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Authors: [1*]**REMI-ESAN, Ifelolu Adeseye and** [2]**ADEBOYE, Love Emmanuel**

POWDERED EXTRACT OF LOPHIRA ALATA (EKKI) AS ANTIBACTERIAL PROPERTIES AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

[1*]REMI-ESAN, Ifelolu Adeseye and [2]ADEBOYE, Love Emmanuel

[1,2,]Environmental Biology Unit, Department of Science Laboratory Technology,
The Federal Polytechnic Ilaro, Ogun State, Nigeria.

Corresponding E-mail: ifelolu.remiesan@federalpolyilaro.edu.ng

ABSTRACT
The phytochemicals saponins, cardiac glycosides, steroids, carbohydrates, quinones, and terpenoids that were contained in the plant extract are primarily responsible for the antibacterial properties of the ethanolic extract of *Lophira alata*. With regard to the two test organisms *Escherichia coli* and *Staphylococcus aureus*, the plant extracts produced notable yields and antibacterial efficacy. At 20 mg/ml to 40 mg/ml concentrations, the extract of *Lophira alata* exhibited considerable efficacy. At 40mg/ml, the zone of inhibition was at its maximum. As concentration of the extract rises, the higher the zone of inhibition. These findings from this research showed that *Lophira alata* possesses potent antibacterial properties that can fight infectious gram positive and negative bacteria organisms.

Keyword: *Lophira alata*, *Escherichia coli*, *Staphylococcus aureus*, Antibacterial, Ethanol

1.0 INTRODUCTION
Lophira alata is also known as Ponhan in Yoruba, ekki or the red ironwood in English, and Azobé in French, according to Jiofack et al. (2010). Local use includes treating malaria, pain, menstruation problems, hernias, stomach problems, renal discomfort, and toothaches. A mixture of powdered bark, mineral salt, and palm oil is used to cure a variety of illnesses, including heart problems, discomfort, blood in the cough, jaundice, seizures, epilepsy, eye problems, yaws, and snake bites (Idu, 2010; Jiofack et al., 2010). In addition, the leaves are used to cure diarrhea, yellow fever, leprosy, and insomnia. For brushing, twig-made brushes are utilized (Idu, 2010). Some of these local uses have been validated by laboratory investigations, such as the antibacterial capabilities of its methanolic stem bark extract (Baldé et al., 2015) and antimalarial activity (Falade et al., 2014). A study by Haliru et al. (2013) found that the plant had properties that have the ability to modify resistance and inhibit the development of antibiotic-resistant strains.

Various communities utilize different medicinal plants as herbal treatments to prevent and treat various illnesses (Sharif & Banik, 2006; Kubmarawa *et al*., 2007). The use of certain of these therapeutic plants as raw materials by science in the hunt for antibiotics is crucial. Only lately have synthetic medications become widely used, and frequently they are exact replicas of substances found in plants. For many years, medicine has only relied on the leaves, flowers, and bark of plants. World Health Organization (WHO) defined medicinal plant as plants that contains compounds that may be used therapeutically or are precursors to the manufacture of effective pharmaceuticals in one or more of its organs (Junaid *et al*., 2006). The majority of homeopathic or ayurvedic medications use medicinal plants, their components, or extracts, and about 30% or more of today's pharmaceuticals are sourced directly or indirectly from plants. Therefore, it is imperative that the hunt for newer sources of antibiotics remain ongoing. According to Pretorious & Watt (2001), Sharif & Banik, (2006, 2007), Doughari *et al*., (2007, 2008), plants are the least expensive and safest alternative sources of antimicrobials.

MATERIALS

Materials: Micropipettes, multiple micropipettes, incubators, inoculating loops, rotary evaporators, petri dishes, conical flasks, aluminum foil, cotton wool, distilled water, spirit swabs, hand gloves, measuring cylinders, electric scales, spatulas, ekki tree backs, ethanol, filter paper, mortals, and pestles are the materials used.

2.0 METHOD

Study Location: The investigation was carried out at the federal polytechnic in Ilaro, Ogun state, in the microbiological laboratory of the science laboratory department.

Plant Collection and Identification: A member of the Lufasi Nature Park crew gathered the plant in February 2022 in the Lekki Urban Forest Animal Shelter Initiative (LUFASI) Lekki - Epe Lagos state, Nigeria. The plant was transported to the department's forest unit for identification, and the specimen was verified.

