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Phytochemical Analysis and Investigation of the Antibacterial and Antioxidant Activities of *Aristolochia albida* Root Extracts

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Authors' contributions

This work was carried out in collaboration among all authorsAll authors read and approved the final manuscript.

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ABSTRACT

Introduction: The increasing prevalence of antibacterial resistance has led to the deliberate laborious exploration of plants for new potent drugs. Over the years, plants have served as rich reservoirs of medicinal components that are used for the management of various ailments because of the belief that they exhibit minimal side effects and improved efficacy than other synthetic counterparts.
Aim: The aim of this research is to evaluate the phytochemical content, antimicrobial and antioxidant activities of the root extracts of *A. albida;* a shrub that is commonly used in North Central Nigeria for the management of some microbial infections, snakebite, stomach ailments and pain.

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Methods: The roots of *A. albida* were harvested, washed with clean water, dried, ground, and extracted with hexane, ethyl acetate, methanol and water using the Soxhlet extractor. The phytochemical screening which indicated the presence of alkaloids, anthraquinones, flavonoids, terpenes, cardiac glycosides and tannins was done using standard methods. The antimicrobial activity was tested using agar well diffusion technique against five human pathogens namely: *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pneumonae* and *Pseudomonas aeruginosa* while the antioxidant activities were tested using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.
Results: All extracts showed the antibacterial activity against all test organisms at all concentrations used. At 50 mg/cm^3, *S. aureus* showed high sensitivities with 23.00 mm, 20.50 mm, 22.00 mm and 18.50 mm diameter of zone of inhibition for hexane, ethyl acetate, methanol and water extracts respectively. The results obtained in this study indicated that the MICs of the four extracts were weak (12.50-200.0 mg/cm^3) when compared to the MIC range of most commonly available antibiotics having a range of 0.015-0.107 mg/cm^3. The extracts demonstrated minimum bactericidal concentration (MBC) range of 400-50 mg/cm^3. All the extracts showed a DPPH scavenging activity which increased with increase in sample concentration. Among the solvents used, hexane extract gave the highest antioxidant activity, IC_{50} = 0.032 mg/cm^3. Ethyl acetate and water extracts of *A. albida* had IC_{50} = 0.05 mg/cm^3.
Conclusion: The results reported in this research work highlighted the potential use of *A. albida* extract as a source of phytochemicals with promising antimicrobial and antioxidant activities.

Keywords: Antibacterial; antioxidant; aristolochia; extracts; phytochemicals.

1. INTRODUCTION

According to the traditional medicinal practices and also from modern scientific studies, medicinal plants are indispensable species that are utilized by man for medicinal purposes to treat diseases and improve human health [1]. These plants are thought of as rich sources of ingredients that can be utilized in the synthesis and production of drugs [2]. Medicinal plants are made up of different kinds of chemical constituents known as phytochemicals [3]. Some of these phytochemicals like flavonoids, anthraquinones, tannins, terpenoid, steroids, alkaloids, saponins, cardiac glycosides etc., elicit certain biological functions that enhance therapeutic activities such as antioxidant, antiviral, antimalarial, analgesic, diuretic, anthelmintic, antibacterial, antifungal, anti-allergic anti-mutagenic, anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties [4,5]. These pharmacological and biological effects of a vast number of plants have been unveiled through the isolation and characterization of the active ingredients [6]. At present, the vast majority of over-the-counter drugs are produced from plant-derived secondary metabolites [2]. It is also a known fact that natural products and their derivatives exhibit minimal side effects and improved efficacy than most of their synthetic counterparts [7]. This, perhaps, explains why the popularity of traditional medicine has greatly increased across the world in both developed and developing nations with the World Health Organization (WHO) estimating that 80% of the rural population in developing countries like Nigeria still patronize practices related to traditional medicine; implying that they still use medicinal plants as remedies or cure for many diseases [8,9].

Despite the vast health benefits derived from the use of plants as medicines, several challenges such as scanty scientific data to give credence to the use of some herbal remedies, lack of standardized formulation of herbal remedies and adulteration of herbal materials still exist [10]. Even with these challenges, plants possessing medicinal values have a promising potential to act as preventive medicine against various diseases as well as complement conventional medical treatments to increase efficacy or reduce adverse effects of conventional therapies [11].

