. The extracts were tested for antimicrobial activity against five organisms, namely *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Salmonella typhi*. The agar well diffusion method was used to carry out this test. The study on antimicrobial activities shows that ethanolic extracts of the six Senna species possess antibacterial activity against human pathogens used in this study. The antibacterial activities of the extracts were more pronounced at higher concentration than at lower concentration in the species of Senna investigated. However, *Senna alata* showed more antimicrobial activity. The minimum inhibitory concentration (MIC) of the plant extracts ranged from 21.5 mg/ml to 62.5 mg/ml with *Senna alata* having the lowest value (31.25 mg/ml) for the pathogen tested except *Klebsiella pneumonia* (65.5 mg/ml) while the other Senna species have similar minimum inhibitory concentration (MIC). The antimicrobial activity of ethanolic extract of *Senna alata* was favourably compared with the standard drug, ciprofloxacin. The antibacterial activities of the plant extract could possibly be due to alkaloids and flavonoids. Bioactive substance from these six Senna species can therefore be employed in the formation of antimicrobial agent for the treatment of various bacterial infections or diseases.

Keywords: Antibacterial, Senna spp., leaf extracts, minimum inhibitory concentration, zone of inhibition.

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INTRODUCTION

It has been estimated that up to 80% of the population of Africa uses traditional medicine in primary health care [1]. Approximately 25 % of all prescription drugs used contain one or more bioactive compounds derived from plants [2]. Yet fewer than 10 % of the plant species have been examined for the presence of bioactive compounds [3]. Hence screenings of antimicrobial plants for new bioactive agents as alternative to conventional antibiotic drugs possess an enormous challenge and are important especially with the emergence of drug resistant disease strains [4, 5]. Such plant products are considered as biodegradable and have fewer side effects than conventional antimicrobial drugs [6].

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [7,41]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re - evaluation of the therapeutic use of remedies from plants [8, 9]. According to [1], medicinal plants would be the best source to obtain a variety of drugs. Medicinal plants are

the richest bio-resource of drugs, modern medicine, and food supplements, folk's medicine, pharmaceutical intermediates and chemical entities [10, 11].

The genus *Senna* comprises shrubs, sub shrubs, and herbaceous perennials with paired-pinnate leaves. *Senna* is native to tropical Africa and cultivated in Egypt and the Sudan and elsewhere; it is native to India and cultivated mainly in India and Pakistan [12]. *Senna* is a stimulant laxative and used for treatment of constipation [13]. Significant inhibitory activity in mice against leukemia has been documented.

In Nigeria, the plants are used for treating eczema and other skin defects caused by fungal infections [14]. The leaves are also prepared into vegetable soup to treat small pox and measles. *Senna podocarpa* leaves are extensively known for their anticonorrhoeal and purgative properties as well as treatment of guinea worm infections and sore-healing remedy among the Igbos in Nigeria [15]. Fresh leave concoction is used for curing syphilis [16], herpes and swine fever [17] or used as purgative and for repelling or killing insects such as termites, bed bugs and mosquitoes [18].

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent [19]. For example Ogundare, [20] have investigated the antimicrobial pattern and phytochemical properties of the leaf extracts of *Senna podocarpa*, Mubashir et al. [21] also investigated the antibacterial activity of whole plant extract of *Marrubium vulgare*; Iwalokun et al. [22] have reported on the comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus* and Ogundare [23] reported on antimicrobial effect of *Tithonia diversifolia* and *Jathropa gossipifolia* leaf extracts.

In Africa, herbal medicines are often used as primary treatments for many diseases including HIV/AIDS and HIV-related problems. These African herbs are being recommended by the ministry of health in South Africa and member states for use in HIV [23]. On this background, the present study was intended to muster information about the antimicrobial properties of six *Senna* species and their potentials in the pharmaceutical industries. The objective of this study to investigate the antimicrobial activity assay of the six *Senna* species using their plant extract to test against clinically isolated microbial samples.

