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Phytochemical screening and antibacterial activity of root and stem bark of *Lannea acida*

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Abstract
Lannea acida is a plant known to have various medicinal and therapeutic properties. The phytochemicals or secondary metabolites and antibacterial activity of its root and stem bark were studied in this research work. The phytochemical screening of the plant revealed the presence of bioactive components comprising flavonoids, alkaloids, saponins, steroids, terpenoids, glycosides and phenols while tannins were not detected in both the root and stem bark of the plant. The plant extracts was subjected to antibacterial activities against *E. coli*, *S. aureus* and *Shigella spp* to confirm its medicinal and therapeutic properties using various concentrations range 50 mg/mL – 6.25 mg/ml. From the study it was observed that the stem bark extract has the highest antibacterial activity against *E. coli* and *Shigella spp* with zone of inhibition of 18.50 ± 0.02 and 14.10 ± 0.12 mm while the root recorded the highest antibacterial activity against *S.aureus* with zone of inhibition of 16.37 ± 0.07 mm. The result indicates that the extracts of root and stem bark of this plant possess antibacterial activities.

Keywords: *Lannea acida*, phytochemical, antibacterial, bioactive compounds and therapeutic

1. Introduction
Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability (Shazadi *et al*., 2010). From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products (Shazadi *et al*., 2010). In the last few decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents (Nascimento *et al*., 2000) [14]. Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs (Srivastava *et al*., 2005) [22, 23].
The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers (Mohanta *et al.*, 2003) [13]. Some medicinal plants have been used in the production of various drugs singly or in combination and even as principal raw material for the production of other conventional medicines (Tahir and Khan, 2012) [24].
Lannea acida is a plant that belongs to the family Anacardiaceae, commonly called Faru in Hausa (Nigeria) and grows in Sub-Saharan Africa. It is a deciduous shrub or tree with a dense, rounded crown. It usually grows 1.5 – 10 m tall, through specimen up to 18 m have been recorded. The bole can be 50 – 70 cm in diameter. Stem barks of *L. acida* are traditional used in Nigeria as anti-bortifacient, vermifuge and to treat anal haemorrhoids, diarrhea, dysentery, malnutrition and debility and in Cameroon to treat dysmenorrhea, amenorrhea and infertility the root is used to treat skin infections while the leaves treat rheumatism. Information provided by the traditional healer in Moutourwa (Far north region of Cameroon) revealed that the maceration of *L. acida* stem bark in local alcoholic drink (palm wine) is used to treat diarrhea and gynaecological complaints (Ahmed *et al.*, 2010) [3].

2. Material and Methods
2.1 Sample Collection and Authentication
Fresh plants of *Lannea acida* was collected from Abubakar Tafawa Balewa University Bauchi, Bauchi State, the plant was authenticated by the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi.

2.2 Preparation of Plant Extract
The method described by Ndip *et al.* (2007) [15] was employed; the plant root and stem were harvested, air-dried under shade for about two weeks and ground to a powder using mortar and pestle as well as laboratory blender. The samples was kept in polytene bag until it is required for analysis. Methanol (100%) and water were used as solvents for the extraction of crude extract. Water is chosen as a solvent so as to mimic the traditional style, since most of the plant parts were administered as either infusions or decoctions. Fifty grams of the powdered plant material was macerated in 400ml of each solvent in extraction pots such that the level of the solvent was above that of the plant material. The macerated mixtures was left for 72 hours at room temperature with proper circular shaking. The insoluble material was filtered using filter paper (Whatman No.1) and evaporated to almost dryness using rotary evaporator at 50^0C. The crude extracts were weighed and placed in a refrigerator at -4°C in sealed conical flask until use.

2.3 Qualitative Phytochemical Analysis of The Plant Extracts
Phytochemical screening of the extracts were carried out

using standard qualitative phytochemical methods described by Trease and Evans (2002) [25], Sofowora (1996) and Harborne (1984) [9].

2.3.1 Test for Tannins
About 0.5 g each extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 15% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

2.3.2Test for Flavonoids
Few quantity of the each extract was dissolved in distilled water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added which produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

2.3.3 Test for Alkaloids
For the test of alkaloids, the Dragendorff and Mayer reagents were used. For the Dragendorff test: in each tube containing 0.2 ml of crude extract is added 15 ml hydrochloric acid (2%), then two to three drops of the Dragendorff or the Mayer reagent. The presence of a red or orange precipitate indicates the presence of alkaloids for the Dragendorff test, while for the Mayer test the precipitate characterizing the presence of alkaloids appears whitish.

