

PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *SENNA ALATA* LEAF EXTRACT

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Abstract

Medicinal plants are gaining acceptance globally, Senna alata being one of the many that herbal practitioners are using to cure a wide range of diseases. This study is aimed to determine the antibacterial effect of Senna alata on multidrug resistant strains of Pseudomonas aeruginosa and Staphylococcus aureus and to determine its antioxidant activity. Cold, Hot aqueous and 80% Methanolic extracts of Senna alata were used for the analysis. Kirby-Bauer disc diffusion method, Agar Well Diffusion (AWD), Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) techniques according to established standards were used to analyze the antibacterial property of the plant extracts on clinical isolates. Antioxidant studies of the Methanol extract component were carried out using 2,2-di-phenyl-1-picrylhydrazyl (DPPH), Total Oxyradical Scavenging Capacity and the Total Phenolic Content of the extracts were estimated. The 80% Methanolic extract showed a better mean diameter zone of inhibition at a statistically significant difference of $P < 0.05$ than the cold and hot aqueous extracts, with a DZI value as high as 17 ± 0.61 at a concentration of 400mg/ml. The MBC/MIC ratio was 2. The phytochemical analyses revealed an abundance of flavonoids, tannins and cardiac glycosides. The antioxidant activity showed abundance of phenols and the IC_{50} was 1.22 and 2.34 for DPPH and TOSC respectively. This study proves the antibacterial property of Senna alata, thus justifying its use among the local herbal practitioners. It is hereby recommended that more researches be carried out on Senna alata to establish its safety in human tissues.

Keywords: Antibacterial activities, *Senna alata*, Disc Diffusion, Agar Well Diffusion, Diameter Zone of Inhibition (DZI), Antioxidant studies, DPPH, Total Oxyradical Scavenging Capacity, Total Phenolic Content.

Introduction: Medicinal plants are gaining wide acceptance globally, even though not as much among the medical practitioners due to a lack of research and scientific validation. The rise in multidrug-resistant bacteria in today's world is a result of widespread use of conventional antibiotics to treat infectious diseases (Mazzariol et al., 2021). The immune system's natural reaction to harm caused by microbial, chemical, and physical agents is inflammation. Excessive inflammation leads to many acute and chronic human diseases like autoimmune diseases, illnesses of the circulatory system, malignancies, and metabolic and neurological disorders (Parveen et al., 2015).

An entire class of extremely reactive molecules produced by the metabolism of oxygen is known as reactive oxygen species (ROS). Normally occurring physiological quantities of ROS are necessary for cellular functions. Higher quantities of reactive oxygen species, however, can result in significant cellular and tissue damage and accelerate the development of illnesses like cataract formation, liver injuries, and aging. Synthetic and natural antioxidants can both be useful in assisting the body in lessening the oxidative damage caused by reactive oxygen species (Sagnia et al., 2014). *Senna alata* contains chemical components that have been shown to have a variety of pharmacological activities. However, the real effects are yet unknown; thus, more research is needed to investigate its medical benefits (Carmona & Pereira, 2013).

Senna alata (L.) **Roxb** is also known as *Cassia alata*, a widely distributed medicinal herb of the Leguminosae. It is commonly known as candle bush, craw-craw plant, acapulco, ringworm bush, or ringworm plant. The plant is commonly found in Asia and Africa. In Nigeria, *Senna alata* has many local names, like Asunrun Oyibo among the Yorubas, and Ogalu in Igbo tribe (Oladeji et al., 2020). *Senna alata* has arrays of bioactive compounds such like the phenolics (rhein, chrysophanol, kaempferol, aloe-emodin, and glycosides), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol). These secondary metabolites are reported to display numerous pharmacological, antibacterial and biological activities (Fatmawati, Yuliana, Purnomo & Abu Bakar, 2020; Otto, Ameso & Onegi, 2014).

