Phytochemical Screening and Antimicrobial Activities of *Lannea acida* (a. rich) Stem Bark Extract

O. A. Olatunji¹, C. I. Ihediuche¹, O. W. Bolaji², A. O. Akala¹, S. E. Edet¹ and A. D. Oladipo¹

¹Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria.
²Federal College of Forestry Mechanization Afaka, Kaduna State, Nigeria.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Author OAO designed the work and he supervised the work. Authors SEE and AOA did the sample collection. Author OWB wrote the introduction. All authors did the lab work. Author ADO wrote the conclusion. All authors read and approved the final manuscript.

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**ABSTRACT**

The persistent resistance of micro-organisms necessitates the need to intensify studies on the use of possible strategies for proper control of pathogens using extracts from natural products. The stem bark of *Lannea acida* was extracted by two methods of extraction: Cold and Soxhlet (continuous) extraction. The extract of the stem bark was screened to confirm the presence of alkaloids, flavonoids, saponins, tannins and cardiac glycosides in all the samples of ethanol and hexane/acetone/methanol mixture extracts. The phytochemicals examined varied among the stem bark extracts of other solvents (hexane, chloroform, acetone, and methanol). The result of the antimicrobial test shows that the stem bark extracts exhibited efficacy against some microbes (*Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa Staphylococcus aureus and Streptococcus pyogenes*) that were employed for the study by exhibiting clear inhibition/clearance zones against these microbes which ranges from 06 mm -21 mm. The plant stem bark could therefore be seen as a potential natural source for useful antimicrobial drugs.

*Corresponding author: E-mail: greatestola@gmail.com;*
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1. INTRODUCTION

The need for research and development in the field of African medicinal plants cannot be over emphasized. Nature has been a source of medicinal agents for years and a massive number of modern drugs have been produced from natural sources, many based their use in traditional medicine [1]. The control of bacteria has become a serious issue because of the emergence of antibiotics resistant bacteria [2,3,4,5]. Many cases of multi drug-resistant bacteria are reported for African countries [6,7,8]. For this reason WHO [9], reviewed the medicinal situation in several developing countries and made some fundamental suggestions aimed at promoting and developing the utilization of traditional medicine in order to contribute to the establishment of health care services in Africa [10]. It has been known that plant extracts which contain phenolic and flavonoid compounds have antioxidant and antibacterial effects [11,12,13].

Lannea acida (A. Rich) is a small deciduous shrub or tree belonging to the family Anacardiaceae [14]. It is a valuable multi-purpose tree widely used by local people. The bark is used in the treatment of stomach troubles, beriberi and haemorrhoids [15]. The leaves and bark have been reported to be useful in the treatment and management of gout, rheumatism, wounds, swelling and burns [16].

This study aimed at extracting, conducting simple analysis to detect the phytochemicals present and carrying out of antimicrobial activity of crude extracts from L. acida stem bark.

2. MATERIALS AND METHODS

2.1 Sample Collection

The fresh stem bark of Lannea acida was collected from Gambari village near ogbomoso town in Oyo State, Nigeria.

All the six bacteria isolates that were employed for the study were collected from the post graduate research laboratory, Microbiology department, University of Ibadan, Nigeria.

2.2 Sample Preparation and Extraction

The fresh stem bark was cleaned and air-dried for a period of three weeks after which it was oven-dried at a temperature of 80ºC until the moisture content was completely dried off. The sample was then crushed into a powdery form using mechanical blender. Two extraction methods were employed: Cold and Sohxlet extractions or Batch and continuous extraction. The cold extraction was performed by weighing out 200 g of the powdered stem bark into container together with 250 ml of the various solvents (ethanol, methanol, chloroform, acetone and hexane) used in the extraction. The sample was allowed to stand for 4 days before it was decanted using Whatmann No. 1 filter paper. The extract was then concentrated by simple evaporation in a water bath at 100ºC.

The continuous extraction on the other hand involved the use of a Sohxlet apparatus where 500 ml of hexane/acetone/methanol (3:1:1) mixture was measured with a measuring cylinder and poured into a round bottom flask. This was followed by weighing out 200 g of the sample into a Sohxlet apparatus, already lagged with glass wool so as to prevent particles of the sample falling into the solvent from the top. The extractor was then fitted with the round bottom flask and the condenser connected to it, with an outlet and the inlet end connected to a water tap. The extraction took about 6 hours. The extract was then concentrated in a water bath at 100ºC before subjecting it to phytochemical examination.