2.3.4 Test for Saponins
About 1 g of each extract was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

2.3.5 Test for Phenols
For the test of phenols, Liebermann's test was performed. To 1 ml of the crude extract 1 ml of sodium nitrite was added, few drops of diluted sulfuric acid and then 2 ml of diluted sodium hydroxide were added. A deep red or green or blue color indicates the presence of phenols.

2.3.6 Test for Steroids
Exactly 20 ml of acetic anhydride was added to 0.5 g of each extract and filtered, 2 ml of concentrated H_2SO_4 was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroid.

2.3.7 Test for Terpenoid
About 0.5 ml of each extract was mixed with 10 ml of chloroform and filtered. 3 ml of concentrated H_2SO_4 was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicated the presence of terpenoid.

2.3.8 Test for Glycoside
A small amount of each extract was dissolved in 1ml of water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

2.4 Antibacterial Activities of the Plant Extracts
2.4.1Test Organisms
The test organisms used for this analysis were clinical isolates of bacteria obtained from Department of Microbiology, Gombe State University. The isolates were; *Staphylococcus aureus, Escherichia coli* and *Shigella spp.*

2.4.2 Preparation of the Standard Inocula of the Organisms
The test organisms were prepared by streaking on a freshly prepared nutrient agar plates to obtain discrete colonies. A colony was picked with a sterile wire loop and transferred aseptically in to a small bijou bottle containing sterile normal saline, it was then shaken to dissolve completely and the turbidity was compared with that of a McFarland turbidity standard scale 0.5 which is equivalent to a bacterial cell density of 1.5 x 108 CFU/ML. (CLSI, 2012).
NB: McFarland turbidity standard is a combination of 1 % $BaCl_2$ and 1 % of H_2SO_4.

2.4.3 Culture Media
The culture media used for the analysis include Mueller Hinton agar (MHA), Mueller Hinton broth (MHB) and nutrients agar. The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). All the media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121ºC for 15 minutes.

2.4.4 Determination of Antibacterial activity of the Fractions Using Agar Well Diffusion Method.
The antimicrobial activity of fractions was evaluated using Agar Well Diffusion Method. One gram each of the Methanol and aqueous fraction were diluted in 20 ml of 20% (v/v) dimethyl sulfoxide (DMSO) giving the concentrations of 50 mg/ml from which serial dilution was carried out to obtained 25, 12.5 and 6.25 mg/ml. The bacterial strains was swabbed with sterile swab stick on the Nutrient agar plates. Wells of 8 mm diameter were punched into the agar plates with the help of sterilized cork borer (8 mm). Using a micropipette, 50 µg of the fractions were added to the wells made in the plate. The plates were incubated aerobically in an upright position at 37°C for 24 h. Antimicrobial activities of the fractions were evaluated by measuring the zone of inhibition (mm) against the tested bacteria. The diameters of the zones were measured with meter ruler in millimeter. Each test were carried out thrice and the mean inhibition zone diameter were recorded as Mean±SEM in millimetre. The test was carried out using Ciprofloxacin and Augmentin as positive controls while DMSO was used as a negative control (Rubina, 2011) [19].

2.4.5 Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts
MIC was determined using broth dilution method. The lowest concentration of the fractions showing inhibition for each organism were serially diluted in the test tube containing Mueller Hinton broth. The bacterial strains were inoculated in tubes with equal volume of nutrient broth and

fractions. The tubes were incubated at 37 °C for 24-48 h. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the fractions that produced no visible growth (no turbidity) when compared with the control tubes were considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 h at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC (Rubina, 2011) [19].

2.4.6 Determination of Minimum Bactericidal Concentration (MBC) of the Plant Extracts.

MBC value was determined by sub-culturing the test dilution [which showed no visible turbidity] on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC (Rubina, 2011) [19].