In Nigeria, various parts of the plant like the leaf, root, and stem decoctions are used in the treatment of many ailments like the skin, wounds, burns, diarrhea, constipation, and respiratory tract infection (Oladeji et al., 2020). Some traditional healers use the fresh leaves of the plant in the treatment of dermatitis, mycosis, and other skin diseases/rashes. The leaves of *Senna alata* are more frequently used than the roots, flowers, and stem bark due to the abundance of more active metabolites in the leaves. Apart from these varied pharmacological activities, the leaves are processed into pellets, capsules, and tea in Nigeria for disease prevention and maintenance of sound health (Oladeji et al., 2016).

Effective treatment of bacterial infection is critically important to prevent undesired side effects. The common use of broad-spectrum antibiotics as a first line intervention in combating bacterial pathogens, often result in unwarranted complications that harm the normal microflora of the host. Nigeria is blessed with several varieties of herbal remedies, like *Senna alata*, with proven efficacy and cost-effectiveness, which are used for various purposes. The aim of this research therefore was to screen the leaf extracts of *Senna alata* for antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and determine the antioxidant activity. This is with a view to finding out the relevance of *Senna alata* in the treatment of bacterial infections.

Materials and Method

Collection and Identification of Plant Materials: *Senna alata* plant (Plate 1) was collected from the Shere Hills area of Jos, Plateau State of Nigeria. It was identified at the herbarium of the Department of Plant Science and Biotechnology, University of Jos, by the Plant Taxonomist. A voucher number: JUHN21000414 was subsequently given.

Processing of Plant Materials: The leaves of *Senna alata* (Plate 1) were handpicked and washed thoroughly with distilled water. It was air dried under a shed at an ambient room temperature, pulverized with the aid of a clean mortar and pestle and sieved to get a fine powder.



Plate 1: *Senna alata*(L.) Roxb Plant.

Extraction Procedure: A mass of 120 g of the plant powder was weighed into 500 mL conical flasks and soaked in hot water at 70–80°C. This was left to stand overnight (24 hours) and shaken for 3 hours on a mechanical shaker. The content was filtered using a ball of non-absorbent cotton wool on a Buchner funnel/flask using a vacuum pump. The residue was then subjected to repeated rinsing and filtration using fresh hot water to attain a level of exhaustive maceration. The collective filtrate was evaporated to dryness under reduced pressure by the

use of a rotary evaporator and a drying cabinet at a controlled temperature of 60°C (30°C-60°C) (Zhang, Lin, & Ye, 2018). The above procedure was repeated with the use of cold water and 80% methanol as alternate solvents for extraction. The percentage yield obtained for the extract (Table 1) was determined according to Abbas et al. (2021), and the extract was transferred into a sterile sample container and preserved in the refrigerator at 4°C (Zhang et al., 2018).

Phytochemical Screening: The cold, hot aqueous and 80% methanolic extracts were screened for their respective phytochemical constituents qualitatively (Table 1) according to the methods of Auwal et al. (2014), and Sheel et al. (2014). The average yields were calculated accordingly. Also, the respective extracts were screened for their respective phytochemical constituents quantitatively (Table 2) according to the methods of Biradar & Racheti (2013); Kaur et al., (2015); Santhi and Sengottuvel (2016) and Yahaya et al., (2013).

Ethical Approval

The study was reviewed and ethical clearance was obtained from both the Jos University Teaching Hospital and the Plateau State Specialist Hospital, Jos Ethical Committees. Each of the patients that were recruited was briefed appropriately. The consented voluntarily signed the consent form and filled the structured questionnaire administered prior to collection of the sputum sample.

Preparation of Sensitivity Disc:

Processing of test substances and cells for susceptibility studies: For the susceptibility testing, the Kirby-Bauer principle of the disc diffusion and the agar well techniques were used according to established standards. A stock concentration of the extract was prepared by dissolving 2g of the extract in 5 ml of 10% (v/v) aqueous Dimethyl Sulfoxide (DMSO) to give an assay stock concentration of 400mg/mL. Gentamycin (from Sigma, USA) served as a positive control. A stock solution of this control was prepared by dissolving 100mg in 50ml of 10% (v/v) DMSO to give a stock concentration of 2000µg/mL. The clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from sputum specimens of hospital patients served as the test organisms for susceptibility to the plant extracts following standard protocols. A 0.5 McFarland turbidity standard (1.0×10^6 CFU/mL) of each bacterium was prepared and used in the study.