2.3 Phytochemical Screening

The qualitative test was carried out to determine the presence or absence of secondary metabolites as described by Harborne [17] and Trease and Evans [18]. The procedures are as follows:

2.3.1 Test for alkaloids

0.5 g of the extract was weighed into a 100 ml conical flask containing 2 ml of 5% H₂SO₄ in ethanol. The mixture was heated to boiling in a water bath. It was allowed to cool and then tested for the presence alkaloids. 2 ml of the filtrate of the heated samples were then used to test for colour change using 2 drops of Mayer’s reagent for a yellow precipitate and 2 drops of Wagner’s reagent for reddish-brown precipitate [17].

2.3.2 Test for cardiac glycosides

Small portion of the extract was boiled in 5 ml of 70% of ethyl alcohol for 2 minutes. The mixture
was filtered and 10 ml of water with 5 ml of chloroform was added to the filtrate and shaken vigorously. The lower chloroform layer was separated off and evaporated to dryness in a water bath. The cooled chloroform residue was dissolved in 3 ml of glacial acetic acid containing 0.1 ml of FeCl₃. The solution was transferred to the surface of 2 ml of sulfuric acid (H₂SO₄) and observed for a reddish-brown layer formed at the interface and also observed for formation of bluish-green coloration at the upper layer [18].

2.3.3 Test for flavonoids

Five (5) milli-litre of distilled water was added to 5 ml of aqueous filtrate of each sample. To this mixture, 2 drops of H₂SO₄ was added and observed for a yellow colouration which would disappear on storage [17].

2.3.4 Test for saponins

About two grams of the sample was boiled in 20 ml distilled water in a water bath. After cooling, the boiled mixture was filtered. 10 ml of the filtrate was mixed with 5 ml distilled water and shaken vigorously for stable froth formation. Then 3 drops of olive oil were added to the frothing solution and the formation of an emulsion confirmed the presence of saponins [17].

2.3.5 Test for tannins

About 0.5 g of the extract was boiled in 20 ml distilled water in a water bath. On cooling, a drop of ferric chloride was added and observed for a brownish-green or blue-black coloration [17].

2.3.6 Bioassay

Bioassay means the study of antimicrobial activity of the crude extract of a plant against microorganism(s). It is important because it will enable us to determine the active components of a plant extract. In this study, the extract of the plant stem bark was subjected to test for the purpose of antibacterial properties. The bacterial assay procedures adopted were in line with recommended standards, which include the preparation of the culture medium and subsequent inoculation. This was aseptically done to avoid any possible contamination.

2.3.7 Preparation of medium

Forty nine grams of Muller Hinton agar powder was weighed and dissolved in 1 litre of distilled water in a volumetric flask and sterilized by autoclaving at a temperature of 121°C and a pressure of 15 pounds per square inch (psi) for 15 minutes. The molten agar was allowed to cool down. Then 15 ml of the molten agar was poured out into sterile petri dishes and allowed to solidify. A standard concentration of the test organisms were uniformly spread on the surface of each solidified agar plate. Then single sterile filter paper discs were impregnated with each extract concentration and dried at 300°C in the static incubator. The discs were carefully placed aseptically on the surface of the Muller Hinton agar plates that were pre-inoculated with the broth culture of bacteria using forceps. The plates were left on the bench undisturbed for few minutes after which the plates were incubated in an inverted position in the incubator for about 24 hours. After incubation, the diameters of zones of growth inhibition were measured and recorded using meter rule.

3. RESULTS AND DISCUSSION

Tables 1 and 2 shows the results of the phytochemical screening of the extract of hexane/acetone/methanol in stem bark of L. acida. It reveals the presence of the 5 phytochemicals that were screened out for in the sample. Alkaloids were found in all the extracts except in the chloroform extract. Alkaloids have been considered the most potent of all substances extracted from plants and this may explain its use in fighting stomach ache, diarrhea and constipation [19]. Tannins were not detected in the stem bark extracts of chloroform but were present in all other extracts. Tannins are complex phenolic polymers which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibits the growth of microorganisms [20]. Flavonoids were detected in all the extracts except methanol extracts. Saponins were not detected in extract of chloroform. Saponins have been reported to have anti-inflammatory properties [21]. Cardiac glycosides were present in all the extracts except extracts of hexane and acetone.