Preparation of Plant Extract: The plant's stem bark was washed, allowed to air dry at 37°C room temperature, then ground to powder using a table sized mortar and pestle. It was marked and maintained in an airtight container before the extraction procedure started. The extraction was done using the maceration method, which involves placing 10 kg of *Lophira alata* bark inside a container with the correct amount of solvents (ethanol and water), with ethanol making up 80% of the mixture and water making up 20%. After using the hydraulic extraction technique for the required amount of time, the mixture was agitated and closely covered for 72 hours. In a conical flask lined with cotton wool and aluminum foil, the mixture was first sieved with a muslin blanket to separate the bark from the solvent. Next, Whattman No. 1 filter paper (24 cm) was used to further filter the mixture. As soon as a dry weight was attained, the solvent from the extract was evaporated using a rotating vacuum evaporator RE52 at a temperature between 30 and 40 degrees Celsius.

Preparation of sample: In 2ml of 10% dimethyl sulphoxide (DMSO), 1000g of the extract was dissolved. Six concentrations of the extract 1000, 500, 250, 100, 50, and 25mg/mg were created by further diluting the standard solution, 1000mg/2ml. For test control, the extracts' solutions were utilized. Tetracycline and Ampiclox, two common antibiotics, were utilized as a positive control.

Preparation of media for Bacteria: In the microbiology lab, nutrient agar powder weighing 28g was suspended in 1L of distilled water, and allowed to thoroughly dissolve the nutrient agar before being used to create two strains of bacteria for the study. After that, it was autoclaved for 15 minutes at 121 ℃ to disinfect it. The liquid is then added to the petri dish, and the medium is allowed to harden before continuing. Gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* are the two different types. The organism was kept alive at 4 °C on nutrient agar medium.

Standardization of Inoculum: Using regular saline solution, the organisms were extracted from an overnight culture. Using a spectrophotometer, the organism was calibrated to 0.5 McFarland turbidity standards at a wavelength of 625 mm and an absorbance of 0.08–0.1.

Assessment of the extracts' antimicrobial activities: The antibacterial activity of the extracts was assessed using the Cheesebrough-described agar well diffusion method. Twenty sterile petri dishes each received 15 mL of the 300 mL nutritional agar, which was then put into each plate and allowed to set. Each bacterial isolate's 2 mL overnight standardized culture was seeded onto the plates. After a gradual rotation of the petri dish and an even dispersion of the bacterial isolate, the seeded plates were left to set. The agar surface was sliced into homogeneous wells using an 8mm diameter standard sterile cork-borer. As a positive control, a standard antibiotic disc (Tetracycline and Ampiclox) was positioned on the nutritional agar. To ensure appropriate extract diffusion, the plates were then let to remain at room temperature for an additional hour. For 24 hours, all of the plates were incubated at 37^0C, and zones of inhibition were looked for. Each well has

a zone of clearance around it that denotes inhibition, and a ruler was used to measure the diameter of these zones in millimeters (mm).

Antibacterial Assay: The Agar cup plate technique (Abalaka, 2003) was used to subject every bacterium to the extracts' effects. Using cork borer, three uniformly spaced holes were drilled into the surface of the agar medium. Melted agar was poured into each hole's bottom to stop seepage. Following hardening, 1 milliliter of each of the four prescribed dosages—5 mg, 10 mg, 15 mg, and 40 mg was added to each of the made cups or holes in the extract solution, and it was let to diffuse fully. The test organism was streaked over the agar surface for confluent growth after it had been previously standardized to 106 and cultivated for 24 hours at 37°C in the incubator.

Minimum inhibitory concentration (MIC): The smallest concentration of plant extract fractions with no visible turbidity was determined to be the minimal inhibitory concentration (MIC) using the tube dilution technique (Hugo & Rusell, 2003). By serially dilution the plant extracts from 101 to 1010, the MIC of the extracts was discovered. 9ml of nutritional broth was added to 1ml of each extract. After that, 0.1 ml of the test organism's culture, which had been previously calibrated to 106 cfu/ml, was added to the mixture. After that, it was kept at 37^0C for 24 hours. The Minimum Inhibitory Concentration (MIC) is determined to be the lowest concentration of plant extract in the test tube without any turbidity.

