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Antimicrobial, Antioxidant and Antiproliferative Properties of the Leaves of *Senna siamea*

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Authors' contributions

This work was carried out in collaboration among all authors. This work was conceptualized by authors KFO and TEA. Authors KFO and AJD carried out the bench work. Author OSO designed and monitored the experimental work, while author YD managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to investigate the antimicrobial, antioxidant and antiproliferative activities of leaves of *Senna siamea*. A sequential solvent extraction procedure was used in extracting powdered *Senna* leaves, starting with hexane, ethylacetate, ethanol and distilled water, respectively. Dry extracts obtained were tested at various concentrations against *Pseudomonas aeruginosa, staphylococcus aureus, Salmonella typhi* and *Candida albicans.* Antioxidant (Qualitative and quantitative) and antiproliferative tests were carried out on samples of the dry extracts. Ethylacetate extract of *Senna siamea* showed the highest antibacterial activity against *S. typhi* (zone of inhibition 15.0 mm) at concentration of 500mg/ml. The aqueous extract had the highest antioxidant activity, evidenced by a low IC_{50} value of 12.89µg/ml. Antiproliferative activity was determined by calculating the percentage inhibition of germination of *Sorghum bicolor L.* seeds under appropriate conditions. Plants that inhibit seed germination may inhibit tumour growth. At 24

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hours, the plant showed strong antiproliferative activity as evidenced by the high percentage inhibition. Ethanolic extract had the highest antiproliferative activity (90%) at 5000µg/ml, followed by the aqueous extract (88.5%). However, at 48 hours, all organic extracts showed very weak antiproliferative activity as evidenced by the low % inhibition. Only the aqueous extract showed considerable antiproliferative activity at 5000µg/ml (45%). This implies that the aqueous extract has considerable antiproliferative activity, hence, it may be a promising anticancer drug or has components that have anticancer properties. This shows that *Senna* leaves could serve as source of bioactive compounds with potential antimicrobial, antioxidant and anticancer properties.

Keywords: Senna siamea; antimicrobial; antioxidant; antiproliferative activities.

1. INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [1].

It is largely recognized that most of the currently available antimicrobials which are mainly synthetic are almost inefficient and most of these agents elicit terrible effects to recipients. More so, in poor countries, health care has been sustained by other practices based on cultural alternatives. Despite availability of modern medicine in some communities, medicinal plants have continued to maintain popularity for historical and cultural reasons, in addition to their efficacy and cheaper cost [2].

Antioxidants can be defined as bioactive compounds that inhibit or delay the oxidation of molecules [3]. Different antioxidants act to diminish oxidative damage *in vivo* and their mechanisms of action are highly varied. Antioxidants could be used as effective agents in preventing damage to lipids, proteins and DNA in neurons as well as subcellular sites [4]. Plants have an almost unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics. The continuing search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention [5].

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity because use of natural products, especially plants, for healing is as ancient and universal as medicine itself [6]. Plants are important for pharmacological research and drug development, not only when bioactive phytocompounds are used directly as therapeutic agents but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. In addition, plants can synthesize a large variety of chemical substances that are of physiological importance [7].

Senna siamea (formerly *Cassia siamea*) belongs to the family Caesalpiniaceae, often treated as a subfamily of the large family Leguminosae which consist of trees, shrubs and a few woody herbs found in the tropics. The English name is 'Cassod', Hausa name- 'bakinraskata', and Yoruba 'Odan'. *Senna siamea* has been reported from the southwest Nigerian ethnobotany as a remedy for febrile illness [8]. The leaf aqueous extract has been reported to have hypoglycaemic effect [9]. It was also reported that the leaf could be suitable candidate in preparation of drugs for treatment of infections caused by *Pseudomonas aeruginosa* [10].

People in Northern Africa and South Western Asia have used senna as a laxative for centuries. It was considered a 'cleansing' herb because of its cathartic effect [11]. In Burkina Faso, a decoction of the leaves with lemon juice is used for treatment of fevers whereas in Northern Nigeria, the tree is very popular for its local usage in the treatment of typhoid fever [12].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [13]. Hence, action must be taken to reduce this problem by developing research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, especially from natural products. The aim of this work was to analyze the antimicrobial, antioxidant and antiproliferative activities of the leaves of *senna siamea.*

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts

The plant leaves were collected from a mature tree in Jos, Plateau State, Nigeria. The plant was authenticated in the Department of Forestry Technology, Federal College of Forestry Jos, while herbarium samples were deposited at the Pharmaceutical Chemistry Department of University of Jos, Nigeria. The leaves were air dried for about two weeks and grinded to powder. A sequential solvent extraction procedure was used in extracting 1 kg of powdered senna leaves by maceration, starting with hexane, at room temperature for 24 hours. The mixture was then filtered using muslin cloth. This same extraction procedure was carried out on the marc using ethylacetate, ethanol and distilled water respectively. The marc was discarded, while the organic extracts were individually concentrated to dryness on a rotary evaporator and the aqueous extract was freeze dried at National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. (NIPRD).

