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Isolation of bioactive constituents from the ethylacetate leaf extract portion of *Gmelina arborea* (Verbenaceae)

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Abstract
The medicinal value of plants has assumed important dimension in the past few decades owing mainly to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with anti-inflammatory, antioxidant and antimicrobial potential. In Nigeria (West Africa), the decoction of the leaf extract of *Gmelina arborea* mixed with trona is taken as a remedy for Malaria fever, Typhoid as well as an antibacterial agent. The main objective of the present research was to carry out preliminary phytochemical and antimicrobial evaluation of the ethyl acetate portion of the leaf extract of *Gmelina arobrea* using standard protocols. Preliminary phytochemical screening revealed the presence of Alkaloids, Flavonoids, Saponins, Phenolic compounds, Triterpenes and Steroids. Compound 1 isolated was determined as 2-(2,4- dihydroxy-5-prenylphenyl)-5,6- methylenedioxybenzofuran, while compound 2 was determined as 5,7,2',4'-tetrahydroxy-8-lavandulyl-5'- methoxy flavone. Determination of the antimicrobial activity of the ethyl acetate portion on some isolates was conducted. The zone of inhibition from the antibacterial activity from the ethyl acetate portion was found to be fairly good against gram (+) bacteria e.g *Staphlococcus aureus*, *Bacillus subtilis* and gram (-) bacteria e.g *Klebsiela pneumonia* and *Escherichia coli*. The antifungal activity of the ethyl acetate portion was found to be active against *Aspergillus niger*, *Fusarium moniliforme* and *Fusarium oxysorum.* This study supports the folkloric claims for the use of *Gmelina arborea* against some bacterial caused infections.

Keywords: phytochemistry, antimicrobial, leaf, *G. arborea*, verbenaceae

Introduction
Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effect. The research into plants with alleged various folkloric uses in the cure of various ailments should therefore be viewed as fruitful and logical for the treatment of various ailments. The World Health Organization (2010) recognized many herbal remedies and hence encouraged the developing countries to incorporate the use of folkloric plants for obvious reasons of their availability and affordability apart from being effective alternative to allopathic practice [1]. Medicinal plants are those in which one or more of its organs contains substance that can be used for therapeutic purposes or which are precursors of the synthesis of useful drugs [2]. Infectious diseases have been observed to be the major cause of death worldwide. This might be due to poverty and incidence of drug resistance [3]. Pharmaceutical research in natural products represents a major strategy for discovery and developing new drugs. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient time. The use of active principles from plants as an antimalarial agent and treatment of other related ailment is well documented traditionally. The fascination of natural products mostly used as a preparation from plant with known medicinal properties goes back to ancient times [4].
Flavonoids are known to be associated with reduced risk for certain chronic diseases [5, 6]. These include the prevention of cardiovascular disorder [7] and other kinds of cancerous processes [8, 9]. Flavonoids exhibit antiviral properties (10/11), anti-microbial [12, 13], anti-inflammatory activities [13], anti-ulcer [14] and anti –allergic properties [15]. Flavonoids are mostly found in fruits, vegetables and cereals [16].
Gmelina arborea Roxb. (Verbenaceae) is native to Asia and known by various names, e.g. Yemane, Gamar and Gumhar. This species has been introduced in several countries, particularly in West Africa and especially in Nigeria and Côte d'Ivoire among others. Ethno botanical studies report that the species is widely used to treat many diseases including diarrhea, hypertension and malaria, among others.

A recent study of the composition of secondary metabolites of *G. arborea* showed its richness in phenolic compounds and natural antimicrobial and antioxidant substances that play an important role in pharmacology [17]. *Gmelina arborea* exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. Thus, *Gmelina arborea* is well anchored in its traditional uses and has now found wide-spread acceptance across the world. In Nigeria (West Africa), the decoction of the leaf extract of *Gmelina arborea* mixed with trona is taken as a remedy for malaria fever, Typhoid as well as an antibacterial agent. The cold ethanol leaf extract of *Gmelina arborea* has shown high Antihypertensive property at different concentration dose dependent on rabbit jejunum as well as strong immunomoduratory agent [18]. So also the aqueous leaf extract of *Gmelina arborea* has been used as a good remedy for gonorrhea and other forms of venereal diseases [19].

Consequent upon this, the research work was aim at isolating an active principles from the ethyl acetate portion of the leaf extract of *Gmelina arborea* (verbenaceae) with a view to ascertain some of the medicinal claims of the folkloric healers using standard chromatographic, spectroscopic and biological techniques.