3. Results and Discussion

3.1 Qualitative Phytochemical Screening of the Plant Extracts

The results for phytochemical screening which shows the presence of flavonoids, alkaloids, steroids and terpenoids glycosides and saponins were presented in table 1 and 2. Similar component except alkaloids were confirmed by (Kone *et al*, 2011) [10] to be present in the stem bark of *L. acida.* This difference may be due to age of the plant, physiological variations, environmental conditions, geographic variations, genetic factors (Figueiredo *et al.*, 2008) [8] or evolutional differences of the plant or the presence of an antagonist in the extract. Alkaloids, the most revered of all the phytochemicals and have bactericidal effects (Okwu and Okwu, 2004) [7] are present in all the extracts, alkaloids are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria (Trease and Evans, 1996). Also flavonoids which are reported to be important antimicrobial component (Chung *et al.*, 1998; Karou *et al.*, 2005) [5, 11] were present in both the root and stem bark of *L. acida*. This explains the anti-bacterial property of the plant. Saponin, which is responsible for numerous pharmacological properties (Estrada *et al.*, 2000) [7] was also tested positive in most of the fractions.

Table 1: Phytochemical Screening of *Lannea acida* Stem Bark.

Phytocompounds	Methanol Extract	Aqueous Extract
Alkaloids	+	+ Flavonoids
	+	+
Glycosides	+	+
Phenols	+	+
Saponins	+	+
Steroids	+	+
Tannins	-	-
Terpenoids	+	+

Keys: The sign (+) indicates detected while the sign (-) indicates not detected

Table 2: Phytochemical Screening of *Lannea acida* Root.

Phytocompounds	Methanol Extract	Aqueous Extract
Alkaloids	+	+ Flavonoids
	-	-
Glycosides	+	+
Phenols	+	+
Saponins	-	-
Steroids	+	+
Tannins	-	-
Terpenoids	+	+

Keys: The sign (+) indicates detected while the sign (-) indicates not detected

3.2 Antibacterial Activity of the Extracts

Table 6, 7, 8 and 9 showed the activity of methanol and aqueous extracts of the plant root and stem bark against the tested organisms (*E. coli, S. aureus and Shigella spp*) respectively. The results from the Tables revealed that the root and stem parts of the plant used showed antibacterial activities against the tested organisms with the methanol extracts of the plants having slightly higher activities in tables 6 and 8, than the aqueous extracts of the plants in table 7 and 9. The broad spectrum activity of the methanolic extracts of *L. acida* reported in this study are similar to earlier reports that showed similar antibacterial activities of guava tree plant against pathogenic bacteria. Okwute *et al.* (2010) [18] and Cruzada *et al.* (2014) [6]. However the results obtained from this study was in contrary to the findings of (Abubakar, 2009) who reported that aqueous extracts were more potent in inhibiting the growth of pathogenic *Proteus mirabilis, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus and pseudomonas aeruginosa* than the organic extracts.

The methanol and aqueous extracts of stem bark of the plant shows higher activities against the tested isolates than the methanol and aqueous extract of the plant root this may be due the existence of more phytocompounds present in the stem bark of the plant. The findings from this research tend to agree with (Lawaly *et al.*, 2017) [12] who reported that methanolic and aqueous extracts of *L. acida* showed antibacterial against diarrhea caused by *E. coli* and *Shigella spp*. The results obtained from this study is also in line with the report of Okoro *et al.* (2014) [16] who reported that the stem bark and root extracts of *A. nilotica* showed strong antibacterial activities against isolates. The findings from this research tend to agree with Olaleyen (2007) who reported that methanolic extracts of alkaloids and Saponins from *Hibiscus sabdariffa* had some pharmacologic actions on bacterial isolates like *E. coli, K. pneumoniae, S. aureus* etc. The results obtained from this study is also in line with the findings of (Abd-Ulgadir *et al.*, 2015) who reported that methanolic extract of *Acacia nilotica* sp. Tomentosa stem bark had higher antibacterial activity against *E. coli*. The findings from this research also agreed with the studies of Banso, (2009) reported that ethanolic extract exhibited antibacterial activity against *Streptococcus viridians, S. aureus, E. coli, Bacillus subtilis* and *Shigella sonnei*. To the best of my knowledge there is no documented work on the antibacterial activity of the plant root extract. The results

from Table 8 and 9 showed that stem bark of both methanol and aqueous extract of *L. acida* had the highest antibacterial activity against *E. coli* and *shigella spp* with zone of inhibition of 18.50±0.03 mm and 14.10±0.06 mm but shows a least antibacterial activities against *S. aureus* with no zone of inhibition at 6.25 mg/ml. The results from Table 6 and 7 showed that root of both methanol and aqueous extract of *L. acida* had the highest antibacterial activity against *S. aureus* with zone of inhibition of 16.50 mm and 15.50 mm respectively but shows a least antibacterial activities against *Shigella spp* with no zone of inhibition at 6.25 mg/ml. Table 5 showed the activities of both the positive and negative controls used in this research work. The table reveal that the Ciprofloxacin and Augmentin shows antibacterial activities against the three isolates while DMSO show no activities against the isolates.

