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FREE-RADICAL SCAVENGING POTENTIALS AND ANTIMICROBIAL PROPERTIES OF *SENNA ALATA* (L.) Roxb. (LEAF, FLOWER AND BARK)

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ABSTRACT

The rapid emergence of microbial resistance to antibiotics by infectious agents has been a continuous challenge calling for the development of an alternative therapeutic agent. This study aimed to evaluate the bioactivities and free-radical scavenging potentials of *Senna alata* (L.) Roxb. leaf, flower and bark, a promising plant source for novel antibiotics. Dried powdered sample of *S. alata* (L.) Roxb. parts were extracted separately to obtain crude methanol, ether, xylene, and water extracts. The antimicrobial effects were determined by agar-well diffusion method, against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi, Candida albicans*, and *Aspergillus niger.* The antioxidant activity of each part was measured by 2, 2 diphenyl -1- picrihydrazyl, spectrophotometrically. The study showed that microorganisms were susceptible to both methanolic and aqueous extracts of *Senna alata* leaf, bark, and flower, although the aqueous extracts of the plant parts showed significantly lower activity. The minimum inhibitory concentrations (in µg/mL) of the flower extracts for individual organisms, were 12.5 (*B. subtilis*), 12.5 (*E. coli*, *S. aureus, S. typhi*) and 25.0 (*P. aeruginosa*). The bark extracts revealed 12.5 (*S. typhi*, 25.0 (*S. aureus, B. subtilis, E. coli, P. aeruginosa*) while the leaf extracts showed 6.25 (*S. typhi*), 25.0 (*C. albicans*), 25.0 (*E. coli, S. aureus, P. aeruginosa*) and 50.0 (*B. subtilis*). For the free-radical scavenging activity, the leaf extract revealed 58.10 ± 0.3, bark 51.91 ± 0.14 and flower 35.10 ± 0.14 while the Ascorbic acid (control) measured 70.86 ± 0.14. The results showed that *S. alata* has the potential as a source of broad-spectrum antimicrobial compounds which could serve as a lead to a new drug.

KEYWORDS: **Antimicrobial; Antioxidant; *Senna alata*; Antimicrobial resistance; Microorganisms.**

INTRODUCTION

The era of antimicrobial chemotherapy now spans almost 80 years. During that time, many microorganisms such as bacteria, fungi and protozoal infections which formerly carried high mortality, such as puerperal sepsis, meningitis, tuberculosis, candidiasis, cholera and amoebiasis, have become amenable to treatment with a range of potent antimicrobial agents [1, 2].

Antimicrobial agents are chemicals or drugs used to inhibit the growth of microorganisms already established in the tissue of the host. The basic principle of chemotherapy is selective toxicity.

Infectious diseases have been reported to be responsible for approximately 66.67% of all mortality in tropical countries [3]. The emergence of antimicrobial resistance to convectional antibiotics by microorganisms pose enormous concern to public health.

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Historically, plants have been proven to be a good source of chemo-therapeutic agents with bioactive compounds, that are highly effective against microbial infections. Medicines of plant origins have shown great promise in the treatment of hitherto, intractable infectious diseases. Plants containing protoberines and related alkaloids, picrolema type indole, alkaloids and garcinea biflavones used in African traditional medicine, have been reported to be effective against a wide range of microbial pathogens [4, 5]. Though, microbes of soil origin, produce most of the clinically used antibiotics, higher plants have also been a major source of antimicrobial agents [6-8]. The use of phytochemicals from plants for the treatment of diseases over the years, has attracted the interest of scientist and researchers worldwide [10, 11]. Many studies are being carried out on medicinal plants on account of the information provided by the local populace with the aim of understanding the phytochemical constituents of such plants, for application in the treatment and management of infectious and non-infectious diseases [12]. The spike of interests in natural products in the developed countries, led to the discovery of several drugs and chemotherapeutic agents from plants, and increase in the traditionally used herbal remedies. Bioactive phytochemicals have great therapeutic potentials, and they can serve the purpose of chemotherapy, without those side-effects often associated with synthetic drugs, and minimal chance of developing resistance [13]. The widely held view amongst stakeholders in the society and the medical community, is that therapeutic agents of natural products especially of plant origin, are healthier, safer, and more reliable than those of synthetic sources [14]. Phytochemicals generally, act to stimulate, strengthen and supplement the body`s healing abilities [1, 15].

