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Phytochemical, Antioxidant and Antimicrobial studies of *Lannea egregia* Engl. & K. Krause (Anacardiaceae) extracts and chromatographic fractions

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ABSTRACT

Screening 'new' medicinal plants of traditional importance for bioactive components is a sure way of discovering novel therapeutic agents to treat diseases. This study, therefore investigated the presence of phytochemical, antioxidant and antibacterial components of the extracts of *Lannea egregia*. Phytochemical screening was done by standard methods. Antibacterial activity of the extracts of *Lannea egregia* was determined by agar well diffusion method while the minimum inhibitory concentration (MIC) was determined by agar dilution method. The antioxidant capacity of the crude extracts was determined through the evaluation of total flavonoid content, total phenolic content, ferric reducing power, total antioxidant capacity and 2,2-diphenyl-1-picryhydrazyl. The phytochemical screening of the different parts of this plant revealed the presence of tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, emodins, phlobatannins, anthocyanins, coumarins and phenolics. Phlobatannins was observed to be absent in the stem bark. The crude extracts obtained from the leaves, stem bark and roots of this plant exhibited good antibacterial activity against typed strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. The diameter of the zone of inhibition ranged from 9.0 to 26.0 mm at 100 mg/mL for all the plant parts. The ethyl acetate leaf extract of this plant possessed the highest antibacterial activity with MIC and MBC values of the range of (3.125 to >50 mg/mL) and (12.5 to >50 mg/mL) respectively. The zone inhibition of the chromatographic fractions of both plants ranged 15-23 mm. Antioxidant study of the extracts of the leaf of *L. egregia* revealed that the ethyl acetate and methanol extracts have good antioxidant potentials comparable to that of ascorbic acid control. This study has revealed that the extracts from different parts of *L. egregia* possess good antibacterial and antioxidant activities which could be a function of the various phytochemicals detected in the plant.

Keywords: *Lannea egregia*, Phytochemical, Antibacterial, Antioxidant, Column chromatography.

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Introduction

Medicinal plants are of great therapeutic value as they are commonly employed in traditional medicine for the treatment of infections and diseases. The wide use of medicinal plants in traditional medicine to treat diseases encourages research studies to verify claims and develop alternative therapeutic approach in the management of human health [1]. Many ethnomedicinal plants have been investigated and reported to possess antibacterial, antifungal, antiviral, antiprotozoal, anticancer and other bioactive properties; hence, their significance in the traditional management of infections cannot be overemphasized. They can be used for a variety of purposes; as complementary and prophylactic medicines [2].

The search for antimicrobials from natural sources has received so much attention that efforts are being made to identify lead compounds that can serve as precursors to developing antimicrobial agents that are not only effective but also active against drug resistant pathogens [3]. The lingering challenge posed by the emergence of drug resistant microbes has encouraged the wide use of medicinal plant extracts as an alternative therapeutic option to combat these issues of public health concerns. Presently, the rate of development of drug resistance in pathogenic microbes worldwide is alarming. The probable cause of this maybe due to the indiscriminate and irrational use of chemotherapeutic agents. The consequences of antibiotics abuse include development of resistance, with accompanied therapeutic failure, morbidity and mortality. It is therefore imperative to source for novel and effective antimicrobial agents or enhance the effectiveness of the existing ones against such resistant pathogens. This justifies the extensive researches being carried out on medicinal plants [4].

Lannea egregia Engl. & A. Krause (Anacardiaceae) is a tropical West-African plant known as 'ekudan' (Yoruba in Nigeria), 'sambituliga' (Ivory Coast) and 'tiuko' (Guinea) [5]. However, it is important to note that in some regions of West Africa (notably in Guinea, Ivory Coast and Dahomy) *L. egregia* share the same local name and uses with a closely related species called *Lannea barteri* [6, 7]. The plant is a shrub of about 3 - 13 meters tall, having a spirally grooved bark and with leaves that are hairy, usually occurring in pairs of 3 to 5 with one at the terminal end [8].