Standardization of Inocula: The inoculum was standardized using the Clinical Laboratory Standard Institute (CLSI, 2018) as adapted by Adeshina et al. (2020). Eighteen-hour broth culture of each test organism was standardized by gradually adding sterile normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 CFU/mL. The turbidity of the cell culture was matched with that of the 0.5 McFarland standard by holding the mixture and the standard in front of light against a white background with contrasting black lines through visual comparison with its density by the addition of normal saline.

Standard McFarland preparation: A 0.5 mL of 0.048 M BaCl₂ was added to 99.5 mL of a 0.18M H₂SO₄ solution and vortex for 2 minutes. A UV-Vis spectrophotometer was used to measure the absorbance at 625 nm. An absorbance of 0.1 was obtained, in the accepted range of 0.08 to 0.13. This standard (0.5) was used to make a visual comparison with the density of the suspension against a white background with black lines according to Onwuliri et al. (2020).

Susceptibility tests and determination of MIC and MBC of the plant extracts

The Kirby-Bauer disc diffusion method, agar well diffusion (hole plate method) and broth micro-dilution methods were used as described by Jorgensen et al. (2009) to assess the antibacterial susceptibility of the plant extracts by measuring the Diameter Zone of Inhibition (DZI) and determining the minimal inhibitory/bactericidal concentrations (MICs and MBCs) according to established standards and methods (CLSI, 2020). The analyses were replicated three times and the observations were recorded appropriately.

Screening of the Extracts for *In-vitro* Antibacterial Activity Using the Disc Diffusion Method

Each of the isolates to be tested was streaked onto Mueller-Hinton Agar (MHA). The media used were freshly prepared and poured to a depth of 4mm with the pH adjusted to 7.2-7.4 at room temperature. After overnight incubation at 37°C, a bacteria suspension was made aseptically and compared to 0.5 McFarland turbidity standard. A lawn of the bacterial suspension was then made with a sterile cotton swab. The extract-impregnated discs alongside the positive control (20µg Gentamycin) and negative (10% [v/v] DMSO) control were then placed on the media and incubated accordingly overnight at 37°C. The DZI were then measured according to CLSI (2020) standard. The DZI of the extracts were reported as mean $\pm$ standard deviation in millimetres (mm). The assay was performed in triplicate for each of the different plant extracts (Hot, Cold aqueous and 80% Methanol) and the results were recorded accordingly.

Screening of the Methanolic Extracts for *In-vitro* Antibacterial Activity Using the Agar Well Diffusion Method

Further analysis of the antibacterial activity was done using the Agar well diffusion technique on the methanolic extracts alone due to the observation of a much better DZI than the hot and cold aqueous extracts. The agar well diffusion analysis (hole plate method) was carried out according to established standards (CLSI, 2020). The test was performed by placing a uniform spread of bacteria suspension (about 100µL corresponding to Mc Farland standard 1) on a Mueller-Hinton agar plate followed by the creation of wells of 6mm diameter on labelled positions of the bacteria lawn and filling respective wells with 100µL of prepared methanolic extracts of different concentrations. Positive control wells contained 20µg/mL Gentamycin while 10% DMSO served as the negative control. Plates were incubated at 37°C for 24 hours and zones of inhibition were measured according to CLSI Standards.

Screening of Methanolic Extracts for *In-vitro* Determination of Their MICs and MBCs

The methanolic extract as a result of a better DZI observed from the disc diffusion and the agar well techniques were further subjected to antibacterial susceptibility test by the determination of the MICs and MBCs using 96-well micro-titer plates as described by Njimoh et al. (2015).