Senna (Linn) is a generic name of members of the legume family, with about 535 known species. It is commonly found and widely distributed in temperate and tropical areas. The genus contains shrubs and trees as well as annual and perennial herbs. The leaves are pinnately compound and are featherlike and have yellow flowers, which are often showy. Many are ornamental, and some woody tropical species are among the most important flowering trees [16, 17]. Some species of *Senna* are notable for being host to a certain butterfly species. For example, the cloudless sulphur-butterflies are usually associated with some species of *Senna* [18]. In addition, the *Senna* species are classified under the *Cassia* species, but change in classification was based on the morphological characteristic, as shrubs.

The economic importance of the genus includes its usefulness to the chemical industries, such as the cosmetic industries. It also serves as source of food, as vegetable [19]. *Senna alata* is a shrub of the tropics, with yellow flowers and large leaves. The juice is traditionally used as a cure for ringworm and anti-venom against poisonous bites [10].

The medicinal usefulness of *Senna alata* has been subjected to several chemical and pharmacological analysis. The plant, apart from its uses as firewood and timber, has very important application in traditional medical practice [10, 20, 21].

Senna alata has been identified as a medicinal plant used in the treatment of many ailments and diseases across the globe. The leaves are administered orally as an effective laxative [21]. In Ghana and Cote D'Ivore, decoctions of parts of the plant, such as the leaves, flowers, bark, and root, are used for the treatment of dysentery, diarrhoea, and other gastrointestinal ailments [22, 23]. The macerated juices of young fresh leaves of *Senna alata* are used to treat eye infection and parasitic diseases [1]. The decoctions of the stem, bark and roots are reportedly used for the treatment of urinary-tract infections (UTI), asthma and bronchitis [1, 21]. Nigerians of Northern extraction, particularly those from Adamawa and Taraba States, use the root, stem and leaves in their herbal medicine practice, for the treatment of burns, wounds, skin diseases such as purities, eczema, and itching. There are also reports that an infusion of the flowers is used for the treatment of asthma and bronchitis [24, 20]. Also reported, was the use of the decoction of the flowers as an expectorant in bronchitis and dysporea, as an astringent, and as a mouth wash in stomatitis [25]. The plant *Senna alata,* has been reported to exhibit antimicrobial activity against *Aspergillus breuipis, Penicillium* species, *Geotrictum candidium* and *Fusarium oxysporium* [26]. There has not been any work that presented the antimicrobial and antioxidant properties of *Senna alata* as an interface in a single report. Therefore, this study was designed to determine the antimicrobial activity in relation to the qualitative phytochemical constituents of *Senna alata*, and determine the free radical-scavenging potentials *in vitro*. It also aimed to ascertain it`s reported uses, as naturally occurring antioxidants in traditional medicine, as to place it in the context of a scientific framework.

MATERIALS AND METHODS

Collection and certification of plant material

The fresh mature leaves, flower, and bark of *Senna alata* were collected randomly in the early hour of the day from the Botanical Garden of University of Ibadan. Ibadan, Nigeria. The plant parts were authenticated by expert`s identification in the University of Ibadan Herbarium [27, 28], where a voucher specimen was also deposited with the number UIH22376. The plant materials (leaf, flower, and bark) were kept in separate polythene bags to prevent mixing up and transpiration.

Preparation and extraction of plant material

Leaves, flower, and bark of *Senna alata* were air-dried separately for about three weeks after which they were crisp-dried in the oven at temperature 40ºC for four days. The plant parts were pulverized with grinding mill separately and kept in an airtight container for further use. One hundred grams each of the powdered plant parts was extracted with 100ml of 70% methanol, ether, xylene, and distilled water by cold maceration, in a bowl and covered for three days with frequent stirring or agitation with mixer. The resulting solutions were filtered off and concentrated to dryness with the rotary evaporator. The extracts were stored in sealed labelled containers and kept in the refrigerator at 4ºC, until needed.

pH of the extracts

The pH of the extracts was determined using a pH meter according to the method of [29].

