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Original Research Article

Aqueous Leaf Extracts of *Lasianthera africana*: Antibacterial activity, Acute Toxicity Testing, Effect on Gastrointestinal Microbiota and CD4+ T-lymphocyte Values of Wistar Rats Infected with Extensively Drug Resistant-*Salmonella typhi*

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ABSTRACT

The increasing resistance of microorganisms to antibiotics has created a need for therapeutic plants with strong antibacterial properties. The study determined the bioactive compounds, antibacterial efficacy, and acute toxicity testing (LD_{50}) of aqueous leaf extracts of *Lasianthera africana* (ALELA) using gas chromatography-mass spectroscopy, disc diffusion technique, Reed and Muench, and Karber's methods, respectively. The effect of ALELA on gastrointestinal microbiota and CD4+ values of Wistar rats with or without oral administration of ALELA and/or infected with extensively drug-resistant (XDR)- *S. typhi* were determined using standard protocol and the Pima^{TM} CD4+ analyzer. The results showed that *S. typhi* exhibited extensively drug resistance. The ALELA had L-lactic acid, pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxypropyl), and stigmastan-3, 5-diene in large amounts. The mean zone of inhibition, the MIC, and MBC values of ALELA ranged from 8.0 ± 0.0 to 18.2 ± 1.2 mm; 6.25 to 50 µg/mL and 6.25 to 100 µg/mL, respectively. The LD_{50} of ALELA was ≤ 2464 mg/kg, and there was a mean microbial load reduction from 4.2 ± 0.1 ×10^{10} to 9.7 ± 0.3 ×10^{8} CFU/g in Wistar rats administered 1000 mg/kg ALELA and infected with XDR-*S. typhi*. The mean CD4+ values of Wistar rats administered with ALELA ranged from 8.2 ± 0.2 to 11.5 ± 0.5 cells/µL. The study has shown the antibacterial efficacy of ALELA against XDR-*S. typhi* and other gastro-intestinal flora in Wistar rats, suggesting its potential in treating bacterial gastrointestinal tract infections. Its mild toxicity suggests safety, while its effect on CD4+ T-lymphocyte values indicates immunomodulatory potential.

Keywords*:* Lasianthera *africana, Inhibition*, Microbial Loads, Median Lethal Dose, *Salmonella typhi*

Introduction

Globally, there is a rapid increase in infectious illnesses and their complications, primarily due to the growing resistance of microorganisms to routinely used antimicrobials.[1, 2] The high rates of antibiotic resistance in developing nations are attributable to lack of antibiotic control, irrational use of antibiotics, poor drug quality, and inadequate healthcare systems. [3, 4] Assessing the prevalence of bacterial resistance to antibiotics in a given location involves examining the rate of multidrug-resistant and extensively drug-resistant (XDR) bacteria in that particular geographical region.[4]

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Salmonella is a Gram-negative, rod-shaped, facultatively anaerobic bacterium in the Enterobacteriaceae family. *Salmonella* spp. are known for their strong pathogenicity and potential for cross-infection between humans and animals. Most human infections with salmonella result from the ingestion of contaminated food and water.[5] A relatively large population still depends on herbal medicine,[6,7] and an increase in the use of herbal remedies has been observed in several parts of Nigeria, and many of these herbal remedies have been incorporated into orthodox medicinal plant practice.[8] Herbal medicine has used plants and their juices or solvent extracts, resins, exudates, or other forms of the plant products to treat, cure, or prevent some diseases in both humans and animals practice.[8] One such indigenous plant is *Lasianthera africana*, found in Akwa Ibom State, Nigeria.[9]

Lasianthera africana is a perennial, glabrous shrub of the family Icacinaceae. It is primarily indigenous to and widely distributed in tropical and subtropical areas of Africa, such as Nigeria, Cameroon, Ghana, Ivory Coast, and Congo.[10] In Nigeria, it is called 'Nkanka' by the Igbos, 'Ako-ijo' or 'Irawo-ile' by the Yorubas, 'Editan' by the Efik and Ibibios,[11] and 'Lasian kwai' by the Hausas. The leaf has been used since prehistoric times for preparing soup and in many traditional concoctions for the treatment of various ailments. *Lasianthera africana* is also commonly used as an antacid, analgesic, anti-plasmodial, laxative, antipyretic, and antiulcerogenic,[12] antimicrobial, [13] and antidiabetic.[14] The study determined the antibacterial activity of

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aqueous leaf extracts of *Lasianthera africana*, the acute toxicity, and the effect on gastrointestinal flora and CD4+ T-lymphocyte values of Wistar rats infected with XDR-*Salmonella typhi.*

Materials and Methods

Collection of Plant Leaves

Fresh leaves of *Lasianthera africana* P. Beauv were collected in April, 2024 from a local market at Nung-Udoe (latitude 4° 54' 59" N and longitude 7° 57' 47" E), Ibesipo Asutan Local Government, Akwa Ibom State, Nigeria. The leaves were transported in zip-lock bags and authenticated as *L. africana* by a taxonomist in the Department of Botany and Ecological Studies with a voucher number (BES/LA2021). Thereafter, the leaves were transferred to the Pharmacognosy and Natural Medicine Laboratory at the University of Uyo for processing. The extraneous and undesirable materials were removed by washing the leaves thoroughly under running tap water and then rinsed with distilled water.[15] The washed leaves were cut into small pieces, air-dried at room temperature under shade (26-28 ˚C) for 15 days, pulverized into fine powdered form using a mortar and pestle, and packed into a plastic bag and stored at room temperature until extraction.[16]