The plant *Aristolochia albida* is of the genus *Aristolochia* which in turn belongs to the family *Aristolochiaceae*. *A. albida* is studied for its wide application in folk medicine, anti-inflammatory, antidiabetic effects, stomach ailments, pain as well as for treating snake bites [12]. Recent study that was focused on the evaluation of the antioxidant activity of different leaf extracts of *A. albida* showed that the ethanolic extract provided the best antioxidant activity [13].

Aristolochia species may grow as climbing vines, as short creeping herbs and a few are shrub-like [14]. *Aristolochia* species are herbaceous perennials, under-shrubs or shrubs, often scendent, scrambling, twinning, sometimes lianas, usually with prostrate, polymorphic or lobed leaves bearing essential oils. *A. albida* is commonly known as “Dutuchman’s Pipe” in English, “alogun in yoruba and “Duman dutse (meaning guard of the rock) or “Madacin kasa” (meaning Medicine of the earth) or fiyaka” in Hausa [15].

A. albida is sparsely distributed and is rapidly disappearing from its natural habitat in North Central Nigeria. This is due to poor harvesting methods and over exploitation by traditional medicine practitioners for application in the treatment and management of various diseases. Though widely used in traditional medicine therapy, there is insufficient published documentation describing the biologically active compounds occurring in *A. albida*.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The fresh roots of *Aristolochia albida* were collected from the plants at Warok village in Langtang-North Local Government Area of Plateau State. The roots were washed with water, chopped into small pieces, air-dried under shed for two weeks and ground into fine powder using a mortar and pestle. The resulting powder was packed into a sterile polythene bag and stored in a moisture-free desiccator until when needed for extraction.

2.2 Extraction Process

The water extract was prepared through cold maceration by the addition of 1,000 cm^3 of distilled water to 100 g of the root powder. The mixture was macerated for 48 hours with intermittent vigorous shaking in the morning and evening and then filtered. The filtrate was evaporated using the heating mantle maintained at 70 ^oC to obtain the extract which was stored in an amber bottle at −4 °C in a refrigerator.

The ethyl acetate extraction was performed by adding 1,000 $cm^3$of the solvent to 100 g of *A. albida* root powder. The mixture was macerated for 48 hours with vigorous shaking in the morning and evening to increase the efficiency of extraction. The mixture was then filtered, and the filtrate was evaporated using a rotary evaporator set at 50 °C to remove excess methanol solvent. The resultant content was then kept in a desiccator to produce a light brown powder which was stored in the refrigerator. This procedure was repeated with methanol and hexane. However, the extracts were recovered using the rotary evaporator at 40 ^oC.

Fig. 1. Picture of *Aristolochia albida*

2.3 Preliminary Phytochemical Screening

The analysis of the phytochemical groups of compounds, namely: flavonoids, tannins, saponins, anthraquinones, terpenes, cardiac glycosides and alkaloids of hexane, ethyl acetate, methanol, and water extracts of *A. albida* root were done using standard phytochemical screening procedures as described by Zaman & Pathak [16].

2.4 *In vitro*-Antibacterial Test Using Agar Diffusion Method: Standardization of inoculums

Five micro-organisms, *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Streptococcus pneumonae* and *Pseudomonas aeruginosa*, were sub-cultured to nutrient agar (NA) slants using a wire loop (done aseptically) and incubated for 24 hours at 37 ^oC for bacteria. Growth of micro-organisms in broth was indicated by turbidity. The turbidity produced was adjusted to match 0.5 Mc Farland standard (10^8 CFU/ml), which was further adjusted to 10^5 CFU/ml. After the incubation period, the diameters of the inhibition zones were measured in millimeters using a transparent ruler. All the tests were done in duplicates and the means were calculated as the results.

2.5 Preparation and Storage of Media

Clean petri dishes sterilized in autoclave were allowed to equilibrate at 48-50 ^{o}C before use. They were labeled, indicating the test organisms and type of extracts. Nutrient Agar was used for bacteria.