Yield of extracts

The percentage yield of each extract was calculated as follows:

$$\%\ Yield = \frac{initial\ weight\ of\ plant\ part - final\ weight\ of\ plant\ part}{initail\ weight\ of\ plant\ part} X100$$

Phytochemical screening

Analysis of the crude extracts was carried out to establish the phytochemical components and bioactive constituents present in them. The phytochemical assay for alkaloids, glycosides, tannins, flavonoids, anthraquinones, and saponins, were carried out according to established procedures [1, 30].

Test microorganisms

Seven microbes were used in this study. They were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan. The five microorganisms which cut across both Gram-positive and Gram-negative bacteria include, *Bacillus subtilis, Staphylococcus aureus,* (both of which are Gram-positive); *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi,* (all of which are Gram-negative). The remaining two were fungi namely, *Candida albicans* and *Aspergillus niger*. The organisms were used only after they have been reconfirmed.

Antibacterial activity

The antibacterial sensitivity assay of the extracts of the various plant parts was done, using the agar diffusion method [31, 32]. Overnight culture of the bacterial isolates in nutrient broth were used after adjusting to 0.5 McFarland standard. The inoculum suspensions were tested against the effect of each extract at different concentrations. Surface spread of the inoculum was done on Muller – Hinton agar. Using sterile cork-borer (6 mm diameter), wells were then bored into the agar. Approximately 100 µl of a given concentration of each extract, were dispensed into the wells, and allowed to diffuse on the bench for an hour, before incubation [26, 31].
The plates were incubated at 37ºC for 24 hours. Plates were observed for clear zones of inhibition around each well. Each test was performed in duplicate.

Antifungal activity

The fungal isolates were grown on a potato dextrose agar (PDA) (Oxoid, U K) at 25ºC until sporulation occurred. Then spores were harvested afterward by pouring a mixture of sterile glycerol and distilled water to the surface of the plates and were scraped with a sterile glass rod and standardised. 100 µl of the standardised fungal spore suspension were evenly spread on the Sabouraud dextrose agar (Oxoid, U.K), using a sterile glass-spreader. Wells of equal diameter (6 mm), were bored into the agar, using a sterile cork-borer. The wells were appropriately labelled and filled with a given concentration of each of the plant extracts, ensuring no spillage of the extracts to the surface of the agar medium. The extracts were allowed to diffuse into the agar, on the bench for an hour before incubation. Plates were incubated at 25ºC for 72 hours and later observed for clear zones of inhibition around each well [33]. The diameter of zones of inhibition was measured.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of various concentrations of the extracts was determined using the agar dilution method. Two-fold serial dilutions of the extracts were done with nutrient broth (macro-broth method), [34]. Half strength (10 mg/mL) and ¼ strength (5mg/mL), of each extract, in addition to the normal strength (20 mg/mL) and solvent control, were used. Each of the concentration was mixed with an adjusted volume of molten Muller-Hinton agar at 45°C, to give the required final concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL, respectively. Overnight culture of the test organisms was streaked on to the surface of the solid agar mixed with the various concentrations of the extracts. The plates were incubated for 24 hours for bacterial and 48 hours, for fungal isolates. The lowest concentration of the extracts that completely prevented the growth of any visible colony of the microorganisms, was taken as the minimum inhibitory concentration (MIC) of the extract. [33].

Determination of the minimal bactericidal/fungicidal concentration (MBC/MFC)

This was determined using the 1:1 or 50 – 50 contact ratios, organism to antimicrobial agent, [35]. To a 0.5 ml at different concentrations as used in the MIC assay that shows no visible growth on the agar plate was added 0.5ml of test organism in tubes. These were incubated at 25°C for 72 hrs and 37°C for 48 hrs, for fungi and bacteria, respectively. Samples were streaked out from the tubes on to Sabouraud dextrose agar for fungi, and Muller Hinton agar for bacteria respectively, to determine the minimum concentration of the extract at which 99.9% kill of the organisms is achieved. These concentrations were established by failure of the organisms to grow on transfer to these media plates. The least concentration that prevented the growth of the organisms after days of incubation, was recorded as the Minimal fungicidal/bactericidal concentration. The entire tests were performed in duplicates to ensure accuracy. Agar plates without extract and another without organism were also incubated to serve as positive and negative controls, respectively.