Preparation of the Extract

The pulverized (powdered) leaves of *L. africana* were weighed by a sensitive digital weighing balance, and an aqueous leaf extract of *L. africana* (ALELA) was prepared by soaking 1 kg of the pulverized leaves in 2 litres of sterile dH_2O for 24 h with occasional shaking at room temperature (26-28 ºC). The ALELA was then centrifuged at 3000 x g for 10 min, and the supernatant was filtered using Whatman filter paper No. 1 (Sigma-Aldrich Inc., St. Louis, USA), and the filtrate obtained was evaporated to dryness with steam in a water bath (Model: HH-4 Digital Thermostat water bath**)** for 48 h. The extraction process was repeated three times, and the filtrates from all portions were combined in one container. The dried extract was weighed and stored in stoppered sample vials at 4 ºC, and the graded concentrations (1.25, 2.5, 5.0, 10, and 20 $mgmL^{-1}$) of the extracts were aseptically prepared using 100 mL of 1% (*v*/v) dimethyl sulphoxide (DMSO, Aldrich, USA) and shaken vigorously to obtain a homogenous mixture.[17]

Gas Chromatography-Mass Spectrometry (GCMS) Analysis of ALELA

The ALELA was subjected to GC-MS analysis using a Hewlett-Packard (HP, Palo Alto, California) HP 7890A system attached with a UV detector and HP-5 capillary column (HP-5MS; 30m length, 0.25 mm ID; 0.25µm film). Injector temperature (230°C), detector temperature (280°C), and electron ionization system with an ionization voltage of 70eV were used for GC-MS operation. Helium gas (99.9% purity) was used as a carrier gas at a constant column flow rate of 1 mL/min and an injection volume of 1 µL. The column oven temperature was initially programmed at 40°C (held for 2 min), then increased to 200°C at 5°C/min, and finally increased to 280°C at 5°C/min. One microlitre (1 µL) of the ALELA diluted with solvent (1/100, v/v) in a test tube and vortexed for 2 min was manually injected into the GC-MS using a Hamilton syringe in split mode in the ratio of 20:1. The relative quantity of each bioactive compound present in the ALELA was expressed as a percentage based on the peak area produced in the chromatogram. The interpretation of the mass spectrum of ALELA was conducted by using the available mass spectral databases of the National Institute of Standards and Technology (NIST) libraries.

Test Organisms

A total of 20 clinical bacterial strains consisting of *Staphylococcus aureus* SA01, SA10, SA23; *Staphylococcus gallinarium* SG34; *Streptococcus pneumoniae* SP06, SP20; *Haemophilus influenzae* HI02, HI11, HI15; *Citrobacter freundii* CF13, CF21; *Shigella flexneri* SF08, SF13; *Escherichia vulneris* EV12; *Escherichia coli* EC28, EC33; *Serratia marcescens* SM25; and *Salmonella typhi* ST07, ST19, ST33 used in this study were obtained from the Department of Microbiology Laboratory, University of Uyo. The bacterial strains were checked for purity, maintained on nutrient agar slant at 4ºC, and re-identified using both conventional biochemical tests,[18] and VITEK 2 automated systems (BioMerieux, Inc., France).

Antibiotic Susceptibility Profile of Test Organisms

The *in vitro* antibiotic susceptibility profiles of bacterial isolates obtained were re-confirmed by the Kirby-Bauer disk diffusion technique[19] and VITEK 2 automated systems. Ten microliters (10 µL) of each pure bacterial suspension, prepared directly from an overnight nutrient agar (NA) plate, adjusted to a 0.5 McFarland Turbidimetric Standard of approximately 1 x 10^6 CFU/mL, was evenly inoculated onto each plate containing Mueller Hinton Agar (MHA, Oxoid, England), and the plates were allowed to dry for 5 min. The commercially prepared, fixed concentration antibiotic discs of Fluoroquinolones (Ciprofloxacin, 5 µg; Levofloxacin, 5 µg; Ofloxacin, 5 µg); Monobactams (Aztreonam, 30 µg; Tigemonam, 30 µg); Cephalosporins (Ceftriazone, 30 µg; Ceftazidime, 30 µg; Cefoperazone, 30 µg); Penicillin (Ampicillin, 10 µg; Piperacillin, 100 µg); Carbapenems (Meropenem, 10 µg; Ertapenem, 10 µg); Aminoglycosides (Gentamycin, 10 µg; Streptomycin, 25 µg) (Oxoid, England) were aseptically placed on the surfaces of the plates. Thereafter, the plates were inverted and incubated at 37ºC for 18 h and the diameters of inhibition zones were observed, measured, and interpreted based on interpretative guidelines by the Clinical and Laboratory Standards Institute.[19] Similarly, antibiotic susceptibilities of bacterial isolates were performed using VITEK 2 automated systems (BioMérieux), following the manufacturer's instructions. Isolates that displayed resistance to ≥ 3 antibiotic classes were considered MDR strains, [19, 20] while bacterial isolates that were non-susceptible to all antibiotics except two were classified as extensively drug-resistant (XDR).[20, 21]

