https://dx.doi.org/10.4314/jpb.v21i3.6
Vol. 21 no. 3, pp. 144-155 (September 2024)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Phytochemical, antioxidant and antibacterial studies of extracts and chromatographic fractions of *Gmelina arborea* Roxb (Lamiaceae)

Philip Adegboyega IDOWU[1*], Abdulazeez Opeyemi ASHIRU[1], Deborah Olanrewaju IDOWU[2], Charles Ojo OLAIYA[3], Kayode KARIGIDI[3]

[1]*Department of pharmaceutical microbiology, Faculty of Pharmacy University of Ibadan, Nigeria.*
[2]*Department of Chemistry, Faculty of Science University of Ibadan, Nigeria.*
[3]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria.*

Received 13th June 2024; Accepted 29th July 2024

Abstract
Antimicrobial resistance and oxidative stress are increasing and researchers are being encouraged to search the natural plant products, due to their popular usage in ethno-medicine, for alternative source of antimicrobial and antioxidant compounds. *Gmelina arborea* used in West-Africa and Ayurveda folkloric medicine to cure several diseases was therefore investigated for phytochemical, antioxidant and antimicrobial activities. Extracts and chromatographic fractions of the root-bark tested for antibacterial activity with the MIC determined on seven selected bacteria using agar dilution method. The antioxidant capacity of the crude extracts was determined by six (6) methods: total flavonoid content, total phenolic content, ferric reducing power, total antioxidant capacity, Trolox equivalent antioxidant capacity and DPPH (2,2-diphenyl-1-picryhydrazyl) properties. Phytochemical screening indicated the presence of saponins, tannins, flavonoids, terpenoids anthraquinones, phenols and alkaloids. The methanol and ethyl acetate extracts of the plant showed good antibacterial activities (18 mm inhibitory zones and MIC and MBC 12.5–100 mg/mL) against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6571. Antioxidant study of the extracts revealed that both ethyl acetate and methanol extracts have good antioxidant capacity comparable to that of ascorbic acid standard. Therefore, *Gmelina arborea* could prove a valuable source of developing new antimicrobial and antioxidant compounds for therapeutic uses.

Keywords: *Gmelina arborea;* Secondary metabolites; Chromatographic fractions; Antibacterial; Antioxidant

INTRODUCTION

Phytochemical constituents with good antimicrobial and antioxidant properties are of potential value in the treatment of microbial infections and oxidative related stress. Medicinal plants are a natural source of bioactive compounds with antimicrobial and antioxidant properties due to the secondary metabolites they produce. Medicinal plants are regarded as nature chemical factory that can generate drug moieties for therapeutic and other health benefits. Many developing countries solve a majority of their health problems through the use of traditional medicine and natural herbs [1]. Significant improvement has been made in controlling the

*Correspondence. *E-mail*: igboyega@yahoo.com *Tel*: +234-8033524399 ISSN 0189-8442

spread of bacterial infections upon discovery of antibiotics. However, most of the progress made in stopping the spread of infections has been lost due to the increase in emergence of multi-drug resistance strains of antibiotics [2]. This has led to increase search and research for more effective antimicrobial agents by exploring medicinal plants with traditional and ethnomedicinal values. Similarly, plant-based drugs are well patronized because their ready availability, usually present little or none side-effect and purported efficacy when compared to modern medicines [3]. Due to the fact that medicinal plants have been found to contain several active compounds, researchers are diverting their attention into natural products research for discovery of antimicrobial drugs [4].

Gmelina arborea Roxb. (Lamiaceae), found in many west African countries including Nigeria, was initially found in Asia but was later transported to Africa as it grows best in well-drained soils [5]. In India, *Gmelina arborea* root decoction has been used to treat abdominal tumours. It is also used traditionally to cure sore throat, snake bite, anasarca, rat bite, bilious disorder, anthrax, cholera, epilepsy, septicaemia, bites, sores, blood disorder, small pox, convulsions, rheumatism, delirium, dropsy, diarrhoea, urticaria, dyspepsia, haemorrhage, fever, swelling, headache, gout, stomachic, intoxication and madness [5,7]. The leaves are utilized for the treatment of insect stings, diarrhoea, scorpion bite, malaria and high blood pressure [6]. The plant root is a laxative and anthelmintic. It is used to treat hallucinations, fever, urinary discharges and piles. Its fruit serves as astringent, tonic and diuretic. Naturopathy practitioners advise their usage for vaginal discharges, thirst, alopecia, leprosy, consumption, strangury, and anaemia. Its flower is employed against leprosy and blood disorders [7].

Most research work reported on the plant has been mainly on the leaves and stem-bark, whereas the roots have significant ethnomedicinal uses. The aim of this study was to investigate the antimicrobial and antioxidant properties of *Gmelina arborea* in line with its phytoconstituents, secondary metabolites and chromatographic fractions.