MATERIALS AND METHODS

Collection of samples

The plant materials used for this project work were six (6) species of *Senna* namely *S. occidentalis*, *S. hirsuta*, *S. siamea*, *S. obtusifolia*, *S. polyphylla* and *S. alata*. The six species were collected from within the campus Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were identified and authenticated at the Herbarium Unit, College of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Extraction of plant extracts

The plant parts used were the leaves and the plant materials were air dried. After properly drying, the plant materials were ground into fine powder using electric blender. Thereafter, 50 g of dried powder was soaked in 200 ml absolute ethanol for 48 hours. The plant extracts were filtered through Whatman Number 1 filter paper into beakers. The filtrates were dried until a constant dry weight of each extract was obtained. The filtrates obtained were concentrated under vacuum with rotary evaporator at 40°C to obtain the crude extracts.

The extracts were subsequently freeze dried to be used for antibacterial screening.

Antibacterial screening

The absolute ethanol extracts of the six *Senna* species were screened against a total of five bacterial strains. The test organisms were *E. coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pneumonia* (clinical isolates) obtained from microbiology laboratory of National Veterinary Research Institute, Umudike, Nigeria. The anti-microbial activity was determined by the Agar well diffusion method [24] using Mueller-Hinton agar plates for all the bacteria.

A standardized inoculum culture is spread evenly on the surface of gelled agar plates, wells of 6 mm are aseptically punched on the agar using a sterile corn borer. On each of the culture plates, previously seeded with bacteria, 1 ml of the plant extracts were introduced into the wells and antibiotic discs of Ciproflaxin (500 g/ml) was used as positive control for the bacteria. The experiment was performed in triplicate. Incubation was at 37^o C for 24 hours. The zones of inhibition formed around the disc were measured with transparent ruler and the mean diameter obtained.

RESULTS

The results of the antibacterial test are summarized in the tables 1 – 6. This study shows that ethanolic extracts of *Senna* species possess antibacterial activity against human pathogens used in this research. *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Salmonella typhi*. The antibacterial activity of this *Senna* species were more pronounced at higher concentration than at lower concentration (Tables 1-6).

The antibacterial activity of ethanolic extract of *Senna alata* was favourably compared with the standard drug-ciprofloxacin (Table 1). Other species like *Senna obtusifolia*, *S. occidentalis*, *S. hirsuta*, *S. polyphylla* and *S. siamea* exhibited similar activities but not as intense as that of *Senna alata*.

The minimum inhibitory concentration (MIC) of the plant extracts ranged from 21.5 mg/ml to 62.5 mg/ml with *Senna alata* having the lowest value (31.25 mg/ml) for the pathogen tested except *Klebsiella pneumonia* (65.5 mg/ml) while the other *Senna* species have similar minimum inhibitory concentration (MIC).

Table-1: Ethanolic extract of *Senna alata* (leaves)

Organisms	<i>Senna alata</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	12.7±0.12	30.0±0.20	10.0±0.10	28.0±0.27	7.3± 0.60	26.±0.12	5.3±0.07	25.0±0.10	3.7±0.06	23.0±0.12	31.25	31.25
<i>Klebsiella pneumonia</i>	10.0±0.10	38.0±0.15	4.7±0.42	35.0±0.15	5.9± 0.15	32.7±0.23	4.3±0.12	30.0±0.27	2.7±0.15	27.0±0.35	62.5	31.25
<i>Staphylococcus aureus</i>	10.0±0.10	30.0±0.10	9.0±0.10	28.0±0.10	7.0±0.10	25.0±0.10	5.3±0.06	22.7±0.06	4.0±0.15	20.0±0.10	31.25	31.25
<i>Streptococcus pneumonia</i>	9.7 ± 0.21	22.3±0.25	7.5±0.15	20.0± 017	5.7± 0.12	18.0±0.17	4.0±0.00	16.0±0.20	3.0±0.00	14.3±0.15	31.25	31.25
<i>Salmonella typhi</i>	11.0±0.10	42.0±0.20	9.0±0.10	39.3±0.25	8.0± 0.10	36.3±0.25	6.0±0.10	33.3±0.21	4.0±0.10	31.3±0.15	31.25	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant.

