Journal of Advances in Biology & Biotechnology

23(7): 21-26, 2020; Article no.JABB.60769
ISSN: 2394-1081

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

O. A. Olatunji[1*], C. I. Ihediuche[1], O. W. Bolaji[2], A. O. Akala[1], S. E. Edet[1] and A. D. Oladipo[1]

[1]Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria.
[2]Federal 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.

Article Information

DOI: 10.9734/JABB/2020/v23i730166
Editor(s):
(1) Dr. Sahib Gul Afridi, Abdul Wali Khan University Mardan, Pakistan.
Reviewers:
(1) Carlos Eduardo Rodriguez Molano, Universidad Pedagogica Y Tecnologica De Colombia, Colombia.
(2) Yesudass Dominic Ravichandran, Wollega University, Ethiopia.
(3) N. L. Prasad, Bangalore University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60769

Original Research Article

Received 07 July 2020
Accepted 12 September 2020
Published 30 September 2020

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;*

Keywords: Extraction; resistance; solvents; microbes; phytochemicals.

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 100°C 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% H_2SO_4 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 $FeCl_3$. The solution was transferred to the surface of 2 ml of sulfuric acid (H_2SO_4) 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 H_2SO_4 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, anaemia 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)

S/N	Phytochemicals	AE	CE	EE	HE	ME
1	Alkaloids	+	-	+	+	+
2	Tannins	+	-	+	+	+
3	Flavonoids	+	+	+	+	-
4	Saponins	+	-	+	+	+
5	Cardiac Glycosides	-	+	+	-	+

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)

S/N	Phytochemicals	Hexane/Acetone/Methanol
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponins	+
5	Cardiac glycosides	+

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

Test organisms	Concentration of extracts				
	0.6 mg/ml	1.0 mg/ml	1.3 mg/ml	Positive control	Negative control
E. coli	00 mm	09 mm	12 mm	27 mm	00 mm
S. aureus	13 mm	15 mm	15 mm	17 mm	00 mm
B. cereus	15 mm	21 mm	20 mm	19 mm	00 mm
S. pyogenes	6 mm	15 mm	19 mm	24 mm	00 mm
P. aeruginosa	11 mm	11 mm	14 mm	38 mm	00 mm
K. pneumonia	07 mm	15 mm	21 mm	29 mm	00 mm

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

1. Nair R, Kalariya T, Sumitra C. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol. 2005;29:41-47.
2. Alfred Maroyi, Lannea Acida. A review of its medicinal uses and phytochemistry and pharmacological properties. Asian J Pharm Clin Res. 2018;11(11):69-74.
3. Ahmed MK, Mabrouk JA, Anuka J. Studies of the effect of methanolic stem bark extract of *Lannea acida* on fertility and testosterone in male Wister rats. Journal of Medicinal Sciences. 2010;7:6-7.
4. Diallo D, Marston A, Terreaux C, Toure Y, Paulsen BS, Hostettmann K. Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. Phytotherapy Research. 2001;15:401-406.
5. Kone WM, Soro D, Dro B, Yao K, Kamanzi. Chemical composition, antioxidant, antimicrobial and acetylcholinesterase inhibitory properties of *Lannea barteri* (Anacardiaceae). Australian Journal of Basic and Applied Sciences. 2011;5:1516-1523.
6. Simon F, Kreamer P, de Pina JJ, Demortiere E, Rapp C. Le risque nosocomial en Afrique intertropicale Partie 2: Les infections des patients. Med. Trop. 2007;67:197-203.
7. Rebaudet S, de Pina JJ, Rapp C, Kreamer P, Savini H, Demortiere E, Simon F. Le risque nosocomial en Afrique intertropicale Partie 4: Prevention. Med. Trop. 2007;68:73-82.
8. Belmonte O, Drouet D, Alba J, Morton MP, Kuli B. Evolution of enterobacteriaceae resistance to antibiotics in Reunion Island: Emergence of extended spectrum betalactamases. Pathol. Biol. 2010;58:18-24.
9. WHO. Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria No 70; 1987.
   Available:https://apps.who.int/pcs/pubs/pub_meth.htm
10. Nakajima H. Inaugural Address. Report of the 2nd Meeting of Directors of WHO Collaboration Center for Tradition Medicine, Beijing China, 16-20 Nov., Geneva WHO. 1987;4:5-7.
11. Da-Silva JF, De-Souza MC, Matta SR, De-Andrade MR, Vidal FVN. Correlation analysis between phenolic levels of brazilianpropolis extracts and their antimicrobial and antioxidant activities. Food Chem. 2006;99:431-435.
12. Majhenic L, Skerget M, Knez Z. Antioxidant and antimicrobial activity of Guarana seed extracts. Food Chem. 2007;104:1258-1268.
13. Pereira JA, Oliveira I, Sousa A, Valentao P, Andrade PB. Walnut leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food Chem. Toxicol. 2007;45:2287-2295.
14. Gill LS. Ethno-medicine uses of plant in Nigeria. Bee Keeping and Community Forest Management, University of Benin Press, Nigeria; 1992.
15. Nguyi AA. Tannins of some Nigerian flora. Niger. J. Biotech. 1988;6:221-226.
16. Cushine TP, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;26(4):343-356.
17. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London; 1973.
18. Trease GE, Evans WC. Textbook of pharmacognosy. 14th Edition. W. B. Sanders, London. 1989;60-75.
19. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. Int. J. Mol. Med. Adv. Sci. 2005;1(4):140-147.

20. Nwogu LA, Igwe CU, Emejulu AA. Effects of *Landolphia owariensis* leaf extract function profile and haemoglobin concentration of albino rats. Afr. J. Biotechnol. 2008;2(12):240-242.

21. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4:685-688.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60769