Fifty microliters of 5% glucose and 1% phenol red-supplemented nutrient broth were pipetted into duplicate wells in each micro-titer plate followed by 50µL DMSO-diluted sample uniquely into the first well of the test wells. The wells were then diluted serially by transferring 50µL from the first well to the next until the eleventh. The 50µL extra was discarded from the eleventh well. Positive control wells were similarly treated. Negative control wells (12th well) had no sample. 50µL of the test organism suspension was added to each well and the plates were incubated at 37°C for 24 hours. Visual observation of growth was based on the colour change of the phenol red indicator from red to yellow depicting acid waste produced by the growth of the microorganism. The concentration of the well

containing the lowest sample concentration that prevented visible growth or changes in colour was considered the MIC according to established standards (CLSI, 2018; Sarker et al., 2017).

Minimum bactericidal concentration (MBC) was recorded as the lowest concentration of the extract killing 99.9% of the bacterial *inocula* after 24 hours incubation at 37°C. The determination of MBC was performed on the extracts using the method described by Njimoh et al. (2015). Ten microliters (10µL) were taken from the well obtained from the MIC experiment (MIC value) and two wells above the MIC value well and inoculated on solid MHA plates. The colonies were counted after 18–24 hours of incubation at 37°C. The concentration of sample that produces < 10 colonies was considered as MBC value. The experiment was replicated three times according to established standards and the results were recorded accordingly.

Antioxidant studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The scavenging activity of the 80% methanol extract against DPPH radical was determined according to the method of McCune and Johns (2002). A 500 µL volume of 0.11 mM methanol DPPH was added to 500 µL of the extract and vitamin C and incubated at room temperature in the dark for 15 minutes. The absorbance of the blank (Ab) and samples (As) was measured at 517 nm. DPPH was calculated as $[(Ab-As)/Ab \times 100]$. The IC₅₀ values for DPPH inhibition were determined by fitting the percentage inhibitions calculated from absorbance data to a sigmoidal dose-response curve using Origin 7.0 software.

Total phenolic content (TPC): The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu reagent (Belguidoum et al., 2015). The test was conducted by adding 0.1 ml of extract sample to 0.5 ml of 10% Folin-Ciocalteu reagent and mixing. A 2.0 mL volume of 20% aqueous Na₂CO₃ solution was added after 5 minutes; the mixture was kept for 30 minutes in the dark at room temperature, and then the absorbance was read at 760 nm versus the prepared blank solution. Results were expressed as mg Gallic acid equivalents/mg of the sample (mg GAE/mg).

Total oxyradical scavenging capacity assay: The total oxyradical (OH⁻) scavenging activity of the extract was measured as described by Smirnoff and Cumbes (1989). The test was conducted by taking 2 mL of the extract at concentrations ranging from 0.31 to 5.0 mg/mL, 0.6 mL of 8 mM ferrous sulfate, 0.5 mL of 20 mM H₂O₂, and 2 mL of 2 mM salicylic acid, mixed and incubated at a temperature of 37°C for 30 min. After this, 0.9 mL of distilled water was added to each vial. The final solution was centrifuged at 4472 ×g for 10 minutes, after which the absorbance was read at 510 nm. The IC₅₀ values for Total Oxyradical (OH⁻) Scavenging activity were determined by fitting the percentage inhibitions calculated from absorbance data to a sigmoidal dose-response curve using Origin 7.0 software.

Statistical Analysis: The data were expressed as mean ± standard deviation (STD). The differences between the groups were determined by two-way analysis of variance (ANOVA). Values P < 0.05 were set as the level of significance. The statistical analysis was performed by the GraphPad Prism software version 5.00 for windows (GraphPad Prism 5.0 (2022)).

Results

Table 1 shows the result of qualitative phytochemical screening carried out on *Senna alata* leaf extracts. Phytochemical compounds such as carbohydrates, tannins, steroids, terpenes, and cardiac glycosides were discovered in all the extracts. However, Saponins were present only in the aqueous extract, while flavonoids were observed in the 80% methanolic extract only. Anthraquinones were not detected in any of the extracts (Table 1). The quantitative analysis (Table 2) reveals varying concentrations of the phytochemicals. The 80% methanolic extract shows the highest concentration level in oxalates, alkaloids, flavonoids, tannins, cyanide, phytate, and total phenol. However, the cold aqueous shows the highest concentration of saponins among the extracts.