Table 2: Ethanolic extract of *Senna obtusifolia* (leaves)

Organisms	<i>Senna obtusifolia</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	8.7±0.06	38.7±0.15	7.0±0.10	37.0±0.20	5.3±0.06	34.7±0.16	3.7± 0.06	32.0±0.20	1.7±0.06	29.7±0.15	62.5	31.25
<i>Klebsiella pneumonia</i>	9.0±0.10	37.0±0.20	7.0±0.10	35.0±0.10	5.7±0.15	33.3±0.12	4.0± 0.10	31.7±0.15	2.3±0.12	29.3±0.12	62.5	31.25
<i>Staphylococcus aureus</i>	7.7±0.06	31.0±0.10	6.0±0.00	28.7±0.12	4.3±0.06	27.0±0.17	3.0± 0.00	25.3±0.23	1.3±0.06	22.3±0.21	62.5	31.25
<i>Streptococcus pneumonia</i>	10.3±0.15	28.0±0.10	8.3±0.15	26.0±0.10	7.0±0.10	24.3±0.15	5.3± 0.15	23.3±0.12	3.0±0.10	20.0±0.10	31.25	31.25
<i>Salmonella typhi</i>	8.7±0.06	29.7±0.15	7.0±0.10	28.0±0.10	5.3±0.06	25.3±0.06	3.7± 0.06	22.7±0.06	2.3±0.06	20.30±0.00	62.5	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant.

Table-3: Ethanolic extract of *Senna occidentalis* (leaves)

Organisms	<i>Senna occidentalis</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	8.0±0.10	29.3±0.06	6.0±0.10	27.±0.17	4.7± .06	24.±0.23	3.0± 0.00	21.0±0.21	0.3±0.06	19.±0.15	62.5	31.25
<i>Klebsiella pneumonia</i>	8.3±0.15	24.3±0.02	6.7±0.21	22.±0.21	5.0±0.17	20.±0.23	3.3±0.315	18.0±0.17	1.0±0.10	16.±0.12	62.5	31.25
<i>Staphylococcus aureus</i>	7.7±0.15	27.0±0.20	6.0±0.10	25.±0.21	4.7±0.15	22.±0.23	3.30±0.10	20.0±0.15	1.0±0.17	18.±0.12	62.5	31.25
<i>Streptococcus pneumonia</i>	8.0±0.10	26.7±0.31	5.7±0.06	25.±0.21	4.3±0.06	23.±0.15	2.3± 0.06	21.0±0.21	1.0±0.00	18.±0.12	125	31.25
<i>Salmonella typhi</i>	9.0±0.10	26.3±0.21	7.0±0.20	25.±0.20	5.7±0.15	32.±0.12	4.0±0.10	20.0±0.10	2.0±0.10	19.± .10	62.5	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant.

Table-4: Ethanolic extract of *Senna hirsuta* (leaves)

Organisms	<i>Senna hirsuta</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	8.3±0.12	35.7±0.15	6.0±0.10	33.3±0.15	4.7±0.12	30.7±0.12	3.7±0.12	29.0±0.10	1.0±0.10	27.3 ±0.06	62.5	31.25
<i>Klebsiella pneumonia</i>	9.3±0.06	28.7±0.06	7.7±0.06	26.3±0.06	5.3±0.06	23.7±0.06	3.3±0.06	22.0±0.17	1.0±0.10	19.7±0.06	62.5	31.25
<i>Staphylococcus aureus</i>	8.3±0.06	29.0±0.10	6.7±0.06	27.0±0.20	4.7±0.06	24.7±0.15	2.7±0.06	22.3±0.21	0.7±0.06	20.3±0.15	125	31.25
<i>Streptococcus pneumonia</i>	18.3±0.12	29.0±0.10	16.0±0.10	27.0±0.17	13.0±0.10	24.7±0.21	10.7±0.12	21.0±0.10	7.7±0.12	19.3±0.12	31.5	31.25
<i>Salmonella typhi</i>	10.0±0.10	26.7±0.15	7.3±0.15	24.7±0.12	5.0±0.10	21.7±0.06	3.3±0.06	20.3±0.06	1.3±0.06	18.3±0.06	62.5	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant.

Table-5: Ethanolic of *Senna polyphylla* (leaves)