Minimum bactericidal concentration (MBC): This was the outcome of the previously established MIC. The lowest concentration of plant extract in the test tube with no turbidity was found to be the minimal inhibitory concentration (MIC). The tubes that had no turbidity were then plated out onto nutrient agar plates, and the absence of growth after a 24 hour incubation period was proof that the minimum bactericidal concentration (MBC) had been reached.

Phytochemical Analysis: Phytochemical screening of the extracts was carried out in accordance with the methods described by Trease and Evans (1989) and Odebiyi and Sofowora (1978) in order to search for active compounds such as saponins, tannins, alkaloids, phlobatannin, and glycosides.
Alkaloids: In a test tube with 3ml of the extract, 1ml of 1% Hydrochloric acid was added. After 20 minutes of heating, the mixture was cooled and filtered. Mayer's reagent is added in 2 drops per milliliter of extract. The presence of alkaloids was indicated by a creamy precipitate.
Tannins: To 1ml of the extract, 1ml of newly made 10% KOH was added. Tannins were visible as a cloudy, white precipitate.
Glycosides: 1ml of the extract was mixed with 10ml of 50% H_2SO_4 and boiled for 15 minutes. Then 10ml of Fehling's solution was added. The presence of glycosides was confirmed by a brick-red precipitate.
The emulsion test involved adding five drops of olive oil to 3 milliliters of extract in the test tube and aggressively shaking it. Absence of saponins was indicated by the absence of a stable emulsion.
Flavonoids: 3ml of the extract were combined with 1ml of 10% NaOH. There was no yellow coloring, which indicates that flavonoids were not present.
To perform the Salkowski test for steroids, one milliliter of the extract was placed in a test tube along with five drops of concentrated H_2SO_4. There was red coloration, which indicates the presence of steroids.
One milliliter of the extract was mixed with one percent HCl phalobatannin. If red precipitate is absent, the findings are negative.
Triterpenes: Five drops of acetic anhydride were combined with one milliliter of the extract and a drop of concentrated H_2SO_4. After heating for an hour, the liquid was added to chloroform after being neutralized with NaOH. Triterpenes are missing when there is no blue-green tint.

Statistical Analysis: Statistics for Windows (StatSoft, Tulsa, OK, USA) was used to do a one-way analysis of variance (ANOVA). The mean values of the data were compared for significant difference (P 0.05) using the Tukey's HSD test.

3.0 RESULTS

Table 4 displays the phytochemical results of ethanol extracts of *Lophira alata* bark extract. The *Lophira alata* of bark extracts indicated the existence of carbohydrate, steroids, cardiac glycosides, quinines, terpenoids, and saponin, according to the qualitative data reported in Table 1.

Table 1: Results showing *Lophira alata* ethanolic extract against *E. coli* and *S. aureus*

Concentration (mg/ml)	***E. coli***	***S. aureus***
5	-	-
10	-	-
15	-	-
20	16.17±2.46	12.33±2.85
25	17.03±3.03	14.33±2.03
30	17.03±4.16	15.17±1.45
35	21.00±6.12	17.00±2.08
40	22.17±2.52	19±1.15
Tetra (0.33mg/ml)	30.03±3.25	35.17±1.73
Amp (10µg/ml)	27±6.24	30.00±3.06

N:B Results are mean of triplicates
Tetra=Tetracycline Amp= Ampiclox

Table 2: MIC of *Lophira alata* against *E. coli* and *S. aureus*

Organisms	**Concentration of extract (mg/ml)**							
	30	25	20	15	10	5	0	**MIC**
E. coli	-	-	-	+	+	+	+	20
S. aureus	-	-	+	+	+	+	+	25

Key: + = Activity, - = No activity

Table 3: MBC of *L. alata* against test organisms

Organisms	**Concentration of extract (mg/ml)**											
	50	**45**	**40**	**35**	**30**	**25**	**20**	**15**	**10**	**5**	**0**	**MBC**
E. coli	-	-	-	+	+	+	+	+	+	+	+	**40**
S. aureus	-	-	-	-	-	+	+	+	+	+	+	**30**

Key: + = Activity, - = No activity

Table 4: Qualitative Phytochemical Screening of *L. alata*

CONSTITUENTS	-VE/+VE
Saponins	+
Cardiac Glycosides	+
Steroids	+
Quinones	+
Carbohydrate	+
Terpenoid	+