Material and Methods

Experimental

General experimental procedure: All melting points (mp) were determined on Gallenkamp melting point apparatus and results are uncorrected. All solvents of analytical grade were purchase from BDH chemical Ltd. Poole England. TLC analysis was carried out using cellulose (TLC) and Silica gel 60 F_{254} (TLC) plates (Merck, Darmstadt, Germany). Polyamide (Roth, England) and Sephadex LH_{20} (Fluka, Switzerland) were used for open column chromatography (CC). Chromatographic system: TLC: Visualization of the plate were performed using visible light U.V fluorescence and or spraying with the following reagent i=2% $AlCl_3$ and ii= 10% H_2SO_4 by heating at 110^{o}c for (5 – 10min). Column chromatography (CC): Chloroform, Chloroform/Ethyl acetate mixture, Ethylacetate, Ethylacetate/ methanol and methanol 100% was used base on increasing gradient polarity system. UV spectra were recorded on specord 40 UV –VIS spectrophotometer (Jena Analytik AG Germany) and HREI-MS was measured on Finnigan MAT GCQ and JOEL JMS-700. Optical rotations were measured with JASCO DIP polarimeter while IR spectra were measured on a Nicolet Avalar 320 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR experiments were performed on Bruker spectrometer 400MH$_Z$ for ^{1}H and 100MH$_Z$ for ^{13}C NMR. Spectra were referenced to the CD_3OD solvent, signals at δ3.30 (`H) and 49.00 (^{13}C) with TMS as an internal solvent standard. Chemical shift – values (δ) were reported in parts per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J – values) were given in Hertz – TOF spectrometer.

Tests for Antimicrobial

Antimicrobial activity of the ethyl acetate portion leaf extract of *G. arborea* was investigated against the following clinical isolates which includes the following bacterial species *Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Bacillus anthracis, Staphlococcus aureus Bacillus subtilis and Streptococcus pyogenes* while the fungal species are *Penicillium digitatum, Penicillium notatum, Fusarium oxysorum, Aspergillus niger, Aspergillus fumigates, Rhizopus oligosporus and Fusarium moniliforme* which were obtained from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Collection of Plant Materials

Leaf portion of *Gmelina arborea* (Verbenaceae) was collected from a farm land in Bomo, a village outskirt of Zaria in Kaduna State of Nigeria in the month of October, 2019. Botanical identification of the plant was performed at the herbarium section of the Department of biological science, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen No. (DC 8643) was deposited.

Extraction and Isolation

Leaf part of *Gmelina arborea* plant was obtained, cut and sliced into pieces. This was then air dried at room temperature for 7 days after which the material was crushed into powder to afford 700g. The powdered material was then subjected to cold maceration techniques at room temperature with MeOH (3.0 L) at 45 ^{0}C for 48hrs with intermittent agitation. The extract was concentrated to dryness under reduced pressure to afford a semi solid material. This was further re-suspended in water (600ml) and partitioned successively with n-hexane, (3×500ml), Ethyl acetate (3×400ml) and N-butanol (5×400ml). The various extracts were concentrated using rotary evaporator to afford n-hexane, (3.52g), ethyl acetate (4.14g), n-butanol (5.22g) and aqueous residue (6.33g) respectively. The various fractions of the extract were subjected to phytochemical screening using standard protocols [20, 21].

The ethyl acetate soluble portion (2.5g) was subjected to column chromatography on a silica gel (70-230 mesh) and the column was eluted with gradient solvent system of n–hexane (100%,400ml), Chloroform/EtOAC (10:1-1:5) and EtOAC/ MeOH (10:1-1:5). Similar fractions from each portion were pooled together on the basis of TLC analysis to afford 62 fractions of 20ml aliquot (F_1 –F_{62}). Fractions ($F_{8\text{-}24}$= 150mg) was further re-subjected to repeated gel filtration techniques using sephadex $LH_{\text{-}20}$ and RP – 18 column chromatography using (100% Methanol) as an eluting solvent to obtain compound 1 (22mg, R_f 0.6).