2.6 Determination of Minimum Inhibition Concentration

The minimum inhibitory concentration MIC of the extract was estimated for each of the test organisms in duplicates. To 0.5 cm^3 of varying concentration of the extract (400,200,100,50 mg/cm^3), 2.0 cm^3 of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 Mc Farland turbidity standard was introduced into the tubes. The procedure was repeated on the test organisms using the standard antibiotic, tetracycline. A tube containing nutrient broth only was seeded with the test organism as described above to serve as control. Tubes containing bacteria cultures were then incubated at 37 ^{o}C for 24 hours. After incubation, the table was then examined for microbial growth by observing for turbidity.

2.7 Determination of Minimum Bactericidal Concentration

To determine the minimum bactericidal concentration, a loopful of broth was collected from each set of test tubes in the minimum inhibition concentration determination that did not show any growth and inoculated on sterile nutrient agar. Nutrient agar was streaked with the test organisms respectively to serve as control. The plates inoculated with bacteria were then incubated at 37 ^{o}C for 24 hours. After incubation, the concentration at which no visible growth was seen and noted as the minimum bactericidal concentration.

2.8 Evaluation of Antioxidant Activity Using the DPPH Radical Scavenging Method

The antioxidant property of the extracts of the *A. albida* was measured spectrophotometrically by 2.2-diphenyl-1-picrylhydrazyl (DPPH) method which was established on the measurement of radical scavenging activity of root extracts of *A. albida* according to the method described by Scherer & Godoy [17]. The following equation, in which Ablank is the absorbance of the negative control, was used to calculate the percentage of inhibition:

$$I\,\% = \frac{(Ablank) - (Asample)}{(Ablank)} \times 100$$

The results were reported as an IC_{50} value expressing the dose required to make a 50 % inhibition. A lower IC_{50} expresses a greater antioxidant capacity.

3. RESULTS AND DISCUSSION

Various species of *Aristolochia* contain different chemical compounds. Those of the therapeutic interests include steroids, cardiac glycosides athraquinones, tannins, terpenoids, alkaloids, flavonoids, lignins, and fatty acids. Preliminary phytochemical screening of the root of *A. albida* (Table 1) showed that the crude root extracts of *A. albida* contained alkaloids, anthraquinones, flavonoids, terpenes, saponins, cardiac glycosides, carbohydrates and tannins.

Table 1. phytochemical analysis of the hexane, ethyl acetate, methanol and water root extracts of *A. albida*

Test	Hexane extract	Ethyl acetate extract	Methanol extract	Water extract
Alkaloids	+	+	+++	++
Saponins	+	+	+++	++
Tannins	traces	traces	+	+
Flavonoids	++	++	-	-
Carbohydrates	-	-	++	+
Terpenes	+++	+++	Traces	-
Anthraquinones	+	+	-	-
Cardiac glycosides	++	+++	+	+

***KEY: (**+) = slightly present, (++) = moderately present, (+++) = highly present, (**-**) = absent*

According to the results, terpenes, flavonoids, alkaloids, saponins, anthraquinones, cardiac glycosides and traces of tannins were present in hexane and ethyl acetate extracts. Methanol and water extracts were found to contain alkaloids, saponins, tannins, cardiac glycosides and carbohydrates with traces of terpenes in both solvents. This agrees with the findings of Yonki et al. [18] who reported the presence of alkaloids, flavonoids, saponins, tannins and terpenoids in methanol crude extract of the root of *A. albida*. Flavonoids have been reported to possess anti-inflammatory, antimicrobial and antioxidant activities [19].