Determination of mechanism of antibiosis (fungicidal or fungistatic, bactericidal or bacteriostatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MFC/MIC or MIC index [36], to elucidate whether the observed antifungal effect was fungicidal or fungistatic. When the ratio of MFC/MIC is ≤ 2.0, the extract is considered fungicidal or otherwise fungistatic. If the ratio is ≥16.0, the extract is considered ineffective.

Antioxidant assay

The free-radical scavenging potentials of extracts of the plant parts were measured in accordance with established standard [37]. About 0.4 mg/mL ethanoic stock solutions of the extract of the leaves of plant *S. alalta,* were prepared and to the various concentrations of the extract, were added. 0.5 methanol solution of DPPH (1mM). The mixture was allowed to stand for 30 minutes, at room temperature (25°C), after thoroughly shaken. The spectrophotometric reading at 517 nm of the absorbance of the resulting solution was taken. Ascorbic acid and dytylated hyroxylanisole were used as standard antioxidants (positive control), while a blank volume of methanol was simultaneously running with each assay. All experiments were performed in triplicates. The same procedure was repeated using control sample (DPPH without the extracts). The percentage inhibition of DPPH by the plant extract, was calculated using the following equation:

$$\%\ Inhibition = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control}\ X\ 100$$

Statistical analysis

Experimental results are presented as the mean ± SEM of triplicate experiments.

RESULTS

The percentage yield and pH of the different plant extract are as shown in Table 1. The pH range of the extracts are between 6.0 – 7.5. The result showed that the extracts were mildly acidic to neutral.

The result of the phytochemical screening of the plant parts (leaf, flower and bark) of *Senna alata* is shown in Table 2. The extracts of flower, leaf and bark were found to contain volatile oil, glycoside, alkaloid, saponin, anthraquinones, flavonoid and tannin. The extracts of the leaf and bark also contain carbohydrate, the leaf extract contain protein.

DISCUSSION

Herbal drugs are in great demand due to their valued pharmacological properties. Plants are

Table 1: pH and Percentage yield of different extracts of the plant parts.

Solvents of extraction	Percentage yield			pH – values		
	Leaf	Bark	Flower	Leaf	Bark	Flower
Water	29.0	13.0	52.0	7.5	6.8	7.0
Methanol	20.0	11.0	50.0	6.8	6.5	6.5
Xylene	21.0	10.0	51.0	6.0	7.5	6.5
Ether	23.0	10.0	54.0	6.5	6.5	6.0

Table 2: Phytochemical analysis of leaf, flower and bark of *Senna alata*

Compounds	Leaf	Flower	Bark
Volatile oil	+	+	+
Glycoside	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Anthraquinones	+	+	+
Flavonoid	+	+	+
Tannins	+	+	+
Carbohydrate	+	_	_
Protein	+	_	_

NOTE: + = present; - = absent

Table 3: Antimicrobial screening of different solvents fractions of flower of *Senna alata* on Test Microorganisms.

Microorganisms	Fraction	200µg/mL	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	G/G
S. aureus	Methanol	14 ±0.00	14 ±0.00	13 ±0.00	13 ±0.00	12 ±0.00	25 ±0.00
	Aqueous	-	-	-	-	-	22 ±0.00
	Ether	-	-	-	-	-	21 ±0.00
	Xylene	-	-	-	-	-	21 ±0.00
B. subtilis	Methanol	12 ±0.00	14 ±0.00	-	-	-	23 ±0.00
	Aqueous	14 ±0.00	-	-	-	-	21 ±0.00
	Ether	-	-	-	-	-	20 ±0.00
	Xylene	-	-	-	-	-	20 ±0.00
E. coli	Methanol	12 ±0.00	-	-	-	-	18 ±0.00
	Aqueous	-	-	-	-	-	21 ±0.00
	Ether	-	-	-	-	-	19 ±0.00
	Xylene	-	-	-	-	-	20 ±0.00
P. aeruginosa	Methanol	18 ±0.00	-	-	-	-	21 ±0.00
	Aqueous	-	-	-	-	-	22 ±0.00
	Ether	-	-	-	-	-	21 ±0.00
	Xylene	-	-	-	-	-	21 ±0.00
S. typhi	Methanol	22 ±0.00	-	-	-	-	23 ±0.00
	Aqueous	-	-	-	-	-	21 ±0.00
	Ether	-	-	-	-	-	21±0.00
	Xylene	-	-	-	-	-	20 ±0.00
C. albicans	Methanol	-	-	-	-	-	18 ±0.00
	Aqueous	-	-	-	-	-	18 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
A. niger	Methanol	-	-	-	-	-	22 ±0.00
	Aqueous	-	-	-	-	-	21 ±0.00
	Ether	-	-	-	-	-	26 ±0.00
	Xylene	-	-	-	-	-	21 ±0.00