In traditional medicine, the genus *Lannea* is locally used to treat infections and diseases. The root and stem bark of members of this genus are used in traditional medicine to treat infections such as diarrhea, stomach pains, rheumatism, gonorrhea, rachitism, chest pain, gastric ulcer, skin and respiratory tract diseases [9]. Other species in this genus, particularly *Lannea welwitschii* is used in traditional medicine to treat wound infections [10] and was reported to possess phytochemicals with antimicrobial properties [11]. *Lannea egregia* is used in Nigeria in the treatment of cancer, menstrual disorders and postnatal problems [12, 13]. The decoction from the leaves of this plant is used in traditional medicine to treat migraine and malaria [14]. The secondary metabolites or phytochemical constituents produced by a plant determine its biological activities and therapeutic potentials. Plants with antioxidant capacity are important in human health as they scavenge and remove free radicals and reactive oxygen species (ROS), which are often implicated in disease processes, from the body systems [15]. Medicinal plants that are rich in phytochemicals and possess both antimicrobial and antioxidant activities could be of therapeutic value in the treatment of infections, and this was the cardinal motivation for this study.

Methodology

Collection of plant

The leaves, stem bark and roots of *Lannea egregia* were collected in June 2018 from Olokemeji Forest Reserve (Latitude: 7° 25' north, Longitude: 3° 32' east) in Ogun State, Nigeria. Authentication of the plant was done at the Forest Research Institute of Nigeria (FRIN), Ibadan herbarium unit where voucher specimen numbered FHI-111937 was deposited.

Extraction of the plant material

The plant parts (700 g of leaves and 200 g of stem bark) were air-dried, ground to coarse powder and extracted in a Soxhlet apparatus with successive solvents selected in the order of increased polarity (n-hexane, ethyl acetate and methanol). The extracts were then concentrated in rotary evaporator and stored at 4^{o}C for use.

Collection of isolates

Typed cultures: *Staphylococcus aureus* NCTC 6571, *S. aureus* ATCC 29213, *Bacillus substilis* ATCC 6638, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *E. coli* 35218 used in this study were collected from the Molecular laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. Validation of samples was done by conventional cultural and biochemical methods (Gram staining, growth on selective media and biochemical tests carried out on each organism).

Antibiogram Determination

The antibiogram profile of the test isolates was determined using the Kirby-Bauer disc diffusion susceptibility test technique with strict adherence to the guidelines stipulated by the Clinical laboratory Standard Institute (CLSI) [16]. Multi-disc antibiotics (Abtek Biologicals), comprising eight antibiotics commonly used for Gram positive bacterial infections was used for this analysis. The concentrations of these standard antibiotics as impregnated on the respective discs were: ceftazidime 30 µg, cefuroxime 30 µg, gentamicin 10 µg, ceftriaxone 30 µg, erythromycin 5 µg, cloxacillin 5 µg, ofloxacin 5 µg and augmentin 30 µg. The turbidity of an overnight broth culture of the isolates was compared to that of 0.5 McFarland standards (1.0 x 10^{8} cfu/mL). An inoculum of the test organism was swabbed on the entire surface of freshly prepared Mueller Hinton agar plates containing 2-4% NaCl. The culture was allowed to soak in for 10 min, and then the antibiotic disc was aseptically placed on the inoculated agar surface and was incubated for 24 hours at 37^{o}C. Susceptibility was determined by the measurement of clear zone of inhibition around the antibiotics with adherence to the standard provided by CLSI for result interpretation.

Phytochemical Screening

The plant samples were tested for the presence of the following 15 phytochemicals: terpenoids, alkaloids, saponins, tannins, phlobatannins, flavonoids, phenols, anthraquinone glycosides, cardiac glycosides, steroids, emodins, carbohydrates, proteins, coumarins and anthocyanins using standard procedures as described by [17, 18].