Various species of *Aristolochia* have been reported to show antimicrobial activities [20]. Table 2 shows a varying degree of antimicrobial activity demonstrated by the four *A. albida* root extracts when investigated against the five microorganisms. All the extracts showed good activity against the bacteria *S. aureus*, *P. aeruginosa, E. coli, S. pneumonae and S. typhi.* The results showed that each extract had a different degree of growth inhibition (Table 2). At 50 mg/cm^3, *S. aureus* showed high sensitivities with 23.00 mm, 20.50 mm, 22.00 mm and 18.50 mm diameter of zone of inhibition for hexane, ethyl acetate, methanol and water extracts respectively. This compared favorably with the positive control (tetracycline) which gave a diameter of 27.63 mm at 20 mg/cm^3. At 400 mg/cm^3, hexane and methanol extracts recorded diameters of inhibition that surpassed TCN with values of 29.50 mm and 28.00 mm respectively. *A. albida* methanol extract also elicited better antibacterial activity than the methanolic extract of *A. longa* on *B. subtilis, M. luteus, S. aureus,* which had zones of inhibition diameter of 11.05 mm, 9.08 mm, 3.11 mm respectively [21]. Ethyl acetate and water extracts only gave values slightly lower than TCN (Table 2). This is in conformity with the findings that methanol extracts of *A. albida* showed good activity against *P. aeruginosa and S. aureus* [19].

Result of minimum inhibitory concentration (MIC) for methanol root extracts revealed that *S. typhi* and *P. aeruginosa* had the highest MIC (50 mg/cm^3) while the lowest MIC (12.50 mg/cm^3) was shown against *E. coli* (Table 3). The results obtained in this study indicated that the MICs of the four extracts were weak (12.50-200.0 mg/cm^3) when compared to the MIC range of most commonly available antibiotics having a range of 0.015-0.107 mg/cm^3 [12].

Table 2. Antimicrobial Activity for Hexane, Ethyl acetate, Methanol and Water Crude Root Extracts of *A. albida* against Test Microorganisms

Microorganisms	Concentrations (mg/ml)	Zone of inhibition (mm)					
		Hexane extract	Ethyl acetate extract	Methanol extract	Water extract	Positive control (TCN)	Negative control
	400	29.50	27.50	28.00	25.00	TCN	
	200	28.00	25.00	25.50	23.00	27.63	0.00
S. aureus	100	25.50	23.00	24.00	20.00		
	50	23.00	20.50	22.00	18.50		
	400	26.00	24.00	25.00	21.00		
E. coli	200	24.00	21..50	23.50	19.50	24.88	0.00
	100	20.00	20.00	20.00	17.00		
	50	18.00	18.00	18.00	15.00		
	400	17.00	18.00	20.00	19.00		
S. typhi	200	14.50	15.00	17.00	17.00	24.00	0..00
	100	13.00	13.50	14.50	15.00		
	50	10.50	11.00	12.00	13.00		
	400	22.00	23.50	26.50	26.00		
S. pneumonae	200	20.00	21.00	25.00	23.50	25.63	0.00
	100	18.00	19.00	22.00	21.00		
	50	16.00	16.00	20.50	18.00		
	400	27.00	21.00	22.00	24.00		
P. auruginosa	200	24.50	18.00	20.00	22.00	24.00	0.00
	100	23.00	16.50	17.50	20.50		
	50	21.00	15.00	15.00	18.00		

TCN=tetracycline

Table 3. Minimum Inhibitory Concentration (MIC) in for Hexane, Ethyl acetate, Methanol and Water Crude Root Extracts of *A. albida* against Test Microorganisms

Test organism	Extracts(mg/cm^3)			
	Hexane	**Ethyl acetate**	**Methanol**	**water**
S. aureus	12.50	25.00	25.00	100.00
E. coli	25.00	12.50	12.50	200.00
S. typhi	100.00	100.00	50.00	100.00
S. pneumonae	50.00	25.00	25.00	50.00
P. aeruginosa	25.00	25.00	50.00	50.00

However, this result indicated that all the extracts had strong antimicrobial activity when compared to the standard drug (Tetracycline) at the same concentration of 50 mg/cm^3 (Table 2). Phytochemicals found in the root extracts of *A. albida* demonstrated the capacity to possess antimicrobial activities and may be explored to develop drugs that can be used to combat antimicrobial resistance [22]. This is attributable to the abundant presence of alkaloids and saponins in the methanol root extract of *A. albida* which is in conformity with the findings of Shuai-Cheng et al. [23] and Abazar et al. [24]. These results were also in agreement with the findings of Owolabi et al. [25] and Bourhia et al. [26] which revealed the presence of steroids, anthraquinones, phenols, flavonoids, alkaloids and tannins in the root extracts of *A. ringens, A. paucinervis* and *A. baetica.*

Also, this result indicated that all the extracts had strong antimicrobial activity when compared with the results obtained from the crude extracts of other plants acclaimed to have antibacterial properties. This is attributable to the abundant presence of alkaloids, flavonoids and cardiac glycosides in the root extracts of *A. albida* which, again, is in conformity with the findings of Shuai-Cheng et al. [23] and Abazar et al. [24].