Testing for Antibacterial Efficacy of Lasianthera africana

The antibacterial efficacy of the ALELA was evaluated *in vitro* using the agar well diffusion method.[22] Twenty bacterial strains listed earlier were used. Each bacterial strain was inoculated onto a nutrient agar plate and incubated at 37°C for 24 h. Ten microliters (10 µL) of each bacterial suspension, prepared directly from an overnight nutrient agar plate and standardized to match 0.5 McFarland turbidity of approximately 1 x 10^6 CFU/mL, was evenly inoculated onto each plate containing MHA, and the plates were allowed to dry for 5 min. Five wells were punched over each culture plate using a sterile cork borer of 6 mm diameter and 100 µL of 1.25, 2.5, 5.0, and 10 mg/mL concentrations of ALELA to give contents of 12.5, 25, 50, and 100 µg/mL concentrations of ALELA, respectively, were dispensed into three (3) labelled wells; 10 µL of 5% DMSO was dispensed into the 4th well (negative control) and 10 µL of 5 mg/mL amoxicillin/clavulanic acid was dispensed into the 5th well (positive control) using a micropipette. All the culture plates were refrigerated for 30 min for pre-diffusion of the ALELA into the agar and thereafter incubated at 37°C for 18 h. The experiments were performed in triplicate. The zone of inhibition (ZI), which corresponded to the antibacterial activity of ALELA after the incubation, around the wells was measured and recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) of ALELA

The MIC of ALELA against MDR-bacterial strains was determined by the macro-broth dilution technique.[19] One hundred microliters of stock solution (20 mg/mL) of ALELA was serially diluted using nutrient broth in test tubes to obtain concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/mL, respectively. In each test tube, 100 µL of each concentration of ALELA was added to 9.9 mL of nutrient broth to give final concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/mL, respectively. Thereafter, 100 µL of each bacterial suspension, containing approximately 10^6 CFU/mL, was added into each test tube. Two control tubes were used: a nutrient broth tube inoculated with bacteria (a positive control) and a nutrient broth tube inoculated with ALELA (a negative control). All test tubes were incubated at 37ºC for 24 h and were examined for bacterial growth. The MIC value was taken as the lowest concentration of the ALELA that visibly inhibited the growth of the test bacteria after 24 h of incubation at 37°C.

Determination of Minimum Bactericidal Concentration (MBC) of ALELA

A loopful from each MIC broth tube without visible bacterial growth was streak-inoculated onto freshly prepared nutrient agar plates. The

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inoculated plates were incubated at 37°C for 24 h, then examined for bacterial growth.[2] The MBC value was taken as the lowest concentration that killed 99.9% of the test bacteria after 24 h of incubation at 37°C.

Screening of Wistar Rats for Colonization by Salmonella typhi

Prior to the initiation of the experiment, all the Wistar rats were bacteriologically screened for *S. typhi* infections. Swab samples obtained from the rectal mucosa of Wistar rats via moistened sterile cotton-tipped applicators were pre-enriched in 5 mL of Selenite F broth (Scharlau Microbiology, Spain) and incubated at 37°C for 18 h. A loopful from the enriched culture was inoculated onto plates of MacConkey agar (MCA) and Salmonella –Shigella agar (SSA) (Oxoid, Basingstoke, England) and incubated at 37°C for 24 h. After incubation, the plates were examined for round, non-lactose-fermenting, and colourless colonies on MCA and SSA. Colourless colonies were subcultured twice, to ensure purity of the colonies, onto nutrient agar plates (Oxoid, Basingstoke, England) and incubated at 37°C for 24 h. Pure culture plates were parafilmed, refrigerated at 4°C, and colonies were Gram-stained, subjected to biochemical tests,[18] and the VITEK 2 automated systems for identification. *S. typhi*-free Wistar rats were used for the experiments.

Antigen (S. typhi) Preparation

The XDR-*S. typhi* suspension was prepared from an overnight Salmonella-Shigella Agar plate and adjusted to a 0.5 McFarland Turbidity Standard of 1.5 x 10^6 CFU/mL.

Ethical Clearance

Ethical approval (UU/FP/2023/11) to carry out this study was obtained from the Animal Care and Use Committee, University of Uyo, Uyo.