Organisms	<i>Senna polyphylla</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	10.0±0.10	29.0±0.10	8.3±0.15	28.0±0.10	6.7±0.15	24.7±0.06	6.3±0.15	22.7±0.06	3.0±0.10	20.0±0.00	31.25	31.25
<i>Klebsiella pneumonia</i>	11.3±0.15	43.3±0.15	9.0±0.10	41.0±0.10	7.0±0.10	38.3±0.06	4.7±0.06	35.7±0.06	2.3±0.06	32.7±0.06	62.5	31.25
<i>Staphylococcus aureus</i>	11.3±0.12	39.0±0.10	9.0±0.10	37.0±0.10	6.7±0.06	34.7±0.15	5.0±0.10	32.5±0.06	2.7±0.06	30.0±0.10	62.5	31.25
<i>Streptococcus pneumonia</i>	10.0±0.10	32.0±0.10	8.0±0.10	29.0±0.12	7.0±0.10	28.0±0.310	4.3±0.12	26.0±0.10	2.7±0.06	23.3±0.06	62.5	31.25
<i>Salmonella typhi</i>	11.0±0.10	26.7±0.12	8.7±0.12	24.3±0.15	7.3±0.15	22.3±0.12	5.7±0.12	20.3±0.12	3.0±0.10	18.7±0.15	31.25	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant.

Table-6: Ethanolic extract of *Senna siamea* (leaves)

Organisms	<i>Senna siamea</i> extract and ciprofloxacin concentration and zone of inhibition (in mm)											
	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		MIC	
	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug	Extract	Drug
<i>E. coli</i>	11.7±0.15	33.3±0.31	9.7±0.06	31.7±0.21	8.0±0.10	29.7±0.15	6.0±0.10	27.0±0.10	4.0±0.10	24.3±0.15	31.25	31.25
<i>Klebsiella pneumonia</i>	10.3±0.15	32.7±0.12	8.3±0.15	30.7±0.12	6.3±0.15	29.0±0.10	3.7±0.12	26.3±0.12	1.7±0.12	24.0±0.10	62.25	31.25
<i>Staphylococcus aureus</i>	10.7±0.12	38.3±0.15	8.3±0.15	36.3±0.15	6.3±0.15	33.7±0.12	3.7±0.12	31.3±0.15	2.0±0.10	29.3±0.12	62.25	31.25
<i>Streptococcus pneumonia</i>	12.3±0.06	31.7±0.15	10.7±0.06	29.0±0.17	8.3±0.06	26.7±0.15	6.3±0.10	24.0±0.17	4.0±0.10	21.0±0.17	31.25	31.25
<i>Salmonella typhi</i>	10.7±0.12	41.3±0.12	9.0 ± 0.10	39.0±0.10	7.0±0.00	36.0±0.10	5.0±0.00	33.7±0.12	3.3±0.06	31.0±0.17	31.25	31.25

P ≤ 0.05 is significant; ≤ 2mm zone of inhibition is not significant

DISCUSSION

The results antibacterial activity of *S. alata* can be favourably compared to that reported by Ugoh and Elibe [25]. The high antimicrobial activity in *Senna alata* may be due to the phytochemical content of the plants such as alkaloids, saponins, flavanoids tannins phytate, oxalate and phenol [4, 26]. Several other workers have reported antimicrobial and antibacterial activity of other *Senna* species [27-29]

Alkaloids and flavonoids had been reported to exhibit antimicrobial activity [30]. Generally, however these active components are responsible for the diverse pharmacological actions of the ethanolic extracts of these *Senna* species. Hence, the presence of these compounds in the six *Senna* species corroborates the antimicrobial activities reported by Igbinsosa et al. [31]. Many scientists have worked on the anti-microbial properties of several plants in Nigeria [32-36]

The test organisms used in this study are associated with various forms of human infections. It has been demonstrated from clinical point of view that *Klebsiella pneumonia* is an important cause of neonatal nosocomial infection [37]. *Escherichia coli* is responsible for most septicemia and can also infect bladders, meninges, surgical wounds, skin lesion and the lung, these are common among debilitate and immunodeficient patients [38]. *Salmonella typhi* is a serious concern for the food industry and poses a serious public health problem in developing countries [39]. *Staphylococcus aureus* causes wound infections and urinary tract infections [40].

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Obi Ogonnaya who assisted in the laboratory work and Mr. C.C. John for carrying out the statistical analysis of the data generated from the study.

CONCLUSION

The antibacterial activity of the six *Senna* species justifies their use in folklore medicine. The fact that the ethanolic extract where active against clinical isolates is an indication that it can be a source of very potent antibiotics that can be used against drug resistant microorganisms prevalent in our society. Bioactive substances from *Senna* species plant can therefore be employed in the formation of antimicrobial agents for the treatment of various bacterial infections/diseases. These bioactive compounds can therefore be source of raw materials for synthesis of effective antibacterial drugs in the pharmaceutical industries.

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