Fraction (F_{26}- F_{57}) with the same TLC pattern were also resubmitted to repeated gel filtration techniques using sephadex $LH_{\text{-}20}$ and RP – 18 column chromatography using (100%) as an eluting solvent to afford 18 (B_1-B_{18}) smaller fractions of 5ml aliquot with three homogeneous spots. Fraction (B_3-B_{12}) was further subjected to PTLC analysis. PTLC was carried out using Fluka silica gel precoated glass plates 20×20cm with layer thickness of 0.25mm. A thin line of about 1.5cm from the bottom of the plate was drawn with a pencil. Pooled sample of (B_3-B_9) was dissolved in methanol solvent to give an approximate concentration of 20mg/ml. It was then applied uniformly along the thin line using capillary tube. The plate was allowed to dry after which it was developed using an appropriate solvent system. The developed plate was air dried in a fume cupboard and the position of the band of interest was marked with pencil and scraped off the back of the plate on to a foil. The scraped sorbent was size reduced using pestle and mortar, transferred to a sintered glass funnel and washed repeatedly with Acetone 100% and the solution obtained was evaporated to give the isolated compound (19mg) and was tag as compound 2. The progress of elution was monitored by TLC analysis using

precoated plate in different solvent system of N-hexane: Ethylacetate (80:20), Chloroform: Ethylacetate (65:45) and EtOAC: MeOH (70:30). The chromatogram was spread with 10% H_2S0_4 and kept in an oven at temperature of 105 ^{0}C for 5min after which it was removed to ascertain the compounds on the plate [20].

Antimicrobial study of Ethyl acetate portion of the leaf extract

The antibacterial and antifungal activity of the Ethyl acetate portion of the leaf extract was tested at various dilutions using methylene glycol as solvent at a concentration of 6mg/ml at phosphate-buffered saline (w/v). The various bacterial species were first incubated at 40^{o}c for 48hrs [22, 23]. The zone of inhibition were recorded at 34 $\pm$ 1^{o}c for 48hrs for bacterial and 33 $\pm$1^{o}c after 24hrs for fungi [24, 25]. The antimicrobial activity was determined by the whatman No. 1 filter paper disc (6mm) method. Paper disc were soaked with various samples tested and were dried at 50^{o}C [26]. The discs were then kept on soft nutrient agar (2%) petri dishes previously seeded with a suspension of each bacterial species. For the fungus, petri dishes were placed on Sabouraund's booth [27, 28] medium (1%). The zone of inhibition was expressed as an average of maximum diameter in the result from the table 4.

Results

The Phytochemistry results revealed the presence of Tannins, Saponin, Sterols, Flavonoids, Alkaloids, Cardiac glycosides, Terpenoids and Flavone glycosides (Table. 1). Compound 1 isolated from the ethyl acetate portion of the extract was determined as 2-(2, 4- dihydroxy-5-prenylphenyl)-5, 6-methylenedioxybenzofuran, while compound 2 was determined as 5, 7, 2', 4'-tetrahydroxy-8-lavandulyl-5'-methoxy flavone (fig.1 and 2).The antimicrobial properties of the ethyl acetate portion of the leaf extract was found to be active against some gram (+) and gram (-) bacteria while the extract was also found to be active against three fungus as could be seen in table 4 and 5 below.

Table 1: Preliminary Phytochemical screening of the Leaf extract of *Gmelina arborea*

Constituents	**Test**	**Observation**	**Portions of extracts**					
Carbohydrate			**H_E**	**M_E**	**CL**	**Eta**	**n-But**	**Aq**
General Test	Molisch	Red colouring	-	+	-	-	-	+
Sugar Test	Aniline	Red colour	-	-	-	-	-	+
Sugar (Monosaccharide)	Barfoed's	Red ppt	-	+	-	-	-	+
Red. Sugar	Fehling's	Red ppt	-	+	-	-	-	+
Tannins	Lead Ethanoate	White ppt	-	+	-	+	+	-
	Iron (III) Chloride	Blue – Black	-	+	-	+	+	-
	Ethanoic acid	White ppt	-	+	-	+	-	-
	Methanol's	Red ppt	-	+	-	-	+	-
Saponins Sterols	Frothing	Persist frothing	-	+	-	+	+	-
	Lieberman B.	Blue or green	+	+	+		+	-
Saponin Glycoside	Fehling's Solution	Red ppt	-	-	-	-	-	-
	Tetraoxosulphate(iv) acid	Brick red	-	+	-	+	+	-
Phlobatannins	Hydrochloric Acid	Red ppt	-	-	-	-	-	-
Carotenoids	Carr price's	Blue to red colour	-	+	-	+	-	-
Emodol	Borntrager's	Red colour	-	-	-	-	-	-
Flavones aglycones	Shibata's	Red to Orange	-	+	-	+	-	-
Terpenoids	Liebermann B.	Pink to Red colour	+	+	+	-	-	-
Alkaloids	Mayer's Wagner's Dragendorff's	Buff ppt Dark brown ppt Redish brown	- -	+ +	+ -	+ +	+ -	- -
Flavonoids	Shinoda	Deep red	-	+	-	+	+	-
	Tetraoxosulphate (vi) acid	Deep Yellow	-	+	-	+	-	-
Cardiac glycoside	Legal's	Deep red colour	-	+	-	+	+	+
	Kedd's	Violet colour	-	+	-	+	+	-
	Keller – kilanis	Reddish brown	-	+	-	+	+	+
	Baljet	Orange to Deep red	-	+	-	+	-	-
	Lieberman	Bluish green	-	+	+	+	+	-