Experimental Design: Treatment of Wistar Rats

Thirty-five (35) apparently healthy *S. typhi* -free Wistar rats, weighing between 150 and 200 g, were obtained from the animal house of the Faculty of Pharmacy, University of Uyo, Nigeria. The Wistar rats had free access to standard animal palletized feed and water ad libitum and were acclimatized to standard environmental conditions for 1 week prior to the experiments.[23] Then, the Wistar rats were deprived of food overnight prior to the start of the experiment. The Wistar rats were divided into 7 groups (5 Wistar rats per group) as follows:

Group 1: Negative control, Wistar rats were given animal palletized feed and 10 mL of water.
Group 2: Positive control, Wistar rats were given animal palletized feed + 10 mL of broth culture of XDR-*S. typhi* (1.5 × 10^6 CFU/mL).
Group 3: Wistar rats were given animal palletized feed + 1000 mg/kg of ALELA.
Group 4: Wistar rats were given animal palletized feed + 2000 mg/kg of ALELA.
Group 5: Wistar rats were orally given animal palletized feed + 10 mL of broth culture of XDR-*S.typhi* (1.5 × 10^6 CFU/mL) (orally) + 1000 mg/kg of ALELA.
Group 6: Wistar rats were given animal palletized feed + 10 mL of broth culture of XDR-*S.typhi* (1.5 × 10^6 CFU/mL) (orally) + 2000 mg/kg of ALELA.
Group 7: Wistar rats were given animal palletized feed + 10 mL of broth culture of XDR- *S.typhi* (1.5 × 10^6 CFU/mL) (orally) + 1000 mg / kg of Ciprofloxacin.

All the Wistar rats in groups 1–7 were kept for 10 days and thereafter anesthetized with an intraperitoneal (I.P.) injection of a diazepam / ketamine mixture. Then, blood pooled from at least three (3) infected Wistar rats in each group was collected for Cluster of Differentiation 4 (CD4+) *T-lymphocyte value.*

Effect of ALELA on CD4+ T- Lymphocyte Values of Wistar Rats

The initial CD4+ T-lymphocyte values (cells / μL) of Wistar rats (n = 35), divided into 7 groups (5 Wistar rats per group), were determined. Then, the CD4+ T-lymphocyte value of Wistar rats with or without oral administration of ALELA (1000 mg/kg and 2000 mg/kg) and/or infected with XDR-*S.typhi* (1.5 × 10^6 CFU/mL) on the 7th and 14th days was determined using the Pima™ CD4 analyzer (Waltham, Maryland, USA). The blood samples were run only after the normal and low-value control cartridges gave acceptable values. Each venous blood sample (25 μL) collected in an EDTA vacutainer tube was loaded into a Pima™ disposable anticoagulant-coated cartridge preloaded with anti-human CD4-dye monoclonal antibodies. Then, the collector was removed, the cartridge was capped, and it was immediately placed in the Pima™ CD4 analyzer for 20 min, and results were obtained.

Effect of ALELA on Bacterial Flora of the Gastrointestinal Tracts of Wistar Rats

The initial bacterial flora of the gastrointestinal tract of Wistar rats (n = 35) were determined using standard bacteriological procedures. Then, fresh stool pellets were collected using sterile, wide-mouthed containers from Wistar rats (n = 35), divided into 7 groups (5 Wistar rats per group) with or without oral administration of ALELA and/or infected with XDR-*S.typhi* (1.5 × 10^6 CFU/mL) on 48, 96, and 144 h. Each stool sample (1 g) was separately homogenized and serially diluted using 9 mL of phosphate buffered saline (pH 7.2). Each aliquot (1 mL) was pour-plated onto NA, MCA, SSA, eosin methylene blue (EMB), and thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Oxoid, England). The plates were incubated at 37°C for 24 h, and CFU/mL were determined. A loopful of the colony obtained was subcultured onto a plate of NA and incubated at 37°C for 24 h. The pure colonies were streaked onto nutrient agar slants and incubated at 37°C for 24 h.[18] The bacterial strains were maintained on nutrient agar slant at 4ºC, and identified using the VITEK 2 automated systems (BioMerieux, Inc., France).

Acute Toxicity Testing (Median Lethal Dose, LD_{50}) of ALELA

Preliminary tests, using Wistar rats (n = 12) randomly divided into 4 groups (3 Wistar rats per group), were conducted to establish the highest and lowest dose of the ALELA that killed either none or all the Wistar rats, respectively. Thus, the dose levels used in this acute toxicity testing ranged between these two dose extremes (1500 and 3500 mg/kg). Thirty-five Wistar rats, weighing between 150 and 200 g, were randomly divided into 5 groups (7 Wistar rats per group) and were handled according to standard guidelines for the use and care of laboratory animals. The Wistar rats had free access to standard animal palletized feed and water *ad libitum* and were acclimatized to standard environmental conditions for 1 week prior to the experiments.[23] Then, the Wistar rats were deprived of food overnight prior to the start of the experiment. The ALELA was dissolved in 10% (*v*/v) DMSO, and 5 groups (A, B, C, D, and E) of Wistar rats were intraperitoneally administered ALELA at dosages of 1500, 2000, 2500, 3000, and 3500 mg/kg body weight, while the Wistar rats (group F) were administered normal saline (negative control). The Wistar rats were closely observed for mortality within 24 h, and the LD_{50} of the ALELA was determined using Karber's Method (Eq. 1) as described by Akinjogunla,[24] and the Modified Arithmetic Method of Reed and Muench [25] as seen in equation 1.

$$LD_{50} = LD_{100} - \frac{\Sigma (a \times b)}{N} \quad (1)$$

Whereas LD_{100} = Lethal dose causing the 100% death of all test Wistar rats.
a = difference between two successive concentrations of extracts injected.
b = average number of dead Wistar rats in two successive concentrations.
N = total number of Wistar rats in a group.