EXPERIMENTAL METHODS

Collection of plants and extraction. The root bark of *Gmelina arborea* was collected from The Botanical Garden of University of Ibadan, Oyo state. Authentication of the plant was done at the Herbarium Unit of Forest Research Institute of Nigeria (FRIN), Ibadan, where voucher specimen numbered FHI-113028 was deposited. The root bark of the plant was shade-dried at room temperature then ground to coarse form. A Soxhlet apparatus and methanol was used to extract a small part of the sample (100 g). A Soxhlet apparatus with three solvents successively in increasing order of polarity (n- hexane, ethyl acetate and methanol) was then used to extract the rest of the plant sample. The extracts gotten from each phase of the extraction (using different solvents) were then concentrated in rotary evaporator and stored at 4°C for use.

Collection of isolates. The following typed cultures namely, *Klebsiella pneumoniae, Acinetobacter baumannii, Salmonella Typhimurium* ATCC 14028, *Escherichia coli* ATCC 25925, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6571 and *Citrobacter freundii* were collected from Molecular laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. Validation of the identity of the isolates collected was done by conventional cultural and biochemical methods (Gram staining, growth on selective media and biochemical tests carried out on each organism).

Antibiotic susceptibility profile. Antibiotic sensitivity test was done for the isolates using Muller-Hinton agar (MHA) medium (Hi-media) by disc diffusion method [8] with strict

adherence to the guidelines stipulated by the Clinical Laboratory Standard Institute [9]. Multi-disc antibiotics (Abtek Biologicals, England), comprising eight antibiotics commonly used for Gram-positive bacterial infections was used for this analysis. The concentrations (µg) of these standard antibiotics as impregnated on the respective discs were Cefixime (30), Ofloxacin (5), Augmentin (30), Nitrofurantoin (30), Ciprofloxacin (5), Ceftazidime (30), Cefuroxime (30), Gentamycin (10). Sterilized nutrient broth in culture tubes was inoculated with few colonies of test organisms. A bacterial suspension was produced by incubating the tubes for 24 hours after which the turbidity of the overnight broth culture of the isolates was compared to that of 0.5 McFarland standards (1.0 x 10^8 CFU /mL). For the sensitivity test the MHA media were prepared and pour in plates after which the plates were dried for 30 minutes before inoculation. The bacterial suspension was swabbed evenly on the whole surface of the medium with sterile cotton swab. After the inoculum had dried, the antibiotic discs were placed on the MHA with sterilized forceps. Then, the plates were incubated at 37℃ for 24 hours. After the incubation period, the zones of inhibition were observed and compared with standard antibiotic sensitivity chart provided by CLSI for result interpretation.

Qualitative phytochemical evaluation. The phytochemical analysis of the whole methanol extract of *Gmelina arborea* was carried out using standard procedures [10]. The extract was chemically tested for the presence of alkaloids, terpenoids, saponins, tannins, flavonoids, anthraquinones, steroids, phenols and cardiac glycosides.

Determination of antibacterial activity. The antibacterial activity of the crude extract of *Gmelina arborea* was done using the agar-well diffusion method [11] with slight modifications. The test organisms were first grown in nutrient broth for 24 hours after which the turbidity of the overnight broth culture of the isolates was compared to that of 0.5 McFarland standards (1.0 x 10^8 CFU/mL) and dilution of overnight phase broth culture of the bacterium to an optical density equivalent to that 0.5 McFarland standard was done. One hundred microlitres (0.1 mL) of the bacterial suspension was inoculated into MHA medium and then poured into a sterile Petri dish and swabbed across the plate with a sterile cotton swab, the plate was allowed to set and wells were then bored into the agar medium using a sterile 8 mm cork borer. One hundred microlitres (0.1 mL) of different concentrations of the extract dissolved in 50% methanol was placed in each hole. The plates were allowed to stand on the laboratory bench for one hour to allow proper diffusion of the extract into the medium and then incubated at 37°C for 24 hours, after which the plates were observed for zones of inhibition. Since extracts were reconstituted in 50% methanol, the diluent was used as a negative control while Ciprofloxacin (5 µg) was used as a positive control to compare the effect of the extracts to those of the standard antibiotics. Each test was done in triplicate.

Determination of the Minimum Inhibitory Concentrations (MICs) of plant extracts. The minimum inhibitory concentration (MIC) of the crude extracts was determined following the stipulated method [12]. The agar was autoclaved at 121°C for 15 minutes and allowed to cool to 50°C in a water bath. Different concentrations (0.5 mL) of the extracts was added to the molten agar (24.5 mL) in the water bath, swirled and poured into Petri dishes, and allowed to cool and solidify. Ten microliter each from both the prepared bacterial inoculum was delivered on the solidified agar surface to give the desired final inoculum of 1×10^4 CFU/spot. The extract concentrations for the MIC ranged from 100 mg/mL to 6.25 mg/mL. Plates were

incubated at 37°C and readings were taken between 16 and 20 hours after incubation.