Determination of the antimicrobial activity of the extracts

The antimicrobial activity of the crude plant extract was determined by agar-well diffusion method [19]. Dilution of overnight phase broth culture of the bacterium to an optical density equivalent to that 0.5 McFarland standard was done. A 0.1 mL of the diluted broth culture was seeded to sterile molten Mueller Hinton agar. The seeded agar was poured into plates and allowed to set. The surface of the agar was dried in a freshly disinfected incubator at 37^{0}C for 30 minutes. A standard cork borer (6 mm in diameter) was used to bore holes equidistant from each other on the agar surface. One hundred microlitre (100 µL) of different concentrations of the extract dissolved in 20% dimethyl sulfoxide was placed in each hole. The inoculated plate was allowed to stand on the bench for 1-hour prediffusion period after which the plates were

then incubated for 24 hours at 37°C. Occurrences of zones of inhibition depicted susceptibility to the plant extract. Since extracts were reconstituted in dimethyl sulfoxide, the diluent was used as a negative control while a chemotherapeutic agent (vancomycin at 30 µg/mL) was used as positive control.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the crude extracts was determined following the method stipulated by Adeniyi and Ayepola [20]. Concentration as high as 10 times the actual test concentration of the extracts was prepared. About 18 mL of homogenized molten Mueller Hinton agar was dispensed and sterilized in McCartney bottles. The agar was allowed to cool to an appropriate temperature that will not denature the active components of the extract before the introduction of 2 mL of the already prepared concentrations of the extract was added to the respective agar in the McCartney bottles clearly labelled according to their concentrations. The mixture was swirled gently for homogeneity and the content was poured into sterile Petri dishes. The plates were left on the bench to set. A suspension of each isolate was made in 0.85% normal saline to match the turbidity of 0.5 McFarland standard and was inoculated by streaking onto the plates. The plates were allowed to stand on the bench for pre-diffusion period of 1 hour after which they were incubated for 24 hours. The plates were then observed for microbial growth and the lowest concentration of the extract inhibiting the growth of the test organisms was recorded as the MIC. For the standard antibiotics (vancomycin), the MIC was determined using broth microdilution technique following CLSI guideline. Suspensions of the test organisms with turbidity equivalent to that of 0.5 McFarland Standard (1.5×10^8 cfu/mL) were inoculated into broth microdilution plates already containing different dilutions of the antibiotics in 100 µL of Mueller Hinton broth. The plates were incubated at 37 °C for 24 hours. The plates were examined for growth by the addition of tetrazolium salt. A change in colour to red or pink is indicative of growth of test organism. The lowest concentration of the antibiotics showing absence of growth is taken as the MIC. Two columns of the titre plate containing broth and antibiotics and broth alone were used as positive and negative control respectively.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by inoculating freshly prepared Mueller Hinton broth in test tubes with inoculum from the plates showing no visible growth. The tubes were then incubated at 37 °C for 24 hours. The tube with the lowest concentration of the extract showing absence of the growth is recorded as the MBC. For the standard antibiotics, the inoculum from titre wells having no change in colour was plated out on freshly prepared Mueller Hinton agar plates. The plates were clearly labelled according to the concentrations of the antibiotics present in the titre wells. The minimum concentration with no visible growth is taken as the MBC.

Bioassay-guided Fractionation

Thin layer chromatography

The constituents of the crude extracts were separated preliminarily by thin layer chromatography. This was done by spotting the extract on pre-coated silica gel plates and placing the spotted plates in various solvent

systems (mobile phase), selecting ones that best separated the extracts into individual constituents. The plates were viewed under UV light at wavelength of 325 nm and iodine tank [21].

Column chromatography

Column chromatography was used to separate the extract into different fractions; silica gel of mesh size 60-120 was used for the fractionation. The dimension of the column is such that the length is 24 times the diameter (3 cm). The column was packed using the slurry method; silica gel was mixed with the mobile phase (n-hexane) and poured into dry glass column clamped in a vertical position. As the solid stationary phase settles down, the column is gently tapped with a spatula until required height was attained. The sample (4 g) was dissolved in a volatile solvent and mixed with a little silica gel to form dry slurry. The sample was then loaded into the column and elution was done with a series of solvent mixtures selected from *n-hexane*, ethyl acetate and methanol. The fractions were collected, concentrated, monitored by TLC and pooled together based on their R_f values [22, 21].