Key: - = Absent, + = Fairly Present.

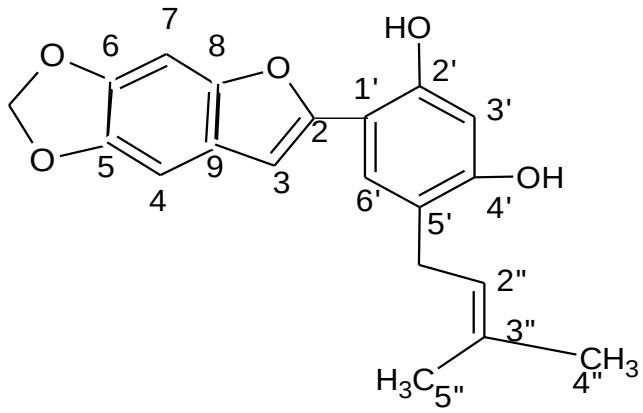
H_E=n-Hex, M_E=Methanol, CL=Chloroform, Eta=Ethyl acetate, n-But=n-Butanol, Aq=Aqueous

Table 2: ^{1}H NMR and ^{13}C NMR spectral data of componud 1 in CD_3OD. (400_{mhz}-^{1}h and 100_{Mhz} for ^{13}c NMR). multiplicity and coupling constant (J, Hz) δ(ppm)

S/no	$\delta_{H\ (ppm)}$	$\delta_{C\ (ppm)}$
1.	----	----
2.	----	154.3
3.	7.16(IH, s)	104.6
4.	7.10(IH, s)	94.02
5.	----	145.20
6.	----	146.30
7.	7.03(IH, s)	99.70(CH)
8.	----	149.50
9.	----	124.30
1'	----	110.8
2'	----	153.8
3'	6.62 (1H, s)	104.2
4'	----	156.3
5'	----	121.3
6'	7.72 (IH, s)	127.4
1"	3.35(2H, d, j=7.5Hz)	29.2
2"	5.35(IH, m)	124.5
3"	----	132.4
4"	1.75(3H, s)	25.7
5"	1.77(3H, s)	18.3
OCH_2O	6.05 (2H, s)	102.4

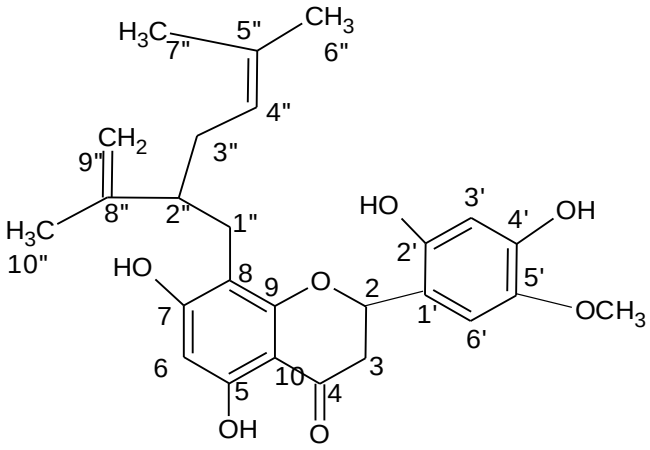
Table 3: ^{1}H NMR and ^{13}C NMR spectral data of compound 2 in CD_3OD. (400_{MHZ}-^{1}H AND 100_{MHZ} for ^{13}C NMR). Multiplicity and coupling constant (J, HZ) δ(ppm).