Determination of Minimum Bactericidal Concentration (MBC). For the determination of MBC, the inoculating loop was used to touch the surface of the agar where the organisms were previously streaked unto and streaked onto fresh nutrient agar plates. The two lowest concentrations from MIC test showing no visible growth were used for this MBC test. The lowest concentration of the extract not showing visible growth after incubation at 37°C for 24 hours was taken as MBC [12].

Fractionation and purification of plant extracts.

Thin-layer chromatography (TLC) method. The extract was applied to commercially precoated TLC plate and different solvent systems were used to separate the components present in the ethyl acetate extract. Visualization was done using iodine tank and UV light (325 nm). After the spots were visualized and labelled, their retention factors (R_f value) were calculated and compared. The R_f values were calculated according to the following formula:

$$\mathrm{Rf} = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

Column chromatography method. Further fractionation using column chromatography silica gel (LAB TECH CHEMICALS, 70-230 Mesh) as stationary phase to isolate the bioactive compounds from the ethyl acetate crude extract (which showed good antibacterial activity) was carried out. A cleaned, dry column was aligned in a vertical position. A beaker was placed under the column outlet. A loose plug of cotton was tamped down in to the bottom of the column. A small layer of clean silica sand was placed over the cotton wool by pouring sand in to the column. The column was tapped gently to level the surface of the sand. The column was then filled with the mixture of silica gel and the first solvent (hexane) and was added carefully from a beaker, while solvent was allowed to flow slowly from the column. The column was tapped as the silica gel was added till a desired height was attained. From dried extract obtained, 5g of extract was loaded on a glass column packed with silica gel (Lab Tech Chemicals, 70-230 Mesh), as a stationary phase. The extract was dissolved in a minimal quantity of methanol and adsorbed on to silica gel G. When the sample had adsorbed to the silica gel, small amount of sand was poured to cover sample. The mobile phase was poured continuously to the top of the column by aid of a funnel. The bottom outlet of the column was opened. The eluates (fractions) were collected in separate test tubes. The column was eluted with different polarity of solvents; first eluted with hexane (100%); graded mixtures of hexane and ethyl acetate (90-10%) till 100% ethyl acetate before the introduction of methanol which used up till 30% was attained. The test tubes were changed after 10 mL of each fraction and each collected fraction was analysed by TLC technique, which was also used to monitor the eluted components. Fractions were collected, pooled together on the basis of R_f values and the solvent was removed. They were then dried, weighed and analysed.

Antimicrobial screening of column chromatography fractions. The pooled column chromatographic fractions were concentrated to dryness after which their antimicrobial activity against some of the test isolates was determined by agar well diffusion method [13]. The fractions were tested at 20 mg/mL as against the extracts that were tested at 50 and 100 mg/mL

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The DPPH radical scavenging activity of the medicinal plants used in this study was determined following the modification of the

established protocol [14]. Different volumes of the prepared sample (100, 200, 300 and 400 µL) were dispensed into four different test tubes. The volume in each tube was made up to 1mL with the appropriate volume of distilled water (900, 800, 700 and 600 µL, respectively). A volume of 3 mL of freshly prepared DPPH reagent (0.03 mg/mL prepared in methanol solution) was added to each test tube. The resulting mixture was agitated and kept in the dark for 35 minutes. The absorbance was measured at 520 nm. Radical scavenging activity of the extract was determined using the equation:

$$Radical\ scavenging\ activity = \frac{\mathbf{Ablank - Asample}}{\mathbf{Ablank}} \times 100$$

A_{bank} is Absorbance of solvent without the sample; while A_{sample} is Absorbance with the sample (extract). Ascorbic acid was used as the standard.

Total phenolic content. The total phenolic content of the plant samples was determined following the described method [15]. The protocol involves the use of spectrophotometer to determine the total phenolic content of a given sample. About 1 mL of plant sample prepared at 1 mg/mL concentration was mixed with 1mL of Folin-Ciocalteu phenol reagent. Subsequently, 10 mL of 7% sodium carbonate and 15 mL of distilled water were added to the mixture after five minutes. The resulting mixture was thoroughly shaken and allowed to stand in the dark for 90 minutes at 25°C Absorbance of the solution was then measured using a spectrophotometer [Biochrom UV-Vis, UK] at 750 nm. A standard curve was derived with gallic acid solution. Gallic acid was used as a standard to plot the graph with equation:

$$Y = 0.920X,\ R^2 = 0.585$$

The equation was derived from the gallic acid calibration curve, with the linearity based on the coefficient of determination R^2 generated for this measurement. The equation of this curve was used to estimate the total phenolic content of each sample expressed in milligram (mg) of gallic acid equivalent per gram (g) of the dried extract.