Statistical Analysis

The data obtained were analyzed using Statistical Packages for Social Sciences software (IBM SPSS, Window software Version 22.0. Armonk, NY: IBM Corp.). Results were expressed as mean value ± standard deviation (S.D), and significant differences were assessed by the Duncan Multiple Range Test. The P-values ≤ 0.05 were considered significant.

Results and Discussion

The morphological and biochemical characteristics, as well as enzymatic reactions (arginine dihydrolase, ornithine decarboxylase, lipase, beta-galactosidase, and hyaluronidase) of twenty isolates

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belonging to the genera *Staphylococcus*, *Streptococcus*, *Salmonella*, *Escherichia*, *Serratia*, *Citrobacter*, *Shigella*, and *Haemophilus*, are presented in Table 1. Microbial resistance to nearly all classes of antibiotics continues to rise despite increasing global awareness and concerns worldwide.[26] Table 2 showed that *S. typhi* (ST19) exhibited extensively drug resistance, *S. typhi* (ST07 and ST33) displayed sensitivity to Ciprofloxacin, Ofloxacin, Levofloxacin, Ertapenem, and Meropenem but were highly (100%) resistant to Tigemonam and Aztreonam. *S. aureus* SA01, *S. pneumoniae* SP06, *E. vulneris* EV12, and *S. marcescens* SL25 were 100% sensitive to Streptomycin, and Gentamycin. *C. freundii* CF21 and *E. coli* EC28 were Ciprofloxacin, Ofloxacin, Aztreonam, and Meropenem resistant. The percentage intermediate susceptibility of *S. aureus*, *H. influenzae*, and *S. flexneri* to Ofloxacin and Ceftriazone ranged from 33.3% to 50.0%, while 100% of *S. gallinarium* SG34 displayed intermediate susceptibility to Aztreonam and Meropenem. The high sensitivity of isolates to Levofloxacin is consistent with findings in Italy on the Levofloxacin activities against 4,003 clinical bacterial isolates.[27] The high resistance to Streptomycin exhibited by *S. typhi* in our study concurs with the findings of Breijyeh *et al.* [28] on the resistance of Gram-negative bacteria to current antibacterial agents.

A total of 59 bioactive compounds were identified in ALELA, and the chromatogram is presented in Fig. 1. Lactic acid (45.7%), Pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxypropyl)- (9.41%), Stigmastan-3,5-diene (4.38%), 4-isobenzofuranol, octahydro-3a,7a-dimethyl (2.11%) and 3-Oxabicyclo[3.3.0]octan-2-one, 7-methylene-4,dimethyl (2.08%) and with retention times of 6.365, 16.008, 21.583, 15.002, and 14.092, respectively, were present in large amounts (Table 3). The bioactive compounds present in an extremely small amount in ALELA were 4-Amino-1,5-pentandioic acid, 1,13-Tetradecadien-3-one, 1-Naphthalene carboxylic acid, decahydro-1,4a-dimethyl-, 5-(.omega.-Aminopropyl)-3-amino-4-cyano- isoxazole, 2H-Pyran-2-one, 5,6-dihydro-4-(2-methyl-2-propen-3-yl)-, methyl ester, 1-Allyl-3-phenyl-2- thiourea, hexadecanoic acid, , and A'-Neogammacer-22(29)-en-3-one. The presence of these compounds in the ALELA corroborated the reports of Aboaba *et al*.[29] Lactic acid can create an acidic environment that inhibits the growth of bacteria and other microorganisms. 5-(ω-Aminopropyl)-3-amino-4-cyano-iso have an impact on the cellular functions of microorganisms. Pent-4-enoic acid disrupts bacterial cell membranes, interferes with cellular processes or structural component synthesis.[30] 2H-Pyran-2-one, 5, 6-dihydro-4-(2-methyl-2-pro) interferes with microbial metabolic pathways, and hexadecanoic acid exerts haemolytic and antimicrobial activity.[31]