S/no	$\delta_{H\ (ppm)}$	$\delta_{C\ (ppm)}$
1.	----	----
2.	5.62 (IH,d,d,j=2.5 13.0Hz)	74.5(CH)
3.	β 3.08(IH, d, d, j=13.0 17.0Hz)	42.5(CH_2)
	α 2.55 (IH,d, d,j=2.5, 17.0Hz)	
4.	---------	196.8
5.	12.40(IH. s)	162.5
6.	5.84(IH, s)	94.8
7.	------	167.01
8.	----	102.20
9.	----	162.40
10	----	107.80
1'	----	115.7(C)
2'	----	149.30
3'	6.35(IH, s)	103.60(CH)
4'	----	148.7
5'	3.68(3H, s, OCH_3)	142.2
6'	6.98(IH, s)	112.01
1"	2.54(2H, m)	26.7 (CH_2)
2"	2.49 (1H, m)	46.8 (CH)
3"	2.05 (2H, m)	31.5 (CH_2)
4"	4.96 (1H, t, j = 2.5Hz)	124.0 (CH)
5"	------	131.02
6"	1.44 (3H, S)	17.3 (CH_3)
7"	1.50 (3H, S)	25.2 (CH_3)
8"	------	148.7
9"	(a) 4.47 (1H, d, j = 1.0Hz)	110.5 (CH_2)
	(b) 4.43 (1H, br s)	
10"	1.58 (3H, d, j = 1.0 Hz)	18.2 (CH_3)
OCH_3	------	56.4 (CH_3)



Compound 1

Fig 1: 2-(2, 4- dihydroxy-5-prenylphenyl)-5,6-methylenedioxybenzofuran



Compound 2

Fig 2: 5,7,2',4'-tetrahydroxy-8-lavandulyl-5'- methoxy flavone

TLC profile of ethyl acetate leaf fraction showing various spots.

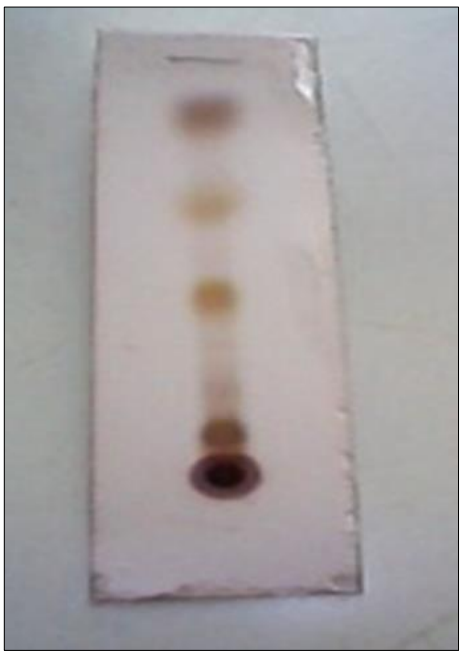


Fig 3: Chromatogram of ethyl acetate leaf fraction using n- hexane: chloroform: methanol (3:3:1) and 10% H_2SO_4 spray reagent.

Table 4: Antibacterial activity of ethyl acetate fraction of the leaf extract. Diameters of Zone of Exhibition (mm)*

Bacterial species	Ethylacetate Fraction	1:4	1:8	1:12	1:16
(-) *Proteus vulgaris*	13.2	10.4	8.2	6.5	0
(-) *Peudomonas aeruginosa*	12.5	11.5	9.8	7.8	0
(-) *Klebsiela pneumoniae*	22.5	15.8	10.3	9.5	7.2
(-) *Escherichia coli*	21.5	20.2	18.7	15.4	8.0
(+) *Bacillus anthracis*	10.5	8.3	7.1	4.0	0
(+) *Staphylococcus aureus*	23.5	19.2	18.2	14.5	10.6
(+) *Staphylococcus pyogenes*	19.5	15.1	12.1	9.3	6.8
(+) *Bacillus subtilis*	20.3	18.4	15.4	12.0	8.6

*The zone of inhibition (mm) taken as average of four determinations in four different directions and whatman No. 1 filter paper (6mm) were soaked with each sample tested for their activity concentration of mg/ml of PBS (w/v)

Table 5: Antifungal activity of ethyl acetate fraction. Diameters of Zone of Exhibition (mm)*