Total flavonoid content. The antioxidant parameter was determined using aluminium chloride following the described method [16]. In a clean and empty test tube, 0.3 mL of the plant sample, 3.4 mL of 30% methanol, 0.15 mL of sodium nitrate (0.5 M) and 0.15 mL of aluminium chloride (0.3 M) were mixed. After five minutes, 1mL of sodium hydroxide (1M) was added. The solution was mixed well and absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids content was plotted using quercetin standard solution following the same procedure as slated above. The quercetin standard calibration curve was used to derive the equation:

$$Y = 0.190X \text{ and } R^2 \text{ value of } 0.998$$

The equation was derived from the quercetin calibration curve, with the linearity based on the coefficient of determination R^2 generated for this measurement.

With this equation, the concentration of the flavonoid content in each extract was computed with respect to their individual absorbance measured at 415 nm. The total flavonoids were expressed in milligram (mg) quercetin equivalent per gram (g) of the dry extract.

Ferric reducing antioxidant power (FRAP). The antioxidant capacity of the extracts was determined by method [17] evaluating their ability to reduce ferric to ferrous ions which is indicated by a change in colour from yellow to pale green or blue depending on the concentration of antioxidants present in a given sample. An increase in the reduction of ferric ions which is indicated by an increase in absorbance is indicative of an increased antioxidant potential of the extract.

A volume of 2 mL of the sample was mixed with 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferric cyanide of concentration 10 mg/mL. The mixture was incubated at 50°C for 20 minutes followed by addition of 2 mL of trichloroacetic acid of concentration 100 mg/mL the mixture was centrifuged at 3000 rpm for 10 minutes and the upper layer of the solution collected. A volume of 2 mL was mixed with 2 mL of distilled water and 0.4 mL of 0.1% fresh ferric chloride. After 10 minutes, the absorbance was measured at 700 nm.

Total antioxidant capacity by phosphomolybdate test. Total antioxidant capacity of the extracts was determined by phosphate molybdenum assay which is based on the potency of antioxidants to reduce phosphate molybdenum (VI) to phosphate molybdenum (V) complex [18]. A volume of 0.1 mL of the sample solution was mixed with 1 mL of reagent solution (0.6 M hydrogen sulphate, 28 mM of sodium phosphate and 4 mM ammonium molybdate). The test tube was capped and incubated in a water bath at 95°C for 90 minutes and allowed to cool. Absorbance of the samples was read at 765 nm against a blank. Ascorbic acid was used as the standard to derive the standard calibration curve with equation:

$$Y = 1.78X, R^2 = 0.998$$

The equation was derived from the ascorbic acid calibration curve, with the linearity based on the coefficient of determination R^2 generated for this measurement.

ABTS (2,2'-azino-*bis*-3-ethylbenzthiazoline -6-sulphonic acid) radical scavenging activity. Free radical scavenging activity of plant extracts was determined by ABTS radical cation decolorization assay [19]. $ABTS^+$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 hrs before use. $ABTS^+$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µL of plant extract to 3.995 ml of diluted $ABTS^{\cdot+}$ solution, the absorbance was measured at 30 minutes after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, $ABTS^{\cdot+}$scavenging effect (%):

$$\frac{A_B - A_A}{A_B} \text{ X } 100$$

where, A_B is absorbance of ABTS radical + methanol; A_A is absorbance of ABTS radical + sample extract/standard. Trolox was used as standard substance.

RESULTS

Yield of extracts. The yields and macroscopic characteristics of the hexane, ethyl acetate and methanolic extracts of *Gmelina arborea* are as follow. The methanol extract gave the highest yield of 24.9 g (3%), ethyl acetate 11.8g (1.5%) while hexane extract had the lowest yield of 7.4 g (1.0%). The hexane extract was oily and greenish in nature while the ethyl acetate and methanolic extracts were sticky and brownish in nature.

Phytochemical screening. The result of the phytochemical screening of the extract showed the presence of certain secondary metabolites in the plant extract: saponins, tannins, flavonoids, terpenoids, phenols, anthraquinones and alkaloids. The result is presented in Table 1.

Antibiotic susceptibility profile of the isolates. The results obtained from the antibiogram of the seven test organisms are presented in Table 2. The resistance (R) and sensitivity (S) were interpreted according to CLSI (2017) standard for Antimicrobial Susceptibility Testing. The results showed that, out of the seven bacteria species tested, six were resistant to Cefixime, while all were

resistant to Augmentin and Cefuroxime. However, most were sensitive to Nitrofurantoin, the Fluoroquinolones and Gentamycin. The nature of the organisms (sensitive or resistant) may affect the activity of the extracts and fractions. The zones of inhibition were interpreted using CLSI (2017) guidelines, on which basis the organisms were classified as Resistant (R), Intermediate (I) or Susceptible (S).