The use of medicinal plants by humans for the treatment of diseases has been in practice for a very long time.[17] In this study, the ALELA exerted antibacterial activity by inhibiting the growth of the tested *S. aureus*, *S. pneumoniae*, *Shigella* spp., *E. coli*, and *S. typhi* (Table 4). The mean ($\bar{x}$ ± S.D) zone of inhibition (Z.I.) of ALELA on the growth of isolates ranged from 7.5 ± 0.5 to 19.5 ± 1.0. The concentration of ALELA at 100 µg/mL had the highest activity (100%) against the isolates, while concentrations of 50 µg/mL, 25 µg/mL, and 12.5 µg/mL inhibited 90% (n = 18), 85% (n = 17), and 75% (n = 15) of isolates, respectively. *S. aureus* SA23 and *E. coli* EC28 were not inhibited by ≤ 50 µg/mL of ALELA. The results of the antibacterial activities of ALELA on bacterial isolates are in agreement with the findings of Ebana et al.[32] on their studies on antimicrobial activity and phytochemical analysis of leaf extracts of *L. africana*. The mean (mm ± S.D) zone of inhibition of ALELA on the growth of isolates ranged from 8.0 ± 0.0 to 18.2 ± 1.2, and this agrees with the results of Andy *et al*.[13] which indicated that ALELA has a high antimicrobial activity (zone of inhibition ≥ 15 mm) against the test organisms. The concentration of ALELA at 100 µg/mL had the highest activity (100%) against the isolates, while concentrations at 25 µg/mL and 12.5 µg/mL inhibited 87.5% (n = 7) and 66.7% (n = 6) of the isolates, respectively. This observation is consistent with the findings of Bernier and Surette, [33] suggesting that bacterial responses to antimicrobials are concentration-dependent. The MIC and MBC values of ALELA for the isolates are presented in Table 5. Of the isolates (n = 20) tested, the MIC value of ALELA for four isolates was 6.25 µg/mL, while the MIC value of ALELA for two isolates was 100 µg/mL. The MBC value of ALELA for three isolates (*S. aureus* SA10, *H. influenzae* HI02, and *S. marcescens* SL25) was 100 µg/mL, while the MBC value of ALELA for *E. coli* EC28 was 200 µg/mL (Table 5). The MIC value of ALELA in our study ranged from 6.25 µg/mL to 50 µg/mL. Our results on the MIC differ from the 10 mg/mL obtained by Ebana et al. [32] in their studies on the antimicrobial activity of the leaves of *L. africana*.

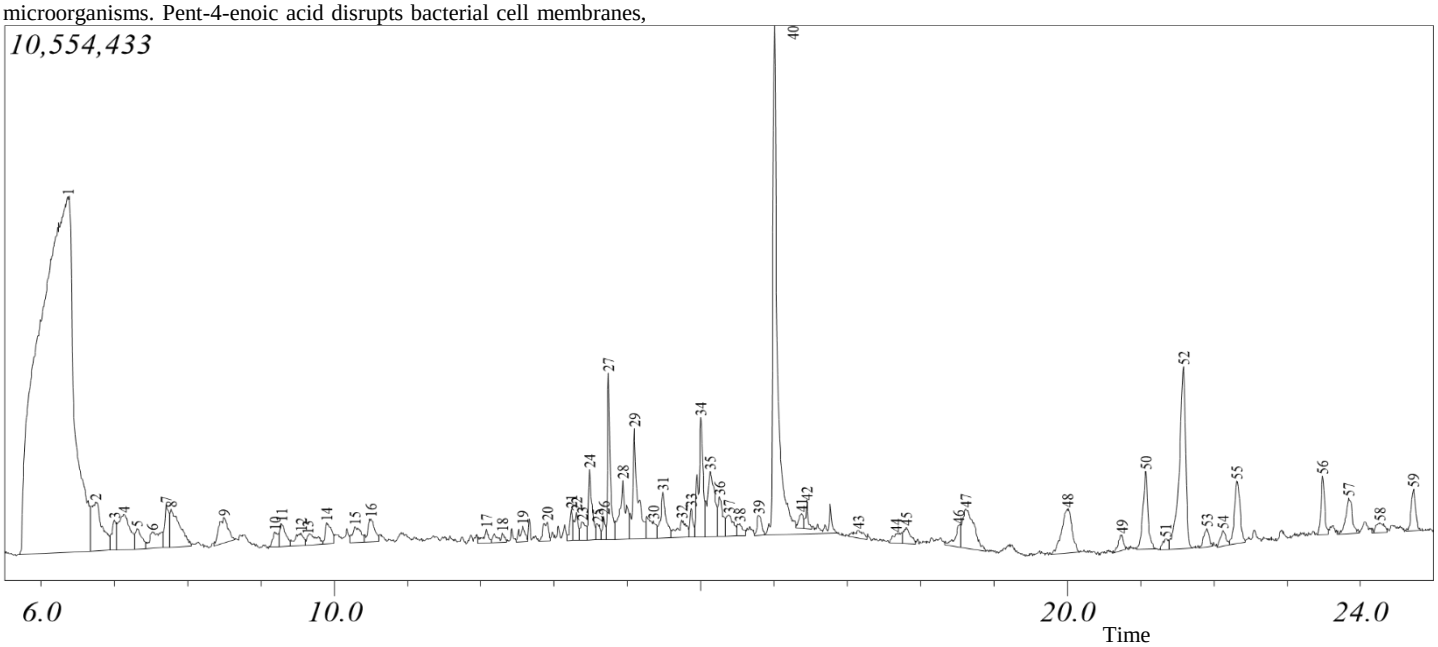

Figure 1: Gas Chromatography-Mass Spectroscopy Analysis of Aqueous Leaf Extract of Lasianthera africana

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Table 1: Morphological and Biochemical Characteristics and Enzymatic Reactions of Bacterial Isolates from Clinical Samples