Fungal species	Ethyl acetate Fraction	1:4	1:8	1:12	1:16
Penicillium digitatum	9.2	8.2	6.3	2.1	0
Penicillium notatum	5.0	3.1	1.0	0	0
Fusararium oxysorum	13.0	10.3	8.2	6.1	4.3
Aspergillus niger	15.3	9.2	8.0	6.3	5.2
Aspergillus fumigatus	9.2	7.1	5.0	3.1	2.0
Aspergillus terreus	8.5	6.2	4.1	3.0	1.3
Trich viride	5.0	4.1	2.1	0	0
Rhizopus oligosporus	10.5	3.2	2.0	1.0	0
Fusarium moniliforme	7.5	5.2	4.0	3.8	2.0

*The zone of inhibition (mm) taken as average of four determinations in four different directions and whatman No. 1 filter paper (6mm) were soaked with each sample tested for their activity concentration of mg/ml of PBS (w/v)

Discussion

Compound 1

Compound 1 was obtained as an amorphous brown solid. The molecular formula was established as $C_{20}H_{18}O_5$ by HREIMS and the m.p point was determined as 246 – 248 ^{0}C. The IR spectrum of compound 1 showed the presence of hydroxyl group (3353 cm^{-1}), an aromatic ring at (1617, 1608, 1498 cm^{-1}) and methylenedioxy (1035, 936 cm^{-1}) groups [29]. The UV spectrum shows an absorption maximum at λ_{max} 322 and 347 nm which was in conformity with 2 –phenylbenzo furan skeleton [30]. The ^{1}H NMR spectrum of compound 1 shows four aromatic singlet signals at 7.16ppm, (IH,s), δ_H 7.10ppm (IH,s), - δ_H 7.03ppm (IH,s), and δ_H 7.72ppm IH,s [31]. The signals observed at δ_H 1.75ppm (3H, s), δ_H 1.77ppm (3H, s), δ_H 3.35ppm (2H, d, j = 7.5 Hz) and δ_H 5.38ppm (IH, m) were all in conformity with 3, 3 –dimethyl group [32]. In the HMBC spectrum, a cross – peaks were observed between H–3 and C–1', C– 4 and C – 8 between H – 6' and C–2, C–2', and C– 4' and C–1" and between H – 1" and C– 4', C– 6' and C– 3' all indicated the linkage by the 3, 3 – dimethylallyl group to C–5' (33). From the above result, compound 1 was determined as 2 - (2, 4-dihydroxy -5-prenylphenyl) – 5, 6 – methylenedioxybenzofuran.

Compound 2

Compound 2 was also isolated as a brown amorphous solid, $(\propto)^{25}\Delta$ + 50 (C – 0.1, CH_3OH); (Log ϵ) 294 (4.41), 232 (Sh), 203 (4.85) nm. IR (KBr), V_{max} 3395, 1636, 1518, 1452, 1376, 1309, 1198, 1157, and 1108 Cm $^{-1}$. The HREIMS of compound 2 indicated a molecular ion peak at m/z 454.1990, which corresponded to the molecular formula $C_{20}H_{30}O_7$, while the m.p was determined at 238-240 ^{0}C [34]. Absorption observed at (3395 cm^{-1} and 1636 cm^{-1}) in IR Spectrum were all due to hydroxyl and carboxyl group [35]. The ^{1}H NMR spectrum of compound 2 showed resonances for a methoxy group at δ_H 3.67ppm and a chelated hydroxyl proton was observed at δ_H 12.40ppm while signals observed at δ_H 2.55ppm (1H, d, d, j = 2.5, 17.0 H_Z, H – 3∝), δ_H 3.08ppm (1H, d, d, j = 2.5, 13.0 H_Z, H – 2) could all be attributed to a typical flavones group (36). The signals exhibited at δ_H 1.44ppm (3H, s, H_3 – 6"), δ_H1.50ppm (3H, s, H_3 – 7"), δ_H 1.58ppm (3H, d, j = 1.0H_Z, H_3 – 10"), δ_H 2.05ppm (2H, m H_2 – 3"), δ_H 2.49ppm (1H, M, H – 2") δ_H 2.54ppm (2H, m, H_2 – 1"), δ_H 4.43ppm (1H brs, H – 9"b), δ_H 4.47ppm (1H, d, j = 1.0 H_Z, H – 9"b) and δ_H 4.93ppm (1H, t, j = 2.5 H_Z, H – 4") could all be attributed to lavandulyl moiety [37]. The HMBC correlation of the hydroxyl group OH –5 (δ_H 12.40ppm / C_6, C_{10} and H-1"/C – 7, C - 9) clearly revealed that the lavandulyl group is linked to C – 8 (37). More so, it was observed that, the two singlet signals at δ_H 6.35ppm (1H, s, H – 3') and δ_H 6.98ppm (1H, s, H –6) suggested that the B ring of compound 2 is 1, 2, 4, 5 tetra substituted [38]. In the NOESY experiment, the irradiation of the methoxy group at δ_H3.68ppm (3H, s) causes the enhancement of H – 6' (δ_H 6.98ppm) correlating with C – 2 in the HMBC spectrum. Thus, the linkage of methoxy group to 5' was confirmed [39, 40]. On the basis of these evidence, compound 2 was determined as 5, 7, 2', 4'-tetrahydroxy – 8 – lavandulyl -5 – methoxy flavone.