2.2 Phytochemical Screening

Phytochemical screening of extracts was carried out according to standard procedure, as described by Sofowora [14] and Harborne [15], to determine the secondary metabolites present in the plant extracts.

2.3 Antimicrobial Screening

The dry extracts obtained were reconstituted in 20% dimethylsulphoxide (DMSO), and subjected to antimicrobial tests using agar diffusion cup plate method as described by Balouiri et al, [16]. Extracts were tested against typed strains of *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 28923) as well as clinical isolates of *Salmonella typhi* and *Candida albicans.* The antimicrobial screening was carried out at Bacterial Research Department of National Veterinary Research Institute Vom, Plateau State, Nigeria. Concentration of extracts tested were 500 milligram, 250 milligram, 125 milligram, 62.5 milligram and 31.25 milligram. 1.25 microgram/ml of Ciprofloxacin was used as positive control, and 20% Dimethylsulphoxide was used as negative control.

2.4 Test for Antioxidant Activity

Qualitative test for antioxidant/radical scavenging properties of extracts was carried out on TLC plates using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Samples were spotted and developed on TLC plates using pet. ether, chloroform and methanol in the ratio 4:5:1, as in normal TLC, ascorbic acid was spotted along as a positive control. After development, plates were air-dried at room temperature and then sprayed with DPPH. Antioxidant spots showed up as yellow against purple background, the time taken for yellow colour to appear was interpreted as an index of antioxidant capability. Only zones that turned yellow within 30minutes of spraying with DPPH were adjudged to have shown positive antioxidant property [16].

2.5 Quantitative Antioxidant Test

Quantitative antioxidant test was carried out as described by Burits and Bucar, [17]. The stock concentration (500mg/ml) was prepared by dissolving 25mg of the extract in 50ml of methanol, from which it was serially diluted to obtain the following concentrations 250, 125, 50, 25, 10, 5, 2.5, 1.25, 0.625 and 0.313 mg/ml respectively. 2mls of each concentration was added to 3ml methanolic solution of DPPH(20µg/ml). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard control; three replicates were made for each test sample. After 30minutes, the absorbance (A) was measured at 517nm and converted into percentage antioxidant activity using the following equation

$$\%\text{Scavenging Activity} = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}}\right] \times 100$$

2.6 Test for Anti-Proliferative Activity

Aim of this test is to find compounds that will inhibit the germination of *Sorghum bicolor L* seeds (Guinea corn), under appropriate conditions. Compounds that inhibit seed germination may possess dormancy inducing properties, inhibit tumor growth and inhibit growth related enzymes in plants and animals [18]. Seeds of *Sorghum bicolor* were sterilized by washing with 95% ethanol for 1minute and then washed with distilled water. 20 seeds of sterilized *S.bicolor* were put in sterile Petri dishes containing cotton wool. 5mls of the test samples each at concentrations of 5000,2500 and 1000µg

was added to the seeds in the petri dishes and incubated at room temperature in the dark for 24 and 48 hours. 5mg of methotrexate was used as control. It was dissolved and made up to 25ml with distilled water giving the concentration of 200µg/ml (stock). 1ml concentrations of 100, 50, 25µg were used in the same manner described above. After incubating for 24 and 48 hours, the number of seeds germinated in each Petri dish was counted and recorded.

$$\% \text{ Germination } = \frac{\text{Number of germinated seeds x 100}}{\text{Total number of seeds used}}$$

%Inhibition of seeds germination = 100-%germination

All experiments were carried out in triplicate.

3. RESULTS AND DISCUSSION

Table 1 shows the phytochemical analysis of *Senna siamea* Extracts.