Gram reaction																												
Gram	Shape	COA	CAT	STA	VP	MR	NIT	IND	URE	MOT	CIT	H_2S	OXI	OPT	DCE	ARD	ODX	LIP	ONPG	HYA	FRU	RAF	MAN	MAL	GAL	LAC	GLU	SUC	Probable Bacteria
+	cocci	+	+	-	+	+	+	-	+	-	+	-	+	nd	-	+	+	+	-	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus* SA01
+	cocci	+	+	-	+	+	+	-	+	-	+	-	+	nd	-	+	+	-	-	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus* SA10
+	cocci	+	+	-	+	+	+	-	+	-	+	-	+	nd	-	+	+	+	-	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus* SA23
+	cocci	-	-	+	-	+	+	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	*Streptococcus pneumoniae* SP06
+	cocci	-	-	-	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	*Streptococcus pneumoniae* SP20
-	rod	-	+	-	-	+	+	-	-	+	-	+	-	nd	-	-	-	+	-	-	-	-	+	+	+	-	+	-	*Salmonella typhi* ST19
-	rod	-	+	-	-	+	+	-	-	+	-	+	-	nd	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Salmonella typhi* ST33
-	rod	-	+	-	-	+	+	-	-	+	-	+	-	nd	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Salmonella typhi* ST07
+	cocci	-	+	-	-	-	+	-	+	-	+	-	-	nd	+	-	+	+	-	+	+	+	+	+	+	+	+	+	*Staphylococcus gallinarium* SG34
-	rod	-	+	-	-	+	+	+	-	+	-	-	-	nd	+	+	-	-	+	-	-	-	+	-	+	+	+	+	*Escherichia vulneris* EV12
-	rod	-	+	-	-	+	+	+	-	+	-	-	-	nd	-	-	-	-	+	-	-	+	+	-	+	+	+	-	*Escherichia coli* EC28
-	rod	-	+	-	-	+	+	+	-	+	-	-	-	nd	-	-	-	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli* EC33
-	rod	-	+	+	+	-	+	-	+	+	+	-	-	nd	-	-	+	-	+	-	+	-	+	+	+	-	+	+	*Serratia marcescens* SL25
-	rod	-	+	-	-	+	+	-	+	+	+	+	-	nd	+	-	-	-	+	-	+	+	+	+	+	+	+	+	*Citrobacter freundii* CF13
-	rod	-	+	-	-	+	+	-	-	+	+	+	-	nd	+	-	-	-	+	-	+	-	+	+	+	+	+	+	*Citrobacter freundii* CF21
-	rod	-	+	-	-	+	+	+	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	+	+	+	-	+	-	*Shigella flexneri* SF08
-	rod	-	+	-	-	+	+	-	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	+	-	+	-	+	-	*Shigella flexneri* SF13
-	C-rod	-	+	-	-	-	+	-	+	-	+	-	+	nd	-	-	+	+	-	+	-	-	-	+	+	-	+	-	*Haemophilus influenzae* HI02
-	C-rod	-	+	-	-	-	+	+	-	-	+	-	+	nd	-	-	-	+	-	+	-	-	-	+	+	-	+	-	*Haemophilus influenzae* HI11
-	C-rod	-	+	-	-	-	+	+	+	-	+	-	+	nd	-	-	+	+	-	+	-	-	-	+	+	-	+	-	*Haemophilus influenzae* HI15

COA: Coagulase; CAT: Catalase; STA: Starch; VP: Vogues Proskauer; MR: Methyl red; NIT: Nitrate; IND: Indole; URE: Urease; MOT: Motility; CIT: Citrate; H_2S: Hydrogen sulphide; OXI: Oxidase; OPT: Optochin; DCE: D-Cellobiose; ARD; Arginine Dehydrolase; ODX: Ornithine Decarboxylase; LIP: Lipase; ONPG: Beta-galactosidase; HYA: Hyaluronidase. FRU: Fructose; RAF: Raffinose; MAN: Mannitol; MAL: Maltose; GAL: Galactose; LAC: Lactose; GLU: Glucose; SUC: Sucrose; nd: Not determined; C-rod: Cocco-bacillus; +: Positive; -: Negative.

Table 2: Antibiotic Susceptibility Profiles of Bacterial Isolates

Bacterial Isolates	Codes	Antibiotic Susceptibility														
		CIP	OFL	LEV	TIG	ATM	CFT	CFP	CEF	PPC	AMP	ERT	MER	GEN	STR	INF
S. aureus	SA01	S	R	S	S	S	S	S	S	R	S	S	S	S	S	NMDR
	SA10	S	I	S	R	S	S	I	S	I	I	S	S	R	S	NMDR
	SA23	S	S	S	R	R	S	R	R	R	S	R	S	S	S	MDR
S. gallinarium	SG34	S	S	S	S	I	S	S	S	S	R	S	I	S	R	NMDR
S. pneumoniae	SP06	S	S	S	S	S	S	S	S	R	S	S	S	S	S	NMDR
	SP20	S	I	S	R	R	S	S	I	S	S	R	I	R	R	MDR
H. influenzae	HI02	I	S	S	R	R	S	S	S	R	R	S	S	S	S	NMDR
	HI11	S	I	R	S	S	S	S	S	S	I	R	S	I	S	NMDR
	HI15	R	R	R	R	S	S	I	I	R	R	S	S	R	R	MDR
C. freundii	CF13	S	I	S	R	R	S	S	R	S	S	S	S	I	R	MDR