Antimicrobial activity of the extracts. The antimicrobial activity of the extract on the test bacteria was represented by the diameter of zone of inhibition produced around the agar wells as shown in Table 3. All the test against negative control (50% methanol) showed no zone of inhibition. The MIC and MBC were presented in form of lowest concentration of extract that inhibit and kill the organisms, respectively (Table 4). Hexane extract did not show antibacterial activity at the tested concentration; therefore, the MIC was not determined.

Characteristics of Column fractions and their antimicrobial activities. Column fractions were of different characteristics (Table 5) and their antibacterial activity on the test organisms is presented in Table 6. A total of 56 fractions were obtained from column chromatography of the ethyl acetate extracts, TLC analysis performed led to pooling together fractions of similar R_f values to obtain 15 fractions as shown in Table 6. The pooled fractions showed different characteristic colouration based on the type of phytochemical constituents (Table 5).

The pooled fractions of the extracts were tested for their antimicrobial activities with the results displayed in Table 6. Fractions 2-14 were active against *S. aureus* ATCC 6571, 9 against *E. coli* ATCC 25922, fractions 2, 3, 6, 8, 9, 12, 13 against *P. aeruginosa* ATCC 27853 and 3, 12, 13 against *S. typhimurium* ATCC 14028, 2-15 against *C. freundii*, 3 and 9 against *A. baummanii* and *K. pneumoniae*.

Table 1: Phytochemical Screening of *Gmelina arborea* whole methanol extract

Phytochemical constituents	Results
Saponins	Present
Tannins	Present
Cardiac glycosides	Absent
Flavonoids	Present
Terpenoids	Present
Steroids	Absent
Anthraquinones	Present
Alkaloids	Present
Phenols	Present

Table 2: Antibiogram of bacteria used showing susceptibility to selected antibiotics

Bacteria	Antibiotics used								
	ATCC	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	GEN
S. typhimurium	14028	R	S	R	S	S	R	R	S
E. coli	25922	I	S	R	S	S	R	R	S
K. pneumoniae	Lab 12	R	R	R	S	S	R	R	R
A. baummanii	Lab 07	R	S	R	R	S	R	R	R
P. aeruginosa	27853	R	S	R	S	S	R	R	S
S. aureus	6751	R	S	R	S	S	R	R	S
C. freundii	Lab 24	R	S	R	S	S	R	R	S

CXM = Cefixime (30 µg), OFL= Ofloxacin (5 µg), AUG = Augmentin (30 µg), NIT= Nitrofurantoin (30 µg), CPR= Ciprofloxacin (5 µg), CRX= Cefuroxime (30 µg), CAZ= Ceftazidime (30 µg), GEN= Gentamycin (10 µg),

Table 3: Antimicrobial activity as diameter zones of inhibition (mm) of extracts of *Gmelina arborea*

Bacteria	ATCC	MeOH	EtOAc	Hex	MeOH	EtOAc	Hex	CPR
		100 mg/mL			50 mg/mL			5 µg/mL
S. typhimurium	14028	18	11	-	16	10	-	30
E. coli	25922	-	12	-	-	11	-	26
K. pneumoniae	Lab 12	-	13	-	-	10	-	20
A. baumannii	Lab 07	-	14	-	-	-	-	30
P. aeruginosa	27853	12	18	-	13	12	-	35
S. aureus	6751	18	15	-	15	13	-	25
C. freundii	Lab 24	11	11	-	10	9	-	30

CPR= Ciprofloxacin (positive control), - = no activity, size of cork borer = 8 mm, MeOH= methanol, EtOAc= ethyl acetate, Hex= hexane

Table 4: MIC and MBC of methanol and ethyl acetate extracts of *Gmelina arborea* leaves

Bacteria	ATCC	MIC (mg/mL)		MBC (mg/mL)	
		MeOH	EtOAc	MeOH	EtOAc
S. typhimurium	14028	>100	50	>100	100
E. coli	25922	>100	50	>100	50
K. pneumoniae	Lab 12	25	50	25	50
A. baumannii	Lab 07	>100	50	>100	100
P. aeruginosa	27853	50	25	50	25
S. aureus	6751	12.5	50	25	100
C. freundii	Lab 24	25	12.5	25	25

Table 5: Characteristics of fractions of ethyl acetate extract from column chromatography

Column fractions	Eluting solvent(s)	Ratio	Volume (mL)	Remark
1	H	100	400	Colourless
2	H: E	90:10	200	Colourless
3	H: E	80:20	150	Light green
4	H: E	70:30	120	Lemon-green
5-10	H: E	60:40	120	Greenish-yellow
11-15	H: E	50:50	120	Brownish-green
16-19	H: E	40:60	100	Light green
20-24	H: E	30:70	100	Yellowish-green
25-27	H: E	20-80	80	BrightYellowish-green
28-31	H: E	10-90	90	Golden-yellow
32	E	100	200	Light army green
33-37	E:M	95-5	75	Greenish-yellow
38-41	E:M	90-10	55	Greenish-yellow
42-45	E:M	85-15	80	Golden-yellow
46-49	E:M	80-20	80	Orange
50-53	E:M	75-25	65	Brown
54-55	E:M	70-30	50	Bright orange