Table 3 shows the antimicrobial activity of extracts of *Senna alata* (leaf) on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extracts were observed to show very low value of DZI at 100 mg/mL and exhibited intermediate DZI at 200 mg/mL concentrations. The antibacterial activity was well exhibited at 400 mg/ml concentrations for the extracts. The DZI of 15 ± 0.45 mm and 17 ± 0.61 mm were recorded at the 400 mg/mL concentration; 10 ± 0.15 mm and 12 ± 0.22 mm at 200 mg/mL concentration; and 6 ± 0.26 mm and 8 ± 0.14 mm at 100 mg/ml concentration of methanolic extract for *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively.

Table 4 was a confirmation of the antibacterial property of *Senna alata* through the Agar well technique showing a DZI of 17 ± 0.44 mm and 17 ± 0.09 mm at a concentration of 400 mg/mL of the extract on *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively.

Table 5 is the MIC/MBC analyses which shows the MIC at 62.5 mg/mL and the MBC at 125 mg/mL. The MIC/MBC ratio is 2.

Table 6 shows that *Senna alata* is rich in phenolic compounds. The sample presents dose-dependent total oxyradical and DPPH scavenging activities (Table 7). The IC_{50} s of the extract for *Senna alata* and vitamin C were 2.34 and 0.17 mg/mL, 1.22 and 0.17 mg/mL respectively.

Table 1
Phytochemical characteristics and Percentage Yield of the *Senna alata* Leaf Extracts

Phytochemicals	80% Methanol	Cold Aqueous	Hot Aqueous
Saponins	+	-	-
Tannins	+++	+	+
Flavonoids	++	-	-
Carbohydrates	++	+	+
Steroids	+	+	+
Terpenes	+	+	+
Anthraquinones	-	+	-
Cardiac glycosides	+++	+	++
Amount Obtained	15.6	12.3	11.8
Yield %	13.0	12.2	59.8

* +++ = abundant; ++ = average; + = in traces; - absent.

Table 2
Quantitative Phytochemical Constituents of *Senna alata* Leaf Extracts

Samples	Oxalate (%)	Saponins (%)	Alkaloids (%)	Flavonoids (%)	Tannins (mg/100g)	Cyanide (mg/100g)	Phytate (mg/100g)	Total Phenol (%)
80% Methanol	24.15±0.35	1.95±0.30	5.75±0.10	6.73±0.05	13.43±0.27	5.69±0.12	283.39±6.55	3.04±0.09
Hot Aqueous	18.85±0.42	1.82±0.11	5.10±0.24	1.59±0.27	10.59±0.33	4.87±0.04	210.53±5.04	2.15±0.11
Cold Aqueous	17.04±0.36	2.64±0.44	4.68±0.14	1.28±0.35	10.06±0.21	5.23±0.43	207.74±7.89	2.00±0.34

Table 3
Mean Diameter of Zone of Inhibition (mm) of Multidrug Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* to Extracts of *Senna alata*, Leaf Using Disc Diffusion

Plant Extracts	Clinical Isolates	Concentration of Extracts			Gen Ctrl 20ug/ml
		100mg/ml	200mg/ml	400mg/ml	
Meth	<i>Pseudomonas aeruginosa</i>	6±0.26 ^c	10±0.15 ^b	15±0.45 ^a	18±0.10 ^d
	<i>Staphylococcus aureus</i>	8±0.14 ^c	12±0.22 ^b	17±0.61 ^a	20±0.38 ^d
Hot	<i>Pseudomonas aeruginosa</i>	3±0.53 ^c	6±0.44 ^b	10±0.24 ^a	18±0.10 ^d
	<i>Staphylococcus aureus</i>	5±0.25 ^c	8±0.22 ^b	11±0.77 ^a	20±0.17 ^d
Cold	<i>Pseudomonas aeruginosa</i>	2±0.40 ^c	4±0.32 ^b	10±0.41 ^a	18±0.48 ^d
	<i>Staphylococcus aureus</i>	4±0.18 ^c	7±0.24 ^b	10±0.41 ^a	20±0.52 ^d