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 as described by Idowu *et al*., [22]. The fractions were tested at 10 or 20 mg/mL (depending on the quantity available) which was lower than that of the extracts that were tested at 50 and 100 mg/mL.

Antioxidantassays

Antioxidant capacity of this plant was determined by five assays: total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), phosphomolybdate test (PMT) and 2,2- diphenyl picrylhydrazyl tests (DPPH)as described in the methods below.

Total Phenolic Content (TPC): The total phenolic content of the plant samples was determined following the method described by Singleton *et al.*,[23]. 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% Na_2CO_3 and 15 mL of distilled water were added to the mixture after 5 minutes. The resulting mixture was thoroughly shaken and allowed to stand in the dark for 90 minutes at 25^0C (i.e. at room temperature). The solution was then placed in a spectrophotometer for the measurement of absorbance 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:

$$\mathbf{Y = 0.920X, R^2 = 0.585}$$

The equation of this curve was used to estimate the total phenolic content of each sample express in milligram (mg) of gallic acid equivalent per gram (g) of the dried extract.

Total flavonoid content (TFC): Flavonoids belong to a group of compounds called the polyphenols notable for their antioxidant properties. This antioxidant parameter was determined using aluminium chloride ($AlCl_3$) following the method described by Aryouet-Grand *et al.*,[24]. In a clean and empty test tube, 0.3 mL of the plant sample, 3.4 mL of 30% methanol, 0.15 mL of $NaNO_3$ (0.5 M) and 0.15 mL of $AlCl_3$ (0.3 M) were mixed. After 5 minutes, 1mL of NaOH (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:

$Y = 0.190X$ and R^2 value of 0.998

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 evaluating their ability to reduce Fe^{3+} to Fe^{2+} which is indicated by a change in color from yellow to pale green or blue depending on the concentration of antioxidants present in a given sample. An increase in the reduction of Fe^{3+} 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 50^0C 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. The higher the absorbance, the higher the reducing power of the plant sample.

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 [25]. A volume of 0.1 mL of the sample solution was mixed with 1 mL of reagent solution (0.6M H_2SO_4, 28 mM of sodium phosphate and 4 mM ammonium molybdate). The test tube was capped and incubated in a water bath at 95^0C 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:

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

Assay for 2,2-diphenyl-1-picrylhydrazyl (DPPH): The DPPH radical scavenging activity of the medicinal plants used in this study was determined following the modification of the protocol established by Kedare and Singh [26]. 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 520nm. Radical scavenging activity of the extract was determined using the equation below:

$$\frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Ascorbic acid was used as the standard DPPH

Column chromatography

The most active extract (ethyl-acetate) was separated into various fractions using column chromatographic

method. The constituents of the most active extract were separated using different solvent systems. The column was packed with silica gel G 60 (mesh size 60 – 120) to about two-third the length of the column. The extract (6.0 g), mixed with silica gel to form a coarse powder was added to the upper layer of silica gel, and was covered with silica sand and cotton wool. Starting with 100% hexane as the eluent, the polarity was gradually increased by gradient addition of ethyl acetate, and then methanol in specific ratio. About 120 fractions of 10mls each were collected, monitored with thin layered chromatographic method (visualized inside iodine tank) and fractions with similar TLC profiles (R_f and colour) were pooled together. A total of 14 fractions were formed and tested for antimicrobial activity.

Antimicrobial screening of the fractions

Antimicrobial activity of the fractions was determined by the agar-well diffusion methods as described above. The fractions were used at lower concentrations compared to the crude extracts.