H= Hexane, E= Ethyl acetate, M= Methanol

Table 6: Antibacterial activity of chromatographic fractions of ethyl acetate extract

Bacteria	ATCC	Chromatographic Fractions 1-15															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	C
K. pneumoniae	Lab 12	-	-	11	-	-	-	-	-	10	-	-	-	-	-	-	-
A. baumannii	Lab 07	-	-	13	-	-	-	-	-	17	-	-	-	-	-	-	-
S. typhimurium	14028	-	-	12	-	-	-	-	-	-	-	-	10	10	-	-	10
E. coli	25922	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-
P. aeruginosa	27853	-	12	18	-	-	18	-	17	14	-	-	12	10	-	-	10
S. aureus	6751	-	13	20	12	13	16	12	11	15	10	10	13	12	10	-	11
C. freundii	Lab 24	-	13	19	12	15	15	18	12	14	10	11	14	12	9	9	14

Numbers in the table represent zones of inhibition in mm. C= Crude Ethyl acetate extract, size of cork borer = 8 mm

Table 7: Antioxidant activity of the root-bark extract of *Gmelina arborea*

Extracts	Antioxidant assays				
	TPC (mgGAE/g)	TFC (mgQE/g)	TAC (mg/g)	ABTS (mg/g dw)	FRAP (mg/g)
Hexane	3.806475	7.153966	3.988764	7.259259	4.205607
Ethyl acetate	30.0249	22.86159	18.05243	42.40741	14.58333
Methanol	37.709	15.39658	3.895131	42.72222	8.936916

TFC= Total flavonoid content, TAC= Total antioxidant capacity, TPC= Total phenolic content, FRAP= Ferric reducing antioxidant power, ABTS (2,2'-azino-*bis*-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, GAE= Gallic acid equivalence, QE= Quercetin equivalence

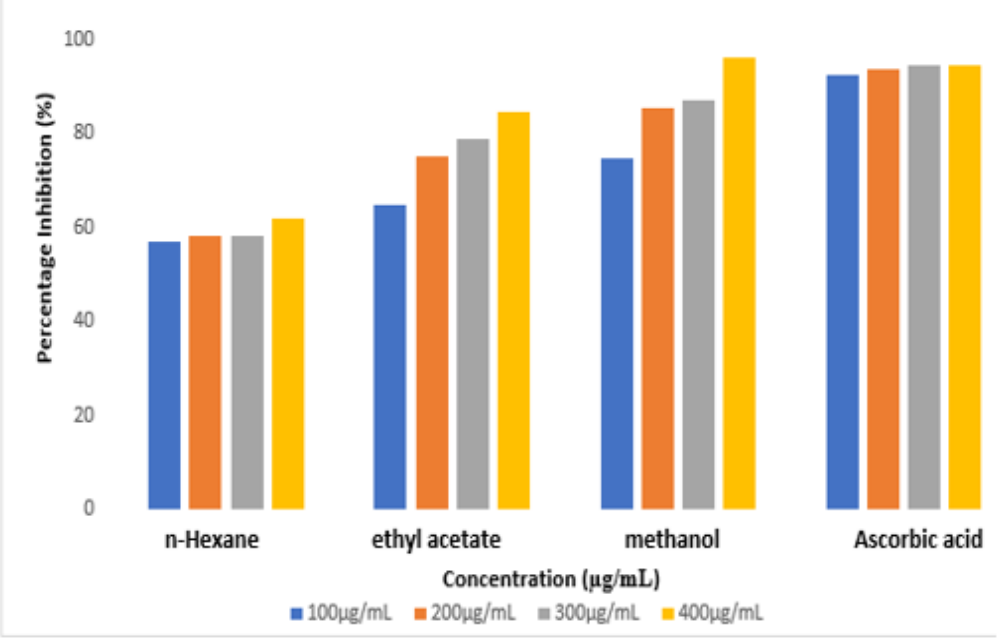

Figure 1. DPPH scavenging activity of the extracts of *Gmelina arborea*

Antioxidant Activities results for five different methods, viz: Total flavonoid content (TFC), Total antioxidant capacity (TAC), Total phenolic content (TPC), Ferric reducing antioxidant power (FRAP) and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity are shown in Table 7. Result of 2,2-diphenyl-1-picryhydrazyl (DPPH) scavenging activity is presented as a bar chart in Figure 1.