Values with different superscripts are significantly different at p<0.05 at a p = 0.0001

*Meth – 80% Methanolic Extract; Hot – Hot Aqueous Extract; Cold – Cold Aqueous Extract

Table 4
Mean Diameter Zone of Inhibition (mm) of Multidrug Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* to Methanol Extract of *Senna alata*, Leaf Using Agar Well Diffusion

Methanol Extracts		MDR Isolates	200mg/ml	400mg/ml	Gen Ctrl 20ug/ml
Plant Extract					
SAL	<i>Pseudomonas aeruginosa</i>		12±0.50 ^b	17±0.44 ^a	18±0.11 ^a
	<i>Staphylococcus aureus</i>		11±0.43 ^b	17±0.09 ^a	20±0.21 ^c

Values with different superscripts are significantly different at p<0.05 at a p = 0.0001

*SAL= *Senna alata*; ± = Standard Deviation; Number of replicates = 3; Gen Ctrl = Gentamycin Control (20ug/ml).

Table 5
Mean MIC, MBC and MBC/MIC Ratio of Multidrug Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* to Methanolic Extracts of *Senna alata*

<i>Senna alata</i>		<i>S. aureus</i>		Gen Ctrl		
<i>P. aeruginosa</i>						
MIC	MBC	MIC	MBC	MIC	MBC	
2000	-	-	-	-	-	-
1000	-	-	-	-	-	-
500	-	-	-	-	-	-
250	-	-	-	-	-	-
125	-	-	-	-	-	-
62.5	-	+	-	+	-	-
31.25	+	+	+	+	-	+
15.6	+	+	+	+	+	+
7.8	+	+	+	+	+	+
MBC/MIC Ratio	2	2	2	2	2	2

*Number of replicates = 3; Gen Ctrl = Gentamycin Control (20ug/ml).

Table 6
Total Phenolic Content (TPC) of *Senna alata*

Sample TPC	mg GAE/mg
<i>Senna alata</i>	26.81±2.54 ^a

Values are mean ± Standard Deviation of triplicate determination
GAE = Garlic acid equivalent

Table 7
DPPH and Total Hydroxyl Radical Scavenging Activity of *Senna alata*

Conct mg/mL	Total Hydroxyl Radical Scavenging Activity		DPPH Scavenging Activity	
	Vit C	<i>Senna alata</i>	Vit C	<i>Senna alata</i>
0.31	96.49±0.74	40.05±1.67	87.50±2.22	15.81±6.82
0.63	96.62±0.21	73.15±2.89	93.56±0.43	26.42±7.45
1.25	97.04±0.25	84.66±0.40	39.96±0.43	39.96±0.43
2.5	97.36±0.49	91.19±0.48	94.41±0.16	45.27±2.52
5.0	97.36±0.42	94.11±0.10	95.64±0.16	51.70±5.83
IC ₅₀	0.17	1.22	0.17	2.34

Values are mean ± Standard Deviation of triplicate determination

*IC₅₀ = half maximal inhibitory concentration

Discussion

The presence of phytochemicals in *Senna alata* extract has been reported by Karthika et al. (2016) and Onyegeme-Okerenta et al. (2017). The variation in the type of phytochemicals present in different solvents as shown in the results of the phytochemical screening might be attributed to the ability of the solvents to dissolve into solution-specific types of phytochemicals as reported by Altemimi et al. (2017). Anyanwu and Okoye (2017) reported that aqueous (water) extraction is commonly used by the local traditional healers, however, it has been shown that plant extracts obtained using organic solvents give more potent and consistent antimicrobial activity results than aqueous extract. Methanol has been proven to be one of the best organic solvents for antibacterial studies. (Anokwuru et al., 2011).