Key:+ = Present, - = Absent

Table 5. Quantitative Analysis of *Lophira alata*

COMPOUND	W1(G)	W2(G)	W3(G)	W3(G)	CONTENT(Mg/G)
Saponin	0.3	52.0925	52.1533	0.0608	0.202666667
Terpenoids	0.1	44.8526	44.9056	0.053	53
Steroid	0.1	49.1034	49.1178	0.0144	14.4

KEY: W= Weight of sample analyzed
W1= Weight of empty crucible
W2=Weight of the filter paper + steroid precipitate

Table 6: Detail of Carbohydrate

Sample	1st reading	2nd reading	3rd reading	Y (average)	M	X=Y/M (mg/g)	conversion to percentage
Carbohydrate	0.23	0.229	0.229	0.229333333	0.0016	143.3333333	143.33333333

4.0 DISCUSSION

Strong antibacterial activity was found in the ethanol extracts from *Lophira alata* stem against clinical strains of bacterial isolates. Even at concentrations as low as 20 mg/ml, the stem extracts' significant anti-*Escherichia coli* and anti-*Staph aureus* actions are highly encouraging (Table 1). *E. coli* and *S. aureus* causes infantile gastroenteritis, travelers' diarrhea wound infection, and opportunistic infection of the urinary tract (UTI) (Dilip, 1985). The discovery of new antibiotics to treat them does not occur at a rate that keeps up with the rate at which bacteria become resistant to antibiotics (Prescott and Klein, 2002). *S. aureus*; a Gram-positive cocci-shaped organism, as opposed to *E. coli;* a Gram-negative rod, the findings in this work are pertinent to achieving good health care delivery in the present day where microbes are quickly developing resistance to antibiotics which were once thought to be "miracle cure all" in the control of diseases. The fact that the extracts contain anti-bacterial action is evidence of the extracts' broad range of activity, which are major qualities of any plant material beneficial in chemotherapy and drug synthesis. These findings suggest that both Gram positive and negative bacteria may cause disease, and that the extract may be used to treat them. For *E. coli* and *S. aureus*, extract at 5 mg/ml, 10 mg/ml, and 15 mg/ml showed no zone of inhibition. For both *E. coli* and *S. aureus*, the zone of inhibition was greatest at a concentration of extract of 40mg/ml. The zone of inhibition was shown to rise as the extract did, meaning that the higher the extract, the higher the zone of inhibition. According to table 4.1, the zone of inhibition for tetracycline was greatest for the test organisms at 30.03 and 35.17mm, respectively.

For stem extracts, the Minimum Inhibitory Concentration (MIC) varies from 20 to 25 mg/ml and the minimum bactericidal concentration (MBC) ranges from 35 to 45 mg/ml (tables 2 and 3). Numerous phytochemicals, such as quinines, saponin, terpenoids, carbohydrates, cardiac glycosides, and steroids, were discovered. Chemical content is higher in the leaves than the stem (Table 4). The ability of certain phytochemicals to modulate resistance in certain strains of bacteria indicates that herbal materials may function as inhibitors of antibiotic resistance (Gibbons *et al*., 2003).

According to Table 4, the plant extract contains phytochemicals such saponins, cardiac glycosides, steroids, carbohydrate, quinones, and terpenoids that are what provide the test organisms' antibacterial activity. According to quantitative phytochemical analysis, there are 0.2027 mg/g, 53 mg/g, and 14.4 mg/g, respectively, of saponin, terpenoids, and steroids. Terpenoids (53 mg/g), which are given in Table 5, were the phytochemicals that were most abundant in the plant extract. According to Table 6, the carbohydrate content was found to be 143.33%.
Although early, the actions of these plant extracts generated invitro against these organisms may be a sign that nature holds the key to improving human health care, and nature is full of these plants.