The antimicrobial activity of *A. albida* extracts against the selected bacterial strains are presented in Table 4 as minimum bactericidal concentration (MBC). The extracts demonstrated bactericidal activities in concentration range of 400-50 mg/cm^3. Result of minimum bactericidal concentration (MBC) for hexane root extracts revealed that *S. typhi* recorded the highest MBC of 100 mg/cm^3 while the lowest MBC of 25 mg/cm^3 was recorded against *S. aureus*.

The remaining test organisms; *E. coli, P. aeruginosa* and *S. pneumonae* recorded a concentration of 50 mg/cm^3, 100 mg/cm^3 and 100 mg/cm^3 respectively (Table 4).

The ethyl acetate extract showed the highest MBC of 400 mg/cm^3 was against *S. typhi* and the lowest MBC of 50 mg/cm^3 against *E. coli*. The bacteria *S. aureus* and *S. pneumonae* recorded MBC of 100 mg/cm^3 each while *P. aeruginosa* showed an MBC of 200 mg/cm^3.

The methanol extract, among all other extracts, demonstrated the best MBC of 25 mg/cm^3 against *S. pneumonae* as shown in Table 4. The highest MBC of 200 mg/cm^3 was recorded against *P. aeruginosa* and *S. typhi.* Both *S. aureus* and *E. coli* recorded an MBC of 50 mg/cm^3 each.

Water extract, among all the extracts, showed the weakest MBC of 400 mg/cm^3 against each of *E. coli* and *S. typhi*. An MBC of 200 mg/cm^3 was recorded against *S. aureus* and *P. aeruginosa* with the lowest MBC of 100 mg/cm^3 recorded against *S. pneumonae*.

Table 4. Minimum Bactericidal Concentration (MBC) in for Hexane, Ethyl acetate, Methanol and Water Crude Root Extracts of *A. albida* against Test Microorganisms

Test organism	Extracts (mg/cm^3)			
	Hexane	**Ethyl acetate**	**Methanol**	**Water**
S. aureus	25.00	100.00	50.00	200.00
E. coli	50.00	50.00	50.00	400.00
S. typhi	400.00	400.00	200.00	400.00
S. pneumonae	100.00	100.00	25.00	100.00
P. auruginosa	100.00	200.00	200.00	200.00