Keys: G= gentamycin; G= griseofulvin, - = no zone of inhibition.
Note: Results are presented as mean + SEM. ($P < 0.05$).

Table 4: Antimicrobial screening of different solvents fractions of bark of *Senna alata* on test microorganisms.

Microorganisms	fraction	200µg/mL	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	G/G (± SEM)
S. aureus	Methanol	22 ±0.00	21 ±0.00	22 ±0.00	20 ±0.00	15 ±0.00	24 ±0.00
	Aqueous	21 ±0.00	18 ±0.00	16 ±0.00	-	-	24 ±0.00
	Ether	-	-	-	-	-	24 ±0.00
	Xylene	-	-	-	-	-	24 ±0.00
B. subtilis	Methanol	22 ±0.00	20 ±0.00	15 ±0.00	-	-	24 ±0.00
	Aqueous	21 ±0.00	19 ±0.00	15 ±0.00	-	-	24 ±0.00
	Ether	10 ±0.00	-	-	-	-	24 ±0.00
	Xylene	-	-	-	-	-	24 ±0.00
E. coli	Methanol	20 ±0.00	18 ±0.00	-	-	-	24 ±0.00
	Aqueous	16 ±0.00	14 ±0.00	-	-	-	24 ±0.00
	Ether	17 ±0.00	-	-	-	-	24 ±0.00
	Xylene	-	-	-	-	-	24 ±0.00
P. aeruginosa	Methanol	16 ±0.00	13 ±0.00	-	-	-	24 ±0.00
	Aqueous	12 ±0.00	-	-	-	-	24 ±0.00
	Ether	-	-	-	-	-	25 ±0.00
	Xylene	-	-	-	-	-	24 ±0.00
S. typhi	Methanol	21 ±0.00	18 ±0.00	14 ±0.00	-	-	24 ±0.00
	Aqueous	-	-	-	-	-	22 ±0.00
	Ether	-	-	-	-	-	24 ±0.00
	Xylene	-	-	-	-	-	23 ±0.00
C. albicans	Methanol	-	-	-	-	-	18 ±0.00
	Aqueous	-	-	-	-	-	18±0.00
	Ether	-	-	-	-	-	19±0.00
	Xylene	-	-	-	-	-	18 ±0.00
A. niger	Methanol	-	-	-	-	-	21±0.00
	Aqueous	-	-	-	-	-	18 ±0.00
	Ether	-	-	-	-	-	20 ±0.00
	Xylene	-	-	-	-	-	18 ±0.00

Keys: G= gentamycin; G= griseofulvin, - = no zone of inhibition
Note: Results are presented as mean + SEM. ($P < 0.05$).

Table 5: Antimicrobial screening of different solvents fractions of *Senna alata* leaf on test microorganisms.