5.0 CONCLUSION

The antibacterial effects of *Lophira alata* ethanolic extract are mostly attributable to the presence of phytochemicals such saponins, cardiac glycosides, steroids, carbohydrate, quinones, and terpenoids. With regard to the two test species (*Escherichia coli* and *Staphylococcus aureus*), the antibacterial extracts produced notable yields. At 20 mg/ml to 40 mg/ml concentraions, the extract of *Lophira alata* exhibited considerable efficacy. At 40mg/ml, the zone of inhibition was at its maximum. As concentration rises, the zone of inhibition expands.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

Abalaka, M. E., Olonitola, O.S., Onaolapo, J.A. & Inabo, H.I. (2009). Investigation On The Bioeffects of Ethanol Extracts Of Leaf And Stem Of Momordica Charantia On Clinical Strains Of *Escherichia Coli* And *Staphylococcus Aureus*. *Bayero Journal of Pure and Applied Sciences*, 2(2): 203 – 206

Baldé, M.A., Camara, A., Berghe, D., Vlietinck, A., & Pieter L. (2015). Ethnobotanical survey, antimicrobial and anti-complement activities of Guinean medicinal plants traditionally used in the treatment of inflammatory diseases in Conakry and Dubreka. *J Plant Sci*., *3* (1– 2): 11–19.

CDC (2016). List of Selected Multistate Foodborne Outbreak Investigations. Available at: www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbre aks-list.html [accessed July 27, 2016]

Doughari, J.H., El-mahmood, A.M., & Manzara, S. (2007). Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afri. J. Microbiol. Res*., 037- 041.Available online (http://www.academicjournals.org/ajmr)

Falade, M.O., Akinboye, D.O., Gbotosho, G.O., Ajaiyeoba, E.O., Happi, T.C., Abiodun, O.O., *et al*. (2014). In vitro and in vivo antimalarial activity of Ficus thonningii Blume (Moraceae) and *Lophira alata*barks (Ochnaceae). *J Parasitol Res.*, *9*: 728–753.

Idu, M. (2010). Documentation of medicinal plants sold in markets in Abeokuta, Nigeria. *Trop J Res.*, *9*(2), 110– 118.

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B.A. *et al*. (2010). Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *Int J Med Med Sci*., *2*(3), 60–79.

Junaid, S.A., Olabode, A.O., Onwuliri, F.C., Okworiu, A.E.J., & Agina, S.E. (2006). The antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. *Afri. J. Biotechnol. 5*(22), 2315- 2321.

Kubmarawa, D., Ajoku, G.A., Enwerem, N.M., & Okorie, D.A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *Afri. J. Biotechnol.*, *6*(14): 90- 1696, (http://www.academicjournals.org/AJB)

Prescott, L., Harley, J., & Klein, D.A. (2002). Microbiology. 5 th edn. McGrawHill. London. Pp 820-950.

Pretorius, C.J., & Watt, E. (2001). Purification and identification of active components of Carpobrotus edulis L. *J. Ethnopharm. 76*, 87-91.

Sharif, M.D.M., & Banik, G.R. (2006). Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. Res. *J. Agric. Biol. Sci., 2*(6): 268-273.

Singh, G., Maurya, S., De-Lampasona, M. P., & Catalan, C. (2005). Chemical constituents, antimicrobial investigations and antioxidant potentials of Anethum graveolens L. essential oil and acetone extract: part 52. *J. Food Sci. 70*, M208–M215. doi: 10.1111/j.1365-2621.2005.tb07190.x

Usman SO, Okoro AU, Oreagba AI, Busari A, & Agbaje EO (2021). Evaluation of Anti-Salmonellal Activities of the Aqueous and Ethanolic Extracts of Selected Nigerian Medicinal Plants used Locally for Typhoid Fever Treatment. *Research gate,*: https://www.researchgate.net/publication/354254919

Valli, M., Pivatto, M., Danuello, A., Castro-Gamboa, I., Silva, D.H.S., Cavalheiro, A.J., *et al*. (2012). Tropical biodiversity: Has it been a potential source of secondary metabolites useful for medicinal chemistry? *Trop Biodivers*., *35*(11), 2278–87

Vashist, H., & Jindal, A. (2012). Antimicrobial activities of medicinal plants – review. Int. *J. Res. Pharm. Biomed. Sci.*, *3*, 222–230.

Vinoth, B., Manivasagaperumal, R., & Balamurugan, S. (2012). Phytochemical analysis and antibacterial activity of *Moringa oleifera* Lam. *Int. J. Res. Biol. Sci. 2*, 98–102.

Zhang, Y., Tang, L., & Gonzalez, V. (2003). Selected isothiocyanates rapidly induce growth inhibition of cancer cells. *Mol. Cancer Ther*., *2*, 1045–1052.