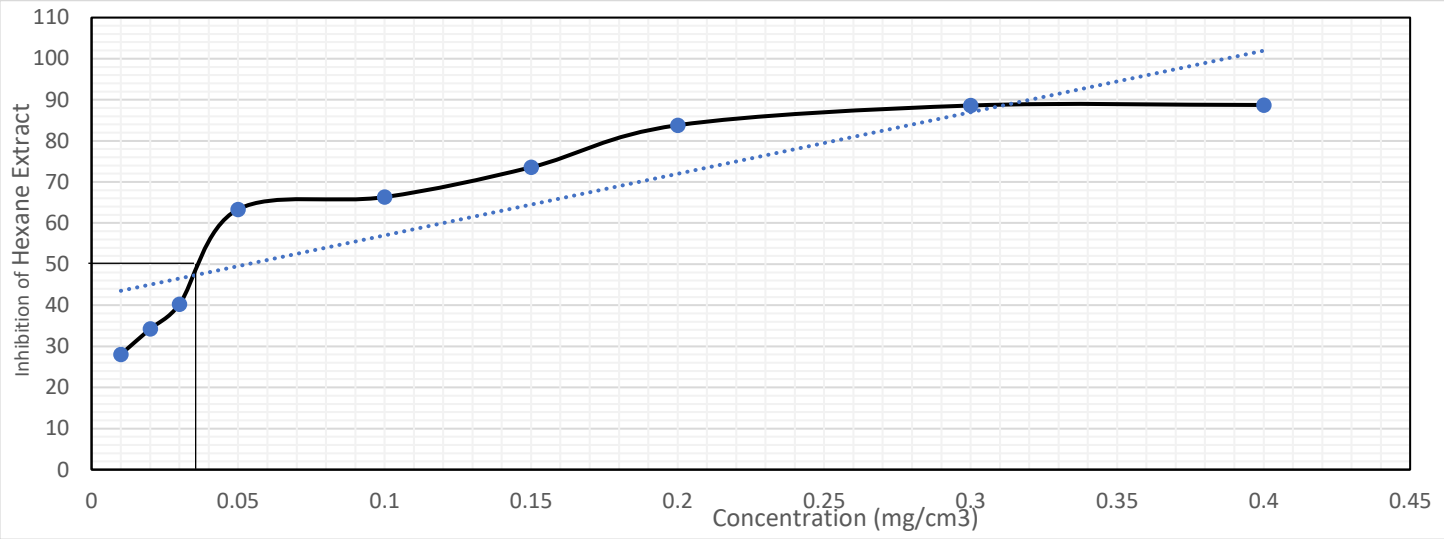

Fig. 2. Graph showing the IC_{50} of *A. albida* hexane root extract

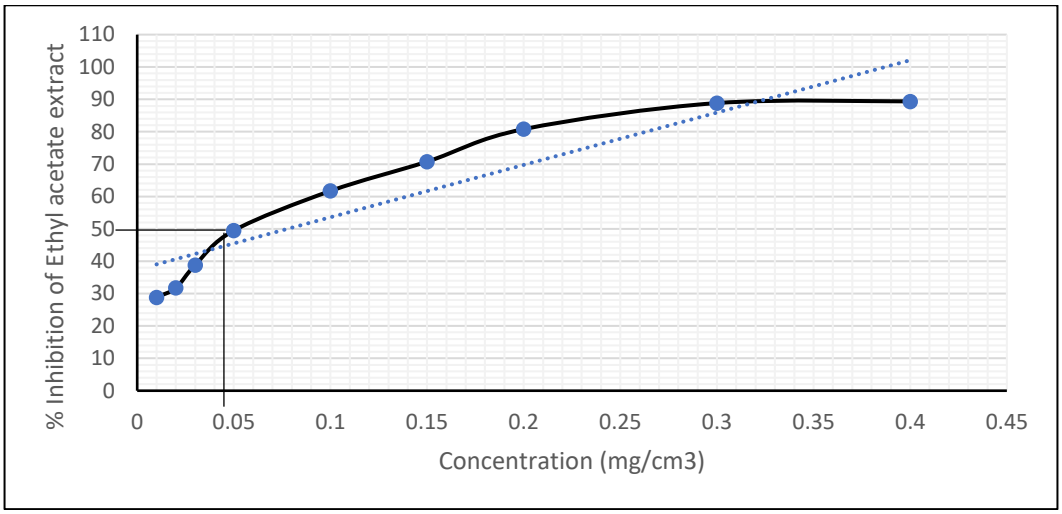

Fig. 3. Graph showing the IC_{50} of *A. albida* ethylacetate root extract

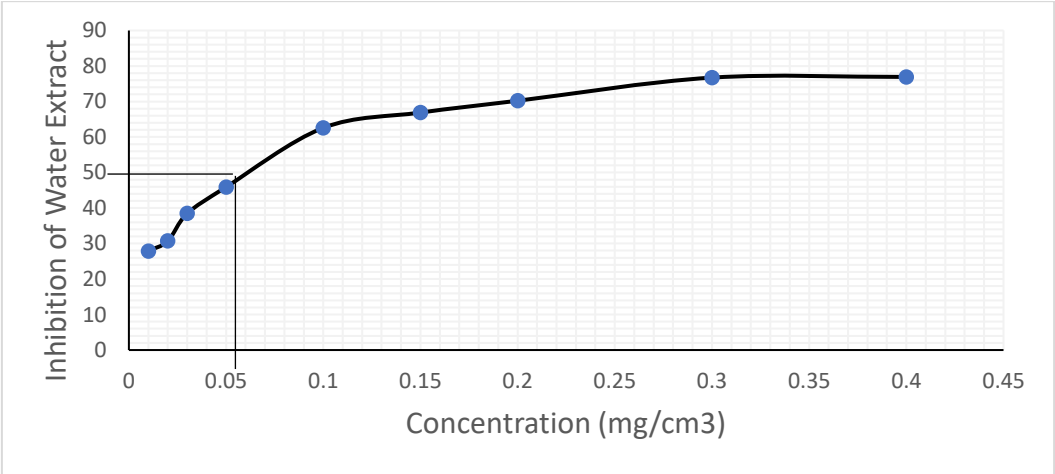

Fig. 4. Graph showing the IC_{50} of *A. albida* water root extract

Table 5. DPPH Radical Scavenging Activities at Various Concentrations of *A. albida* Root Extracts and Ascorbic Acid

		% DPPH inhibition		
Volume(µl)	**Concentration (mg/cm³)**	**HE**	**EAE**	**WE**
AA 10	0.10	95.79201	94.00873	93.93745
200	0.40	88.73215	89.37563	76.92296
150	0.30	88.59800	88.86025	76.72790
100	0.20	83.80331	80.80948	70.20770
75	0.15	73.57579	70.76447	66.88415
50	0.10	66.31459	61.70965	62.58046
25	0.05	63.33747	49.48678	45.89315
15	0.03	40.28036	38.84083	38.46212
10	0.02	34.25142	31.75531	30.70277
5	0.01	28.04596	28.84794	27.89865
0	0.00	0.000000	0.000000	0.000000

AA=Ascorbic acid, HE= Hexane extract, EAE= Ethyl acetate extract, WE= Water extract

On the whole, the results obtained in this study indicated that the MBCs demonstrated by all the extracts of *A. albida* were weak (25-400.0 mg/cm^3) when compared to the MBC of 8µg/cm^3 for doxycycline, a common broad-spectrum antibiotic [27]. However, in comparison with the results obtained from the crude extracts of other plants acclaimed to have antibacterial properties, the extracts of *A. albida* fared better. This can be attributed to the abundant presence of alkaloids, flavonoids, terpenes and cardiac glycosides in the root extracts of *A. albida* which is corroborated by the findings of Shuai-Cheng et al. [23] and Abazar et al. [24].