Microorganisms	Fraction	200µg/mL	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	G/G (± SEM)
S. aureus	Methanol	22 ±0.00	22 ±0.00	15 ±0.00	-	-	28 ±0.00
	Aqueous	20 ±0.00	10 ±0.00	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
B. subtilis	Methanol	22 ±0.00	22 ±0.00	23 ±0.00	-	-	28 ±0.00
	Aqueous	21 ±0.00	20 ±0.00	-	-	-	28 ±0.00
	Ether	15 ±0.00	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
E. coli	Methanol	21 ±0.00	20 ±0.00	-	-	-	28 ±0.00
	Aqueous	14 ±0.00	14 ±0.00	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
P. aeruginosa	Methanol	18 ±0.00	-	-	-	-	28 ±0.00
	Aqueous	14 ±0.00	-	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
S. typhi	Methanol	22 ±0.00	-	-	-	-	28 ±0.00
	Aqueous	-	-	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
C. albicans	Methanol	20 ±0.00	-	-	-	-	28 ±0.00
	Aqueous	14 ±0.00	-	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	28 ±0.00
	Xylene	-	-	-	-	-	28 ±0.00
A. niger	Methanol	14 ±0.00	-	-	-	-	18 ±0.00
	Aqueous	-	-	-	-	-	28 ±0.00
	Ether	-	-	-	-	-	18 ±0.00
	Xylene	-	-	-	-	-	18 ±0.00

Keys: G= gentamycin; G= griseofulvin, - = no zone of inhibition
Note: Results are presented as mean + SEM. ($P < 0.05$).

Table 6: The Minimal Inhibitory Concentrations (MIC) of the various parts of *Sena alata*, against the test organisms.

Plant parts/Solvent	Flower		Bark		Leaf	
Organisms	Methanol (µg/mL)	Water (µg/mL)	Methanol (µg/mL)	Water (µg/mL)	Methanol (µg/mL)	Water (µg/mL)
Bacillus subtilis	12.5± 0.05[a]	12.5± 0.03[a]	25.0± 0.01[b]	12.5± 0.02[a]	50.0± 0.02[c]	25.0± 0.01[b]
Escherichia coli	12.5 ± 0.03[a]	25.0± 0.04[b]	25.0± 0.05[b]	12.5± 0.03[a]	25.0± 0.02[b]	25.0± 0.05[b]
Staphylococcus aureus	12.5± 0.05[b]	25.0± 0.06[c]	25.0± 0.04[c]	12.5± 0.02[b]	25.0± 0.03[c]	6.25± 0.03[a]
Pseudomonas aeruginosa	25.0± 0.04[b]	25.0± 0.04[b]	25.0± 0.03[b]	6.25± 0.01[a]	25.0± 0.02[b]	25.0± 0.02[b]
Salmonella typhi	12.5± 0.02[b]	12.5± 0.03[b]	12.5± 0.02[b]	6.25± 0.02[a]	6.25± 0.03[a]	6.25± 0.01[a]
Candida albicans	12.5± 0.01[b]	12.5± 0.01[b]	12.5± 0.04[b]	12.5± 0.01[b]	12.5± 0.04[b]	6.25± 0.02[a]
Aspergillus niger	12.5± 0.03[a]	12.5± 0.03[a]	12.5± 0.01[a]	12.5± 0.02[a]	12.5± 0.01[a]	12.5± 0.02[a]

Note: Data here, were presented as Mean ± SEM, n = 3. Statistical tool used: One -way Analysis of variance (ANOVA), followed by Duncan post-hoc test. Values with different superscripts are significantly different for a given organism, at $P < 0.05$ level of significance.

Table 7: The Minimal Bactericidal / Fungicidal concentrations (MBC / MFC) of the various parts of *Sena alata*, against the test organisms.

Plant parts/Solvent	Flower		Bark		Leaf	
Organisms	Methanol (µg/mL)	Water (µg/mL)	Methanol (µg/mL)	Water (µg/mL)	Methanol (µg/mL)	Water (µg/mL)
Bacillus subtilis	6.25 ± 0.02[a]	6.25±0.01[a]	12.5± 0.03[b]	6.25± 0.03[a]	25.0± 0.02[c]	12.5± 0.01[b]
Escherichia coli	6.25± 0.04[a]	12.5± 0.02[b]	12.5± 0.02[b]	6.25± 0.01[a]	12.5± 0.03[b]	12.5± 0.02[b]
Staphylococcus aureus	6.25± 0.03[a]	12.5± 0.01[b]	12.5± 0.01[b]	6.25± 0.04[a]	12.5± 0.02[b]	6.25± 0.03[a]
Pseudomonas aeruginosa	12.5± 0.01[b]	12.5± 0.02[b]	12.5± 0.02[b]	3.125±0.03[a]	12.5± 0.01[b]	12.5± 0.01[b]
Salmonella typhi	6.25± 0.02[b]	6.25± 0.01[b]	6.25± 0.03[b]	3.125±0.01[a]	3.125± 0.02[a]	3.125± 0.01[a]
Candida albicans	6.25± 0.03[b]	6.25± 0.02[b]	6.25± 0.01[b]	6.25± 0.02[b]	6.25± 0.01[b]	3.125± 0.03[a]
Aspergillus niger	6.25± 0.02[a]	6.25± 0.04[a]	6.25± 0.01[a]	6.25± 0.03[a]	6.25± 0.01[a]	6.25± 0.04[a]