Table 3: Control for Antibacterial Sensitivity Test of *Lannea acida* Root and Stem Bark against *E. coli, S. aureus and Shigella spp.*

Test microorganism	Positive Control CPX	Negative Control AU	DMzO
E. coli	14.50 ± 0.06	20.03 ± 0.03	0.00
S. aureus	22.27 ± 0.06	29.50 ± 0.05	0.00
Shigella spp	24.27 ± 0.03	14.20 ± 0.06	0.00

Key: CPX= Ciprofloxacin, AU = Augmentin, DMSO= Dimethyl sulfoxide
The value of average zones of inhibition ± Standard
Error of the Mean in millimeter (mm)
0.0= No Activity

Table 4: Antibacterial Sensitivity Test of *Lannea acida* Root Methanol Extract against *E. coli, S. aureus and Shigella spp.*

Test Microorganisms	Concentration (mg/mL)			
Zones of inhibition	50	25	12.5	6.25
E. coli	12.37 ± 0.07	9.33 ± 0.03	5.77 ± 0.15	2.37 ± 0.03
S. aureus	16.37 ± 0.07	13.77 ± 0.06	7.47 ± 0.03	4.73 ± 0.15
Shigella spp	11.20 ± 0.06	8.47 ± 0.03	4.37 ± 0.07	0.00

The value of average zones of inhibition ± Standard Error of the Mean in millimeter (n=3) = No Activity

Table 5: Antibacterial Sensitivity Test of *Lannea acida* Root Aqueous Extract against E. coli, S. aureus and Shigella spp.

Test microorganism	Positive Control CPX	Negative Control AU	DMSO
E. coli	14.50 ± 0.06	20.03 ± 0.03	0.00
S. aureus	22.27 ± 0.06	29.50 ± 0.05	0.00
Shigella spp	24.27 ± 0.03	14.20 ± 0.06	0.00

The value of average zones of inhibition ± Standard Error of the Mean in millimeter (n=3) 0.00 = No Activity

Table 6: Antibacterial Sensitivity Test of *Lannea acida* Root Methanol Extract against *E. coli, S. aureus and Shigella spp.*

Test Microorganisms			Concentration (mg/mL)	
Zones of Inhibition	50	25	12.5	6.25
E. coli	12.37 ± 0.07	9.33 ± 0.03	5.77 ± 0.15	2.37 ± 0.03
S. aureus	16.37 ± 0.07	13.77 ± 0.06	7.47 ± 0.03	4.73 ± 0.15
Shigella spp	11.20 ± 0.06	8.47 ± 0.03	4.37 ± 0.07	0.00

The value of average zones of inhibition ± Standard Error of the Mean in millimeter (n=3) 0.00 = No Activity

Table 7: Antibacterial Sensitivity Test of *Lannea acida* Root Aqueous Extract against *E. coli, S. aureus and Shigella spp.*

Test Microorganisms			Concentration (mg/mL)	
Zones of Inhibition	50	25	12.5	6.25
E. coli	13.10 ± 0.06	10.43 ± 0.07	5.33 ± 0.03	2.07 ± 0.06
S. aureus	15.07 ± 0.07	12.33 ± 0.18	7.27 ± 0.15	5.33 ± 0.03
Shigella spp	11.03 ± 0.03	7.23 ± 0.12	4.70 ± 0.10	0.00

The value of average zones of inhibition ± Standard Error of the Mean in millimeter (n=3) 0.00 = No Activity

Table 8: Antibacterial Sensitivity Test of *Lannea acida* Stem Bark Methanol Extract against *E. coli, S. aureus and Shigella spp.*