Results

The antibiogram determination showed that some of the test organisms were highly resistant as represented by *Bacillus subtilis* and *Pseudomonas aeruginosa* which were 100% resistant to the tested antimicrobials (Table 1). Using CLSI (2016) guidelines, the organisms were classified using the zones of inhibition as Resistant (R), Intermediate (I) or Susceptible (S).

Phytochemical screening showed the presence of certain secondary metabolites in the plant extract as presented in Table 2. Activity of the extracts on the test bacteria was represented by the zone of inhibition produced around the agar wells as shown in Table 3. The MIC and MBC were presented in form of lowest concentration of extract that inhibit and kill the organisms respectively (Table 4). Column fractions (Table 5) and their antibacterial activity on *S. aureus* and *E. coli* is presented in Table 6. However, the results of antioxidant assay are presented in tabular (Table 7) and bar chart in Figure 1.

Table 1: Susceptibility of the test isolates to antimicrobial drugs (Antibiogram)

Organisms	**Antibiotics, zones of inhibition (mm) and CLSI Interpretation**							
Gram positive	**CAZ**	**CRX**	**GEN**	**CTR**	**ERY**	**CXC**	**OFL**	**AUG**
S. aureus NCTC 6571	R (0)	R (0)	S (23)	S (27)	S (21)	R (16)	S (33)	R(12)
S. aureus ATCC 29213	R (0)	R (0)	S (24)	R (0)	S (21)	R (0)	S (25)	I (14)
B. subtilis ATCC 6638	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Gram negative	**CAZ**	**CRX**	**GEN**	**CTR**	**NIT**	**CXC**	**OFL**	**AUG**
E. coli ATCC 25922	R (0)	R (0)	S (17)	S (30)	S (30)	R (0)	S (26)	R (0)
E.coli 35218	R (0)	R (0)	R (0)	I (15)	R (12)	R (0)	I (14)	R (0)

*P. aeruginosa*ATCC 27853	R (0)	R (0)	R (0)	R (0)	R (10)	R (0)	R (9)	R (0)

Key: R= (resistance), I= (intermediate), S= (susceptible), CAZ = (ceftazidime), CRX= (cefuroxime), GEN= (gentamicin), CTR= (ceftriaxone), ERY= (erythromycin), CXC= (cloxacillin), OFL= (ofloxacin) and AUG= (augmentin)

Table 2: Phytochemical screening of the leaf and bark samples of *Lannea egregia*

Parameters	Leaf	Stem bark
Saponin	++	++
Tannin	++	++
Flavonoid	++	++
Alkaloid	+	+
Cardiac glycoside	-	-
Terpenoid	+	++
Anthraquinone	-	-
Anthocyanin	++	++
Coumarin	++	+
Phenol	++	-
Steroid	++	+
Phlobatannin	++	-
Carbohydrate	-	-
Emodin	++	++
Protein	-	-

Key: ++ (present and pronounced), + (present but not pronounced) and – (absent)

Table 3: Antimicrobial activity of the crude extracts of *Lannea egregia* against typed cultures

Plant parts	Extracts	*S. aureus* ATCC 29213			*B. subtilis* ATCC 6638			*P. aeruginosa* ATCC 35217			*E.coli* ATCC 27853			
		Concentrations of extracts in mg/mL (Gen in µg/mL; DMSO 20 %v/v)												
		100	25	Gen	100	25	Gen	100	25	Gen	100	25	Gen	DMSO
Stem	HE	NZ	NZ	23.0	NZ	NZ	NZ	NZ	NZ	16.0	NZ	NZ	16.0	NZ
bark	EAE	17.0	11.0	24.0	16.0	12.0	NZ	13.0	10.0	17.0	16.0	11.0	18.0	NZ
	ME	26.0	20.0	24.0	14.0	8.0	NZ	20.0	14.0	17.0	15.0	11.0	18.0	NZ
Root	HE	NZ	NZ	23.0	NZ	NZ	NZ	NZ	NZ	16.0	NZ	NZ	16.0	NZ
	EAE	26.0	14.0	24.0	9.0	NZ	NZ	20.0	11.0	17.0	17.0	NZ	18.0	NZ
	ME	NZ	NZ	24.0	NZ	NZ	NZ	NZ	NZ	17.0	NZ	NZ	18.0	NZ
Leaf	HE	13.0	NZ	23.0	NZ	NZ	NZ	NZ	NZ	16.0	NZ	NZ	16.0	NZ
	EAE	22.0	20.0	23.0	9.0	NZ	NZ	21.0	14.0	16.0	13.0	NZ	16.0	NZ
	ME	22.0	16.0	23.0	12.0	NZ	NZ	14.0	NZ	16.0	12.0	NZ	16.0	NZ