Steroids found in the Phytochemistry of the leaf extract of *Gmelina arborea* as secondary metabolites are found to increases nitrogen level in the body there by producing protein that helps in the production of muscles. Steroids were found to enhance metabolism and thus inhibit the accumulation of fat, adjust disorders like aneamia by increasing the production of red blood cells in the treatment of arthritis, asthma, brain injury and other type of cancer [41]. However, steroids play an important role in the body as it enhances the onset and progression of cardiovascular and liver diseases as well as acne (by stimulating the serum to produce oil). In the present study, steroid was found to be present in n -hexane, methanol and ethyl acetate extract which could be preferred in the management of cardiovascular and liver ailment [42].

Flavonoids are ubiquitous in plants, they are known to be common part of human diet and could significantly inhibit microbes which are known to be resistance to the conventional antibiotics [43]. Some of the recently isolated flavonoids were reported to exhibit antimicrobial activities [44]. In addition, flavonoids through their free – radical scavenging activity have evoked multiple biological functions which include anti-inflammatory, anti-bactericidal, anti-carcinogenic, Vasodialotory, immune stimulatory, anti-allergic and anti-viral functions [45]. Consequent upon this, the presence of flavonoids in the ethyl acetate and n-butanol extract in our study has exhibited the therapeutic efficacy of the extract and has accounted for the folkloric use of the plant in the treatment of related ailment especially the inhibitory effect of *S. aureus*, *S. typhi*, *E. coli* and *P.aeruginosa.*

Tannins are known to be antimicrobial agent that tends to inhibit the growth of microorganism by precipitating out the microbial protein and thus depriving them of development [46]. In the present study, tannins were detected in the methanol and n-butanol extract fraction of the extract. This can also explain the comparatively better antimicrobial activity of the extract on tested pathogens [47]. This seems further to highlight the limitation of ethyl acetate, n-butanol and residual aqueous portion of the extract in traditional management of those ailment which include diarrhea,

inflammation of the mouth and throat that were reported to be treated with tannins [48]. The presence of carbohydrate and reducing sugars in the leaves extract indicate high energy content of the leaves that could be exploited as a source of edible food or raw materials for industries that utilized carbohydrates reducing sugars to produced food, drugs or biodiesel. However, the antifungal activity of saponin has been reported [49], though saponins has been detected in the methanol extract but was found not to elicited antifungal activity against the fungi especially *C. albican.* This might be due to the tested concentrations of the solvent extract and as such were not lethal enough to elicit a measurable fungi static effect [50].

The antibacterial activity of the ethyl acetate fraction of the leaf extract was found to be fairly good against gram (+) bacteria e.g. *Staphylococcus aureus,* and *B. subtilis* and gram (-ve) bacteria e.g. *Klebsiella pneumonia* and *E. coli*. The antifungal activity of ethyl acetate fraction of the leaf extract was found to be greater activity against *A. niger, Fusarium oxysorum and Fusarium moniliforme.*

Conclusion

The study was able to validate the therapeutic potency of the nutraceuticals embedded in the mid polar region of the leaf extract of *Gmelina arborea*. The ethyl acetate portion of the leaf extract was found to possess some secondary metabolites and demonstrated significant antimicrobial activity against some tested clinical isolates both gram positive and gram negative thus, support the ethno medicinal claim by the traditional healers for the treatment of microbial based infections. This could now be concluded that, the result obtained from this study shows that *Gmelina arborea* possess compounds with antimicrobial properties that can be used as antimicrobial agents in the production of drugs for the therapy of infectious diseases caused by some of the aforementioned pathogens.

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