Test Microorganisms			Concentration (mg/mL)	
Zones of Inhibition	50	25	12.5	6.25
E. coli	18.50 ± 0.03	14.13 ± 0.06	8.47 ± 0.03	4.17 ± 0.06
S. aureus	11.27 ± 0.15	6.03 ± 0.03	2.43 ± 0.13	0.00
Shigella spp	14.10 ± 0.12	10.50 ± 0.03	7.13 ± 0.07	4.47 ± 0.03

The value of average zones of inhibition ± Standard Error of the mean in millimeter (n=3)

Table 9: Antibacterial Sensitivity Test of *Lannea acida* Stem Bark Aqueous Extract against *E. coli, S. aureus and Shigella spp.*

Test Microorganisms			Concentration (mg/mL)	
Zones of Inhibition	50	25	12.5	6.25
E. coli	17.47 ± 0.06	14.10 ± 0.12	10.50 ± 0.03	6.10 ± 0.06
S. aureus	10.47 ± 0.09	6.5 0± 0.03	3.47 ± 0.06	0.00
Shigella spp	13.10 ± 0.03	10.47 ± 0.06	7.50 ± 0.03	4.33 ± 0.03

The value of average zones of inhibition ± Standard Error of the mean in millimeter (n=3) 0.0= No Activity

3.3 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Extracts

The results of the MIC and the MBC for individual sensitivity testing conducted were presented in Table 10, 11, 12 and 13. The results obtained showed that the growth of the three isolates were inhibited at concentrations ranging from 25 mg/ml to 50 mg/ml respectively and were completely killed at concentrations of 50 mg/ml for the methanol and aqueous extracts of the plant root as illustrated in table 10 and 11. Table 12 and 13 shows that the three isolates were inhibited at concentrations ranging from 12.5 mg/ml to 25 mg/ml respectively but were completely killed at concentration of 25 mg/ml. This results obtained from the study is similar to the findings of Okoro *et al.*, (2014) [16] and Gislene *et al.*, (2000) who reported MIC and MBC of *A. nilotica* extracts and other plants on bacteria isolates to be 12.5mg/ml to 50mg/ml.

Table 10: Minimum Inhibitory Concentration (MIC) of *Lannea acida* Root Aqueous And Methanol Extract against *E. coli, S. aureus* and *Shigella spp.*

Concentrations (mg/mL)	*E. coli*	*S. aureus*	*Shigella spp*
50.00	No turbidity	No turbidity	No turbidity
25.00	No turbidity	No turbidity	No turbidity
12.50	Visible turbidity	Visible turbidity	Visible turbidity

Table 11: Minimum Bactericidal Concentration (MBC) of *Lannea acida* Root Aqueous And Methanol Extract against *E. coli, S. aureus* and *Shigella spp.*

Concentrations (mg/mL)	*E. coli*	*S. aureus*	*Shigella spp*
50.00	No growth	No growth	No growth
25.00	No growth	No growth	No growth
12.50	No growth	No growth	No growth

The Minimum Bactericidal Concentration is 50.00 mg/mL

Table 12: Minimum Inhibitory Concentration (MIC) of *Lannea acida* Stem Aqueous And Methanol Extract against *E. coli, S. aureus* and *Shigella spp.*

Concentrations (mg/mL)	*E. coli*	*S. aureus*	*Shigella spp*
50.00	No turbidity	No turbidity	No turbidity
25.00	No turbidity	No turbidity	No turbidity
12.50	No turbidity	No turbidity	No turbidity
6.25	Visible turbidity	Visible turbidity	Visible turbidity

The Minimum Inhibitory Concentration is 12.50 mg/mL

Table 13: Minimum Bactericidal Concentration (MBC) of *Lannea acida* Stem Bark Aqueous and Methanol Extract against *E. coli, S. aureus* and *Shigella spp.*

Concentrations (mg/mL)	*E. coli*	*S. aureus*	*Shigella spp*
25.00	No growth	No growth	No growth
12.50	No growth	No growth	No growth

The Minimum Bactericidal Concentration is 25.00 mg/mL

4. Conclusion

From this research work it was discovered that the therapeutic potential of the root and stem bark of *Lannea acida* is attributed to classes of bioactive constituents present in the root and stem bark of the plant such as alkaloid, flavonoids, saponins, steroids, glycosides, terpenoids and phenolic which may be acting in synergy or individually. The antibacterial activities of the plant as observed in this study lend credence to the traditional claim about it medicinal properties.

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