DISCUSSION

The percentage yield on successive extraction showed an increase with increasing polarity of the solvents from n-hexane through ethyl acetate to methanol. This method of extraction always separates the non-polar

constituents from polar ones, thus facilitating the location and isolation of bioactive phytoconstituents using chromatographic techniques. Natural plant products are known to contain several phytochemicals, some of which are their own naturally occurring phytoalexins, which protect them from other organisms and animals. In this study, phytochemical screening showed that *Gmelina arborea* roots contain saponins, tannins, flavonoids, terpenoids, anthraquinones, phenols and alkaloids (Table 1), which have been reported to exhibit good antimicrobial activities on microorganisms similar to a published report [20] which also showed that saponins, alkaloids, phenols, tannins and anthraquinones were present in *Gmelina arborea*. These phytochemicals are most likely responsible for the detected antioxidant and antimicrobial properties of this plant extracts (Tables 3 and 7). Tannins, phenolics and flavonoids are well known for their antimicrobial activity and the presence of many hydroxyl group in their chemical structures greatly favours antioxidant capacity and antimicrobial activity [3, 15].

Bioassay-guided fractionation, in which all the pooled column fractions (Table 5) were tested for antimicrobial activity (Table 6) facilitated the location and isolation of the antimicrobial active fractions of the ethyl acetate extract which was separated by column chromatography. The antibacterial constituents are relatively polar in nature as confirmed by inactivity of hexane extract (non-polar) in contrast to ethyl acetate and methanol extracts (polar) which showed good antimicrobial activity (Table 3). The chromatographic fractions of the ethyl acetate extract had greater antibacterial activity than the crude extracts. This can be seen in the size of the diameter of the zone of inhibition observed for the fractions, even at lower concentrations tested (Table 6). Plant extracts contain several compounds which utilize several mechanisms of action to overcome resistance making it difficult for resistant organisms to develop resistance to them.

Antibacterial activity testing on plants is a critical step in the process of antimicrobial drug discovery. Significant antibacterial activity was observed in *G. arborea* extracts against *K. pneumoniae*, *S. typhimurium*, *A. baumannii*, *P. aeruginosa*, *S. aureus* and *C. freundii* and this was in line with a previous report [21], where *G. arborea* showed significant antimicrobial activities against gram positive and gram-negative organisms, including *E. coli*, *K. pneumonia*, *S. typhi* and *P. mirabilis*. Also, in another report on the extracts of leaves of *Gmelina arborea* [22], the ethyl acetate extract was found to possess the highest antibacterial activity against various organisms. This is in agreement with the results in this present study indicating the relatively polar nature of the antimicrobial constituents. It is noted that the ethyl acetate extract (Table 3) as well as the chromatographic fractions 3, 9 and 12 (Table 6) were active on most of the bacteria including the multidrug resistant (MDR) species shown by the antibiogram (Table 2). It is difficult for microbes to develop resistance to plant extracts and phytochemicals, due to complex nature of phytoconstituents, their multiple antimicrobial mechanisms and combination synergistic effects of the phytochemicals. It was also noted that some of the chromatographic fractions were more active than the parent ethyl acetate extract (Tables 5 and 7); this may be attributed to the separation of the active phytochemicals from inactive phytoconstituents by the chromatographic process involving adsorption and partition mechanisms. Some ‘inactive’ phytoconstituents may mask or inhibit the antimicrobial potential of the active components; this problem is often overcome by chromatographic separation or solvent partitioning.

According to the MIC and MBC of the active extracts, which were ethyl acetate and

methanolic (Table 4), high concentrations (12.5 – 100 mg/mL) of the extracts were required to exhibit the recorded bacteriostatic and bactericidal activities on the test organisms.

Phenols and their derivatives are groups of secondary metabolites in plants and are commonly responsible for the colour of compounds, smell, nutritional values and antioxidant property and antimicrobial activity of the plant [23]. They were found abundant in *G. arborea* roots.

The antioxidant results are not only concentration dependent but also depend on the polarity of the extracts (Table 7). The Total flavonoid content (TFC), Total phenolic content (TPC), Total antioxidant capacity (TAC), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity (ABTS) and Ferric reducing antioxidant power (FRAP), all showed that the polar fractions (Ethyl acetate and methanol) possessed better antioxidant capacity and are rich in phenolics and flavonoids than the non-polar fraction (n-Hexane). The antioxidant results of the methanol and ethyl acetate extracts (Table 7) are comparable with those of standards like gallic acid and quercetin. The Total flavonoid content (TFC), showed ethyl acetate extract to have the value 22.86159 mgQE/g while methanol has 15.39658 mgQE/g (Table 8), these values indicate that the extract and fractions are rich in flavonoids. Flavonoids are secondary metabolites reported to possess antioxidant potentials, the potency of which depends on the number and position of free hydroxyl group present on the structure of the compound [17]. The FRAP antioxidant assay (Table 7) showed that the higher the absorbance, the higher the reducing power of the plant extract.