Note: Data here, were presented as Mean ± SEM, n = 3, Statistical tool used: One-way Analysis of variance (ANOVA), followed by Duncan post-hoc test. Values with different superscripts are significantly different for a given organism, at $P < 0.05$ level of significance.

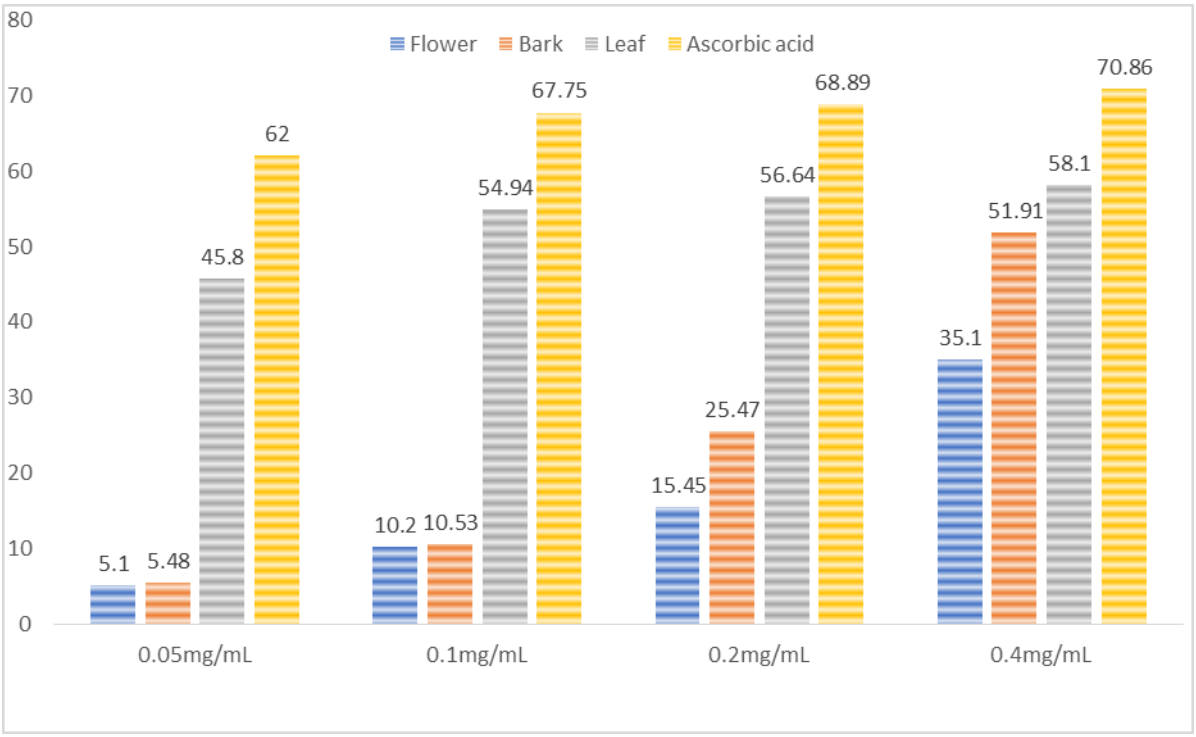

Figure 1: Shows the percentage of free-radical scavenging activity of *Sena alata* plant parts.

explored as a source of novel bioactive compounds for drug discovery. *Senna alata* (leaf, flower, and bark) was selected for evaluation in this study due to the numerous ethnomedicinal claims on its uses.