Key:

HE= (*n-hexane* extract), EAE= (ethyl acetate extract), ME= (methanol extract), Gen= (gentamicin; positive control), DMSO= (dimethyl sulfoxide; negative control) and NZ= (no zone of inhibition)

Table 4: MIC and MBC of Leaf Extracts of *Lannea egregia*

Organisms (Typed)	Concentrations of Extracts (mg/mL)	
	MIC	MBC
S. aureus ATCC 29213	25.0	50.0
S. aureus NCTC 6571	6.3	25.0
B. subtilis ATCC 6638	25.0	>50.0
E. coli ATCC 25922	12.5	50.0
E. coli ATCC 35218	25.0	>50.0
P. aeruginosa ATCC 27853	25.0	>50.0

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

Fraction	Pooled ranged	Polarity range	Description
L1	LE (0-21)	(100% H) – (90%H - 10% E)	Light yellow
L2	LE (22)	(90%H – 10%E)	Deep yellow
L3	LE (23)	(90%H – 10%E)	Deep yellow
L4	LE (24 – 26)	(90%H – 10%E)	Light green
L5	LE (27 – 31)	(90%H – 10%E)	Dark green
L6	LE (32 – 41)	(90%H – 10%E) - (80%H – 20%E)	Dark green
L7	LE (42 – 56)	(80%H – 20%E) – (70%H – 30%E)	Dark green
L8	LE (57 – 63)	(70%H – 30%E)	Deep yellow
L9	LE (64 – 71)	(70%H – 30%E) - (60%H – 40%E)	Green
L10	LE (72 – 77)	(60%H – 40%E)	Dark green
L11	LE (78 – 81)	(60%H – 40%E) – (50%H – 50%H)	Dark green
L12	LE (82 – 103)	(50%H – 50%E) – (10%H - 90%E)	Deep yellow
L13	LE (104 – 117)	(90%E – 10%M) – (80% E – 20%M)	Deep yellow
L14	LE (118 - 129)	(80%E – 20%M) – (70%E – 30%M)	Deep yellow

Key: H = (*n-hexane*), E = (ethyl acetate) and LE = (*Lannea egregia*)

Table 6: Antibacterial activity of chromatographic fractions of ethyl acetate leaf extracts

Column Fractions	Concentration (mg/mL)	Diameter of the zone of inhibition (mm)	
		S. aureus NCTC 6571	*E. coli* ATCC 25922
L1	10.0	-	-
L2	10.0	-	-
L3	10.0	-	-
L4	10.0	15	-
L5	10.0	-	-
L6	10.0	-	-
L7	10.0	13	-
L8	10.0	22	-
L9	10.0	23	-
L10	20.0	21	-
L11	20.0	16	-
L12	20.0	16	-
L13	20.0	16	-
L14	20.0	-	-
GEN	10.0 µg/mL	25	18
DMSO	20.0 %v/v	-	-

Key: - = (no zone of inhibition); L 1-14 = Column fractions; GEN = Gentamicin

Table 7: Antioxidant potential of the leaf of *Lannea egregia* by four different methods

Plant extract	Antioxidant assays			
	TFC (mgQE/g)	TPC (mgGAE/g)	TAC (mg/g)	FRAP (mg/g)
Hexane	2.560±0.450	0.149±0.028	0.338±0.012	0.013±0.002
Ethylacetate	5.153±0.143	0.514±0.004	0.672±0.083	0.293±0.016
Methanol	5.454±0.927	0.497±0.031	0.638±0.014	0.321±0.002