3.1 Antimicrobial Susceptibility Test

The result of antimicrobial test allows the best selection of antibiotic treatment needed at a particular moment, so all the isolates (Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa Staphylococcus aureus and Streptococcus pyogenes) employed
in the study were subjected to antimicrobial susceptibility test, using the convectional agar disc diffusion method on Muller Hinton agar.

The antimicrobial extracts (stem bark extract) were used with different disc concentrations introduced which were 0.6 mg/mL, 1.0 mg and 1.3 mg/ml. The positive control used here is a 30 µg concentration of Ciprofloxacin. Then the measured zone of inhibition/clearance of the isolates was recorded.

The application of the plant stem bark extracts on E. coli showed that only the higher concentrations had sensitivity against the microbe. It therefore tells that the high concentrations (1.0 mg/mL and above) of the stem bark of L. acida may be used in the treatment of diarrhea, urinary tract infection, anemia as well as other diseases that has E. coli as their main cause.

The extracts of the plant stem bark exhibited a more significant potency against S. aureus meaning that it may be useful in the treatment of staphylococcus infection. Also, the extracts of the stem bark demonstrated considerable sensitivity on B. cereus. S. pyogenes was found to be inhibited by the extracts as seen from the zone of inhibition recorded in Table 3. The extracts of the plant stem bark were also active against P. aeruginosa. The positive control here clearly shows higher sensitivity against this microorganism. The high concentrations of the stem bark extracts showed appreciable levels of inhibition on K. pneumonia. Considering the three concentrations (0.6 mg/ml, 1.0 mg/ml and 1.3 mg/ml) of the extracts used for the antimicrobial test, the stem bark extracts were more potent in order of increasing concentrations. This could mean that concentrations of the extract higher than those used for the study may give a more appreciable result.

### Table 1. Results of phytochemical screening of L. acida stem bark extracts using various solvents (cold extraction)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>AE</th>
<th>CE</th>
<th>EE</th>
<th>HE</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AE-Acetone Extract, CE-Chloroform Extract, EE-Ethanol Extract, HE-Hexane Extract, ME-Methanol Extract

### Table 2. Results of phytochemical screening of stem bark extracts of Lannea acida using hexane/acetone/methane mixture (continuous extraction)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>Hexane/Acetone/Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 3. The antimicrobial activities of the stem bark extracts of Linnea acida

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Concentration of extracts</th>
<th>0.6 mg/ml</th>
<th>1.0 mg/ml</th>
<th>1.3 mg/ml</th>
<th>Positive control</th>
<th>Negative control</th>
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</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
<td>00 mm</td>
<td>09 mm</td>
<td>12 mm</td>
<td>27 mm</td>
<td>00 mm</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>13 mm</td>
<td>15 mm</td>
<td>15 mm</td>
<td>17 mm</td>
<td>00 mm</td>
</tr>
<tr>
<td>B. cereus</td>
<td></td>
<td>15 mm</td>
<td>21 mm</td>
<td>20 mm</td>
<td>19 mm</td>
<td>00 mm</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td></td>
<td>6 mm</td>
<td>15 mm</td>
<td>19 mm</td>
<td>24 mm</td>
<td>00 mm</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td></td>
<td>11 mm</td>
<td>11 mm</td>
<td>14 mm</td>
<td>38 mm</td>
<td>00 mm</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td></td>
<td>07 mm</td>
<td>15 mm</td>
<td>21 mm</td>
<td>29 mm</td>
<td>00 mm</td>
</tr>
</tbody>
</table>
4. CONCLUSION
This study revealed that the extracts of *L. acida* employed in the study possess bioactive constituents and they also exhibit antibacterial properties. The plant stem bark could therefore be seen as a potential source for useful medicine/drug. Also, the continued medicinal use of the plant stem extract is therefore encouraged.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES


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