The antibacterial activity of *Senna alata* leaves could be due to the phytochemicals present, such as saponins, alkaloids, tannins, etc. Oladeji et al. (2020) in their study reported phytochemicals to act as plant protectants against pathogens. The same is equally experienced in humans when plant parts were taken as concoctions and decoctions in ethnomedicine (Proestos, 2020). This study shows a dose-dependent antibacterial activity for the extracts. The DZI for cold and hot aqueous solvents were significantly lower than that of 80% methanol across the different concentrations of the extract. Chatterjee and Dutta (2012), Ehiowemwenguan et al. (2014), and Oladeji et al. 2020, have all previously reported the antibacterial activity of *Senna alata* and associated it with the rich phytochemicals present in this plant. Toh et al. (2023) on their own account reported the dose-dependent antibacterial activity of *Senna alata*. The result from this study also showed that the 80% methanolic extract possessed higher antibacterial activity than cold and hot aqueous extracts. Cold water extract exhibited the least activity. The high antibacterial activity of methanol extracts over the other extracts could be attributed to their possession of more phytochemical constituents, which include tannins and flavonoids. Flavonoids were absent in hot and cold aqueous extracts. Harborne (1998) and Neumann et al. (2022) in their separate works reported that the presence of tannins and flavonoids in a plant extract could increase its antibacterial activity. The zone diameter of inhibition of the methanol extract at 400 mg/mL (15±0.45mm and 17±0.61mm) almost reaches that of the positive control disc (gentamycin) at 20µg/ml (18±0.10mm and 20±0.38mm), which is an indication of its potential as a strong antibacterial agent.

Recently, the extraction of plant parts for antibacterial activity is gaining more attention due to the increase in multiple drug resistance bacteria such as *Pseudomonas aeruginosa*, *Staphylococcal aureus* (Sisay et al., 2019). Medicinal plants in Jos, Plateau State, Nigeria,

reported to have antibacterial activity according to some researchers includes: *Senna alata*, *Acalypha wilkesiana*, *Moringa oleifera*, *Allium sativum*, *Aloe vera*, *Bryophyllum pinnatum*, *Cassia occidentalis*, *Citrus sinensi*, *Euphorbia hirta* and *Mangifera indica* (Gotep et al., 2010; Itelima et al., 2017; Lar et al., 2011). This presents a supportive evidence of this study's prove of antibacterial activity of *Senna alata*.

The result from this study indicated that the oxyradical and DPPH scavenging activities of *Senna alata* are significantly lower than that of vitamin C, which was used as a reference. This means that the reference antioxidant (Vitamin C) showed significantly higher free radical scavenging activity than the *Senna alata* plant extract. However, the mean antioxidant activity values of the extract exhibited free radical scavenging activity in a concentration-dependent manner. This agrees with the findings of Udegbumam et al. (2015). The antioxidant properties are the responsibility of some phytochemicals present in the plants, like flavonoids or phenolics. These are also responsible for the observed antibacterial activities of the plant extract (Kasim et al., 2014). In standard practice, Vitamin C is only used as a reference. The good antibacterial activities observed meant that the extracts have enough antioxidant properties but at a lower rate than Vitamin C. Some researchers have reported that some compounds have biotransformation activity *in vivo* after ingestion. This biotransformation could either increase the antioxidant properties or decrease them (Bharat & Ram, 2020; Viny et al., 2014).

Conclusion: From the result of this study, it could be concluded that the 80% methanolic extract of *Senna alata* leaf contains many phytochemicals with antibacterial and antioxidant activities that justified the plant's potential for therapeutic use in the management of bacterial infections by the herbal practitioners. The hot and cold aqueous extracts are the least potent in antibacterial activity as compared to 80% methanolic extract used in this study. The antibacterial activity exhibited by the leaf extracts of *Senna alata* could thus be attributed to the abundance of phytochemicals as revealed from this study. It is thereby recommended that further research be carried out on the safety of *Senna alata* in human tissues.

CONFLICT OF INTEREST The authors (Ebenezer Kolawole Adeosun*, Patience Ogbenyeonu Nwadiaro and Festus Chukwuemeka Onwuliri) declare no conflict of interest/competing interests.

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