From the results of antimicrobial screening (Table 2), it was observed that ethylacetate extract has the highest anti-bacterial activity against *Salmonella typhi* (Zone of inhibition 15.0mm), followed by ethanolic extract (14.0 mm) and then hexane extract (10.0 mm). Preliminary Phytochemical analysis showed that the ethyl acetate and ethanolic extracts contain tannins, saponins, cardiac glycosides and flavonoids while the hexane extract contains mainly flavonoids. Phytoconstituents such as saponins, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections [12]. Hence, it may be deduced that the low concentration of most of these secondary metabolites (saponins and glycosides) in hexane extract of Senna leaves, account for its lower antibacterial activity against *Salmonella typhi*. The aqueous extract showed a low antimicrobial activity (Zone of inhibition 11.0 mm at 500 mg/ml), this agrees with the findings of Doughari and Okafor [12], who reported that the aqueous leaf extract of the plant showed lowest activity (3.50±0.001 mm) at 40mg/ml. This may explain why the use of the plant locally in treatment of typhoid is prolonged probably to achieve the desired effect, since there is low concentration of the metabolites in the aqueous extract. The percentage yield of the aqueous extract is the lowest (1.63%) compared to other organic extracts. Senna showed very little activity against *Pseudomonas aeruginosa*, a pathogen capable of causing life threatening diseases such as pneumonia, urinary tract infections and clinically significant infections such as wound and burns infections [13]. The plant extracts showed very little or no activity against *S. aureus* and clinical isolates of *Candida albicans.* This also agrees with the assertions of Kamal *et al*, who reported that the n-hexane, chloroform and ethylacetate fractions of cassia senna leaves have moderate to less antibacterial activity against *S.aureus* and less potent antifungal activity [19].

Table 3 and 4 show the qualitative and quantitative antioxidant activity test result respectively. Aqueous extract of Senna showed very strong activity as evidenced by its immediate reaction to DPPH spray reagent, indicating strong antioxidant activity. In quantitative antioxidant activity, the lower the IC_{50} value, the higher the antioxidant activity. Ascorbic acid which is the control has the highest antioxidant activity (IC_{50} 11.12µg/ml). Senna aqueous extract had the highest antioxidant activity (12.89µg/ml) after ascorbic acid. Phytochemical analysis of the aqueous extract shows presence of flavonoids. Flavonoids have been reported in several studies to have a strong relation to the antioxidant activity of plant extracts [20]. Flavonoids are vital secondary metabolites of plants due to its strong antioxidant activity. According to Luciana et al, fruits rich in bioactive phenolic compounds such as flavonoids, phenolic compounds, stilbenes and tannins, have important roles in free radical scavenging [21]. The aqueous extract may have a higher concentration of flavonoids, which suggests that the aqueous extracts of the plants have better antioxidants than organic extracts of the plants, hence may be employed as source of antioxidants.

Table 5 shows the result of the antiproliferative activity test. At 24 hours, the plant showed strong antiproliferative activity as evidenced by the high percentage inhibition. Ethanolic extract had the highest antiproliferative activity (90%) at 5000µg/ml, followed by the aqueous extract (88.5%). However, at 48 hours (Table 6), all organic extracts showed very weak antiproliferative activity as evidenced by the low % inhibition, only the aqueous extract showed considerable antiproliferative activity at 5000µg/ml (45%), implying the aqueous extract has considerable antiproliferative activity. Several reports have demonstrated that anti-oxidative activities such as free radical scavenging and lipid peroxidation from natural

extracts can enhance anti-cancer drugs [22]. Hence, the antioxidant property displayed by the aqueous extract could be responsible for its antiproliferative potentials, therefore, it may be a promising anticancer drug or has components that have anticancer properties.

Table 1. Phytochemical analysis of *Senna siamea* extracts

	Hexane	Ethylacetate	Ethanolic	Aqueous
Alkaloids	-	-	-	-
Tannins	-	+	+	+++
Saponins	-	+	+	+
Cardiac Glycosides	-	+	+	-
Steroids	-	-	-	-
Flavonoids	+	+	+	+

KEY: -= Not present += Present

Table 2. Antimicrobial screening zone of inhibition of senna extracts in mm, cup size = 6mm

Sample	Conc.(mg/ml)	*S.typhi* ATCC28923	*S.aureus*	*Candida albicans*	*P.aeruginosa* ATCC27853
Hexane Extract	500	10.0	13.0	12.0	12.0
	250	8.0	9.0	10.0	10.0
	125	8.0	6.0	8.0	8.0
	62.5	7.0	6.0	6.0	6.0
	31.25	6.0	6.0	6.0	6.0
Ciprofloxacin	1.25 µg	32.0	38.0	41.0	36.0
DMSO	20%	6.0	6.0	6.0	6.0
Ethylacetate Extract	500	15.0	11.0	11.0	12.0
	250	12.0	13.0	8.0	10.0
	125	10.0	11.0	7.0	8.0
	62.5	9.0	9.0	6.0	7.0
	31.25	7.6	6.0	6.0	7.0
Ciprofloxacin	1.25µg	37.0	33.0	40.0	37.0
DMSO	20%	6.0	6.0	6.0	6.0
Ethanolic Extract	500	14.0	10.0	9.0	10.0
	250	12.0	12.0	12.0	11.0
	125	10.0	11.0	9.0	10.0
	62.5	9.0	8.0	8.0	9.0
	31.25	8.0	6.0	7.0	8.0
Ciprofloxacin	1.25µg	41.0	38.0	36.0	35.0
DMSO	20%	6.0	6.0	6.0	6.0
Aqueous Extract	500	11.0	13.0	13.0	10.0
	250	8.0	11.0	11.0	8.0
	125	6.0	9.0	8.0	6.0
	62.5	6.0	9.0	8.0	6.0
	31.25	6.0	6.0	6.0	6.0
Ciprofloxacin	1.25µg	35.0	30.0	40.0	37.0
DMSO	20%	6.0	6.0	6.0	6.0