Antioxidant activity was performed using the *in vitro* DPPH method. The results are expressed as percentage DPPH inhibition. The DPPH radical scavenging activity of different extracts of *A. albida* at various concentrations are presented in Table 5. All the extracts showed a scavenging activity which increased with increase in sample concentration [28,29]. The IC_{50} was determined by finding the median of the plot of extract concentration against % DPPH inhibition. A lower value of IC_{50} indicated greater antioxidant activity. Among the solvents used, hexane extract gave the best inhibition concentration, IC_{50} = 0.032 mg/cm^3 (Fig. 2). Ethyl acetate and water extracts of *A. albida* had IC_{50} of 0.05 mg/cm^3 (Fig. 3) and 0.06 mg/cm^3 (Fig. 4) respectively. The percentage DPPH inhibition of ascorbic acid at a concentration of 0.1 mg/cm^3 was 95.03 (Table 5). High antioxidant activity of ascorbic acid compared to the crude extracts of *A. albida* could be explained by its being a pure molecule unlike the extracts which contained several molecules that do not possess antioxidant activities [30]. Many phytochemistry studies revealed that phenol and flavonoid content of plant extracts elicit antioxidant potential due to their hydroxyl groups [31].

4. CONCLUSION

The study explored the phytochemical composition, antimicrobial activity and antioxidant potential of *A. albida* crude extracts. The assay data obtained from this research proved that the medicinal plant *A. albida* contains several secondary metabolites like alkaloids, anthraquinones, flavonoids, steroids, cardiac glycosides and tannins, which are responsible for the antibacterial activities. The presence of flavonoids and tannins might account for the antioxidant activities exhibited by the plant extract. Findings from this study have the capacity to serve as a potential basis for discovery of novel bioactive natural products.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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