Keys: TFC= Total flavonoid content TPC= Total phenolic content,

TAC= Total antioxidant capacity FRAP= Ferric reducing antioxidant power

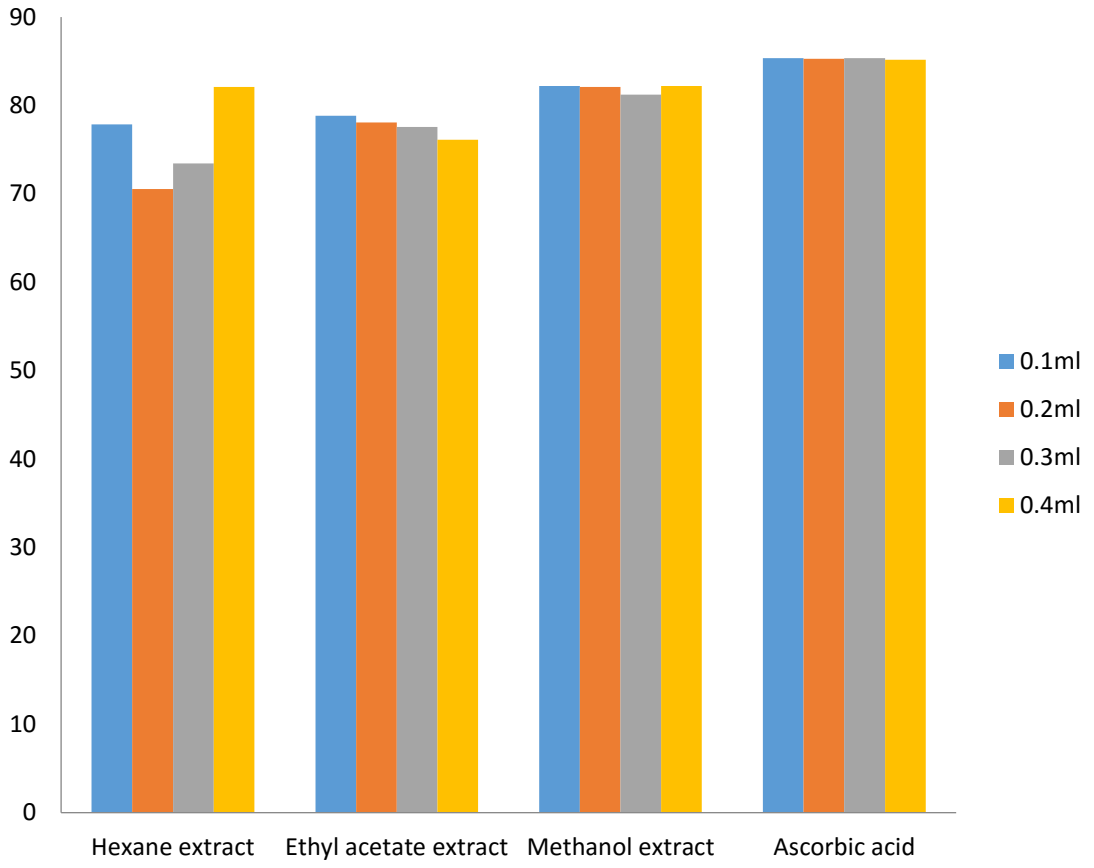

Fig. 1: DPPH of the leaf extracts of *Lannea egregia*vesus Ascorbic acid

Discussion

The antibiogram result (Table 1) showed the resistance status of the organisms used. The Gram-positive *Bacillus subtilis* was found resistant to all the tested antimicrobial agents while the *Staphylococcus aureus* were resistant to the β-lactam antimicrobials (penicillins and cephalosporins). As for the Gram-negative organisms, *Pseudomonas aeruginosa* strain was 100% resistant to all the antimicrobial agents while the *Escherichia coli* strains were also resistant to the β-lactams but susceptible to gentamicin, ofloxacin and ceftriaxone to a certain degree. The antibiogram has clearly shown that the organisms used in this study were mainly resistant strain, and as such required higher concentrations to inhibit (MIC) or kill (MBC) the tested organisms.