The pulverized leaf, flower. and bark of *S. alata* were extracted with methanol, xylene, ether, and water, to ensure that every compound present in the plant parts were extracted on the bases of their polarity and solubility in the various solvents. The highest percentage yield recorded in the flower part across all the solvents used, may be due the fine nature of the pulverized flower sample, which allowed for the in-depth permeability of the solvents of extraction; high concentration of the compounds (secondary metabolites) in the flower, as well as a better solubility coefficient of the compounds in the solvents. This agrees with a reported overview of the phytochemical analysis of bioactive compounds in *S. alata*, [21]. The pH values of the extracts ranged from 6.0 to 7.5, which is in the neighbourhood of neutrality, considered normal for the living system [38].

The preliminary phytochemical screening revealed that *Senna alata* contains some secondary metabolites like volatile oil, saponins, glycosides, alkaloids, anthraquinones, flavonoids, and tannins. The significant antimicrobial activity of *Senna alata*, may be as a result of the presence of these phytochemicals, since it has been reported that secondary metabolites present in plants are responsible for their therapeutic activities [21]. The greater zones of growth inhibition recorded in the aqueous and methanol extracts, is an indication that most of the bioactive compounds in these plants, were more soluble in these solvents [1, 39]. And the reverse may be the case in the xylene and ether extracts, which yielded little to no zones of growth inhibitions against the test organisms. From our results, *Salmonella typhi,* appears to be the most susceptible of the pathogens tested. Its susceptibility, cuts across the crude extracts of the various solvents and the plant parts. With the average MIC value of 6.25 µg/mL, this result supports the ethnomedicinal use of decoctions from this plant in the treatment of some water-borne diseases like typhoid fever. Other researchers have also reported similar antimicrobial activity against common bacterial pathogens like *Streptococcus pyogenes* and Staphylococcus *aureus* [40]. Also, the fact that both Gram-positive, such as *B. subtilis*, (ave. MIC, 22.9 µg/mL), *S. aureus*, (ave. MIC, 17.7 µg/mL) and Gram-negative organisms such as *E. coli*, *P. aeruginosa, S. typhi,* (ave. MIC, 20.8 µg/mL, 22.9 µg/mL, and 9.4 µg/mL), respectively; and some fungi, eg. *C. albicans*, and *A. niger* (ave. MIC, 11.5 µg/mL, and 12.5 µg/mL), respectively, were susceptible to these extracts, points to its broad spectrum of activity, and justifies the use of the plant parts in ethnomedicine, against bacterial infections like dysentery, diarrhoea, urinary tract infections (UTI), bronchitis etc; and some fungal infections like eczema. The MBC/MFC, the concentrations at which the extracts had 99.9% kill of the test pathogens, followed the same trend, thus lending credence to this position [41]. However, the MBC/MFC values for different solvents are significantly different, at $p < 0.05$, when compared with others, for the same tested organisms (Table 7). This could be as a result of the differences in the ability of the individual solvents to extract the bioactive compounds present in the different parts of the plant. This trend was also observed in the MIC values of the various solvent fractions of the plant parts (Table 6).

The positive result on flavonoid tests, encouraged the subsequent assay on the antioxidant activity of the plant alongside with information from literature. The result shows that the leaf extract has the best antioxidant activity. Its free radical inhibition compared relatively well with ascorbic acid, which was used as the standard. This points to the immune-modulating and anti-ageing potentials of the plant, [26].

However, the performance of the flower and bark extracts of *Senna alata* improved with increase in concentration.

CONCLUSION

This study has revealed that crude extracts, of *S alata* possessed significant activity against some bacterial pathogens, and therefore, has high potential as antibacterial agent. The findings of this study, provides a clue to, and justifies the use of these plant parts for the treatment of common health disorders in traditional medicine. *Senna alata* can be locally sourced since it grows well in any Nigeria soil. The effect of this plant against a wide range of bacterial and fungal pathogens, is commendable. Hence, *Senna alata* plant parts hold a great promise as a potential source of new antioxidant and antimicrobial drugs, especially with increasing incidence of drug resistance among pathogen against commonly used drugs. However, there is still the need for further research, to fully understand the mechanism of action of these plant parts.

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