Table 3. Qualitative antioxidant activity test

Sample	Antioxidant activity	Time taken for colour development (DPPH)
Hexane Extract	Moderate	15seconds
Ethylacetate Extract	Moderate	15 seconds
Ethanolic Extract	Strong	10 seconds
Aqueous Extract	Very strong	Immediate

Table 4. Quantitative antioxidant activities of extract

Sample	IC_{50} Value (µg/ml)	Antioxidant activity
Ascorbic acid	11.12	High
Hexane Extract	35.41	Moderate
Ethylacetate Extract	32.77	Moderate
Ethanolic Extract	25.73	Moderate
Aqueous Extract	12.89	High

Table 5. Anti proliferative activity of *senna siamea* plant extracts and control drug(Methotraxate) at 24 hours

Concentration of test sample (µg/ml)	Mean±SEM	% Germination	% Inhibition
Methotraxate	15.00±2.00	75.0	25.0
25			
50	9.30±1.77	46.5	53.3
100	6.00±0.58	30.0	70.0
Hexane Extract	10.70±0.33	53.5	46.5
1000			
2500	8.70±1.45	43.5	56.5
5000	4.00±1.73	20.0	80.0
Ethylacetate Extract	8.30± 1.33	41.5	58.5
1000	5.70± 0.88	28.5	71.5
2500	7.30± 2.41	36.5	63.5
5000			
Ethanolic	8.30± 1.33	41.5	58.5
1000			
2500	8.00± 1.00	40.0	60.0
5000	2.00± 1.16	10.0	90.0
Aqueous 1000	9.30± 1.86	46.5	53.5
2500	5.30± 1.33	26.5	73.5
5000	2.30± 0.88	11.5	88.5

Table 6. Anti proliferative activity of senna siamea plant extracts and control drug(Methotraxate) at 48 hours

Concentration of Test sample (µg/ml)	Mean±SEM	% Germination	% Inhibition
Methotraxate			
25	18.70 0.33	93.5	6.5
50	13.70 1.45	31.5	68.5
100	12.30 1.45	38.5	61.5
Hexane extract			
1000	17.60±0.88	86.5	13.5
2500	17.70±0.67	81.5	18.5
5000	16.30±1.77	81.5	18.5
Ethylacetate extract			
1000	17.70±0.33	88.5	11.5
2500	16.00±0.58	80.0	20.0

Concentration of Test sample (µg/ml)	Mean±SEM	% Germination	% Inhibition
5000	16.70±0.33	83.5	16.5
Ethanolic extract			
1000	17.70±0.33	88.5	11.5
2500	19.30±0.33	96.5	3.5
5000	15.30±2.03	76.5	23.5
Aqueous extract			
1000	16.70±1.20	83.5	16.5
2500	14.00±1.00	70.0	30.0
5000	11.00±1.00	55.0	45.0

Table 7. Anti proliferative Activity of Control (Distilled Water) NB: 5mls of distilled water was added

24 Hours			48 Hours		
Mean±SEM	**% Germination**	**% Inhibition**	**Mean±SEM**	**% Germination**	**% Inhibition**
16.00±0.58	80	20	20±0.00	100	0

4. CONCLUSION

From the result of the biological investigation of *Senna siamea* leaf extracts, it is evident that the plant possesses some antibacterial activity against *Salmonella typhi,* a gram-negative bacterium responsible for typhoid fever, which has been a serious health threat to populace in most developing nations for generations. The leaves of this plant may be used to treat infections caused by *S. typhi* such as typhoid, can be employed as source of antioxidants and also has promising anticancer properties.

However, further studies could be carried out to further ascertain antitumor properties of *senna siamea* plant, using in vitro experiments, as in cultured cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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