Phytochemical screening of these plant parts revealed presence of saponins, tannins, flavonoids, alkaloid, terpenoids, anthocyanins, coumarins, phenols, steroids, phlobatannins and emodins (Table 2). The detection of tannins, flavonoids, steroids and saponins agrees with the findings of Idowu and Idowu [11] on *Lannea welwitschii*, and with Kone *et al*. [7] on *Lannea barteri*. These phytochemicals are most likely

responsible for the detected antioxidant and antimicrobial properties. Tannins, phenolics and flavonoids are well known for their antimicrobial activity and the presence of many hydroxyl group in their chemical structures greatly favors antioxidant capacity.

The extracts of *L. egregia* exhibited moderate antibacterial activity against typed strains of *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* despite the fact that many of them were resistant strains (Table 3). A comparison of the antibacterial activity of the different parts of this plant showed that the leaf extract was the most active. Amongst the crude extracts obtained from the leaves of *L. egregia*, the ethyl acetate extract exhibited the highest antibacterial activity, indicating the relative polarity of the antimicrobial constituents.

Bioassay-guided fractionation was conducted to facilitate the isolation of the antimicrobial constituents of the extract; hence the most active ethyl acetate extract was separated by column chromatography. The antibacterial constituents are relatively polar in nature; fractions 7-13 were eluted by polar solvent mixtures (Tables 5 and 6). The chromatographic fractions of the ethyl acetate leaf 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 5). However, lack of activity of these fractions against *E. coli*, a Gram-negative bacterium presents interesting suggestions; that the extract exerts activity more against Gram positive than Gram negative bacteria or that the fractions are Gram positive specific while the whole extract was active on both. This trend seems to be general as reported by Rabe and Van Staden [27]. One major mechanism by which plant extracts do overcome resistant bacteria is by many active fractions coming together to produce additive or synergistic effects on the microbe. Further, an extract may present many structurally complex constituents which require different biochemical pathway of metabolism, and as such are difficult for the organisms to code for resistant genes.

Total flavonoid content of the most active part of this plant (leaf extracts) showed that the methanol extracts had the highest concentration (5.454 mgQE/g) of the flavonoid content followed by the ethyl acetate extracts (5.153 mgQE/g), then the hexane extract (2.56 mgQE/g) as shown in Table 7. However, the ethyl acetate extract had the highest concentration of phenolic content (0.514 mgGAE/g), followed by the methanol extract (0.497 mgGAE/g) and the hexane extract (0.149 mgGAE/g). The ferric reducing power showed that the methanol extract had the greatest ability to reduce Fe^{3+} to Fe^{2+}. The concentration of Fe^{2+} observed for each extract is as follows: hexane extracts (0.013 mg/g), ethyl acetate extract (0.293mg/g) and methanol extract (0.321 mg/g). The determination of the total antioxidant capacity of the extracts by phosphomolybdate assay revealed that the ethyl acetate extract was the most active followed by the methanol. The hexane extract showed the least capacity (Table 7). The free scavenging (DPPH) ability of the ethyl acetate and methanol extracts was found to be similar to that of ascorbic acid as seen in Fig 1. Hence, it can be deduced that the ethyl acetate and the methanol extract of the leaf of *L. egregia* have good antioxidant potential than the hexane extract. These findings are in line with the active antioxidant constituents being the relatively polar constituents like phenolics, flavonoids and tannins as previously reported [23, 25].

Conclusion

The study has established the antibacterial activity of extracts and fractions of *L. egregia*, thus justified the traditional use of the plant to treat infections. The phytoconstituents possessed antimicrobial and antioxidant activities, hence can be of high therapeutic value and a source of new compounds or lead compound to treat infectious diseases.

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