According to Figure 1, the DPPH radical scavenging capacity of the extracts increased with increasing polarity from n-hexane through ethyl acetate to methanol. Indeed, the results of the DPPH antioxidant activity of the polar extracts were comparable to that of ascorbic acid standard. Generally, it can be deduced that the ethyl acetate and the methanol extract of *Gmelina arborea* have good antioxidant properties than the hexane extract. The current findings further justified the previous report [17] that plant-soluble constituents or metabolites may either be non-polar or polar. The phenolic constituents in plants generally are easily solubilised in polar solvents as a result of the presence of hydroxyl group(s). Therefore, these findings are in line with previously reported work [24] that there was a direct correlation between antioxidant property and antibacterial activity and confirmed that plants with high antioxidant activity usually have high antimicrobial activity as well.

Conclusion. The study has established the antibacterial activity of extracts and fractions of *Gmelina arborea*, thus justifying the trado-medicinal use of the plant in the treatment of infections. This research has also established that the plant contains various antimicrobial and antioxidant phytoconstituents that can be explored therapeutically or as a possible source of compounds to treat various illnesses.

REFERENCES

1. World Health Organization. WHO traditional medicine strategy: 2014-2023. World Health Organization. 2013;1-6.

2. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Journal of Traditional, Complementary and Alternative Medicine. 2013;10:210–229.

3. Daglia M. Polyphenols as antimicrobial agents. Current Opinion in Biotechnology 2012;23:174-181.

4. Srinivasan D, Nathan S, Suresh T, Perumalsamy PL. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine, Journal of Ethnopharmacology. 2001;74:217-220.

5. Duke JA, Wain KK. Medicinal plants of the World Computer Index with more than 85,000 entries, 1983;3.

6. Michael A. Trees, shrubs and lianas of West African dry zones. 2002;11:506.

7. Warrier RR, Priya SM, Kalaiselvi R. Gmelina arborea- an indigenous timber species of India with high medicinal value: A review on its pharmacology, pharmacognosy and phytochemistry. Journal Ethnopharmacology. 2021;267:113593.

8. Kirby MM, Bauer AW, Sherris JC, Truck M. Antibiotic susceptibility testing by a standard single disk method. American Medical Journal of Pathology 1966;45:493-496.

9. CLSI. Clinical and Laboratory Standard Institute: Performance standards for Antimicrobial Susceptibility testing (26th Ed.) Wayne PA: 2016. Supplement M100s.

10. Ayoola GA, Coker H, Adesegun S, Adepoju-Bello A, Obaweya K, Ezennia E, Atangbayila, T. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008;7:3-7.

11. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biologiae et Medicinae experimantalis. 1990;15:113-115.

12. Ohikhena FU, Wintola OA and Afolayan AJ. Evaluation of the Antibacterial and Antifungal Properties of Phragmanthera capitata (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. The Scientific World Journal 2017; Epub 9658598 doi: 10.1155.

13. Idowu PA, Adeniyi BA, Moody JO. Cytotoxicity and Antimicrobial activity of methanol extract and fractions of Entandrophragma angolense (Welw.) C. DC. leaves. Nigerian Journal of Science 2010;44:57-64.

14. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology. 2011;48(4):412-422.

15. Singletonn VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagents. Methods in Enzymology. 1999;299 152-178.

16. Aryouet-Grand A, Vennat B, Pouratt, A, Legret P. Standardization dun extra it de propolis at identification des proncipaux constituents. Journal de pharmacie de Belgique. 1994;49:462-468.

17. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019;8(4):96.

18. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of Antioxidant Potential: Total Phenolics and Flavonoids of different solvent fractions of Monotheca buxifolia Fruit. Osong Public Health and Research Perspectives. 2013;4(5):246-254.

19. Re R, Pellegrini N, Proteggente A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26:1231–7.

20. El-Mahmood M, Doughari JH, Kiman HS. In vitro antimicrobial activity of crude leaves and stem bark extracts of Gmelina arborea against some pathogenic species of Enterobacteriaceae. African Journal of Pharmacy and Pharmacology. 2010;4(6):355-361.

21. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from Indian Journal of Ethnopharmacology. 1997;58(2):75-83.

22. Auxilia A, Francisca P, Ramachandran K, Thendra, R. Assessment of Gmelina arborea Roxb. for their antibacterial activity against clinically important Bacterial pathogens. Journal of Emerging technologies and Innovative Research 2019;6: 407-415.

23. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004;29:1054(1-2):95-111.

24. Fazeli-Nasab B, Rahnama M, Mazarei A. Correlation between antioxidant activity and antibacterial activity of nine medicinal plant extracts. Mazandaran Journal of Medical Sciences. 2017;27:149:63-78.