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	CF21	R	R	R	R	R	S	R	S	S	I	R	R	S	S	MDR
S. flexneri	SF08	S	S	S	S	S	I	I	R	R	R	S	S	I	R	NMDR
	SF13	S	I	S	R	S	S	S	S	S	I	S	S	R	R	NMDR
E. vulneris	EV12	R	R	S	S	S	S	S	I	R	R	R	R	S	S	MDR
E. coli	EC28	R	R	R	R	R	S	S	R	R	S	R	R	S	S	MDR
	EC33	S	S	S	R	R	S	S	R	S	S	R	R	R	R	MDR
S. marcescens	SL25	I	S	S	S	S	S	S	I	I	R	S	R	S	S	NMDR
	ST07	S	S	S	R	S	S	S	S	S	I	S	S	R	R	NMDR
S. typhi	ST19	S	S	S	R	R	R	R	R	R	R	S	S	R	R	XDR
	ST33	S	S	S	R	R	I	R	R	R	R	S	S	I	S	MDR

CIP: Ciprofloxacin, OFL: Ofloxacin; LEV: Levofloxacin; TIG: Tigemonam; ATM: Aztreonam; CFT: Ceftazidime; CFP: Cefoperazone; CEF; Ceftriazone; PPC: Piperacillin; AMP: Ampicillin; ERT: Ertapenem; MER: Meropenem; GEN: Gentamycin; STR: Streptomycin; R: Resistant; I: Intermediate; S: Sensitive; NMDR: Non- multidrug resistant; MDR: Multidrug resistant; XDR: Extensively drug resistant; INF: Inference.

Table 3: Bioactive Compounds in Aqueous Leaf Extracts of *Lasianthera africana* using GCMS

Peak	RT (min.)	Peak (%)	Area	Chemical Compound Name	Mol. Weight
1	6.365	45.72		L-Lactic acid	90.07
2	6.742	1.95		1,3-Dioxolane, 2-(2,4-dimethylphenyl)-2,4,5-trimethyl	220.01
3	7.000	0.51		Pantolactone	130.01
4	7.133	1.48		Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside	194.18
5	7.308	0.48		2-Trichloromethyl-1-bicyclo[2.2.1]heptane	130.62
6	7.517	0.71		3-Dimethylsilyloxytetradecane	312.60
7	7.707	0.66		1,3-Dioxolane, 2-cyclohexyl-4,5-dimethyl-	184.27
8	7.775	1.39		Levoglucosenone	126.11
9	8.496	0.87		5-Butyldihydro-2(3H)thiophenone	142.19
10	9.192	0.31		Benzoic acid	122.12
11	9.278	0.49		Isosorbide	191.14
12	9.547	0.40		Cyclopropane, 1,1-dichloro-2,2-dimethyl-3-(2-methylpropyl	195.13
13	9.642	0.40		Benzofuran, 2,3-dihydro-	120.15
14	9.894	0.48		Cyclohexane, (1-methylethylidene)-	124.22
15	10.285	0.51		5-Octenoic acid, 6-methyl-	156.23
16	10.500	0.61		2-Methoxy-4-vinylphenol	150.17
17	12.076	0.26		4-Amino-1,5-pentandioic acid	132.11
18	12.293	0.26		1-Naphthalenecarboxylic acid, decahydro-1,4a-dimethyl-	302.45
19	12.574	0.32		1-Naphthol, 4-amino-2-methyl-	173.21
20	12.907	0.39		Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	160.25
21	13.234	0.29		3-Cyclopentylpropionic acid, 2-isopropoxyphenyl ester	226.31
22	13.303	0.43		3-Phenylpropanoic acid, dodec-9-ynyl ester	150.17
23	13.379	0.38		Cyclohexanol, 1-(2-nitropropyl)-	187.24
24	13.484	0.89		5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	150.21
25	13.592	0.24		5-(.omega.-Aminopropyl)-3-amino-4-cyano-isoxazole	194.62
26	13.677	0.25		2H-Pyran-2-one, 5,6-dihydro-4-(2-methyl-2-propen-3-yl)-	152.23
27	13.740	1.71		Dispiro[2.0.2.1]heptane-1-carboxylic acid, 1-methyl-	94.15
28	13.937	1.45		Cyclohexanecarboxylic acid, 2-(2-propenyl)-, methyl-	128.17

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29	14.092	2.08	3-Oxabicyclo[3.3.0]octan-2-one, 7-methylene-4,dimethyl	138.16
30	14.350	0.61	1-(4-Methyl-2-pyridyl)-1-pentanone semicarbazon	157.21
31	14.483	0.91	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl	210.31
32	14.741	0.60	2-Furanacetaldehyde	124.09
33	14.867	0.32	3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone	168.23
34	15.002	2.11	4-Isobenzofuranol, octahydro-3a,7a-dimethyl-	319.36
35	15.128	1.78	9-Oxa-bicyclo[3.3.1]non-6-ene-3-carboxylic acid, methyl	221.68
36	15.257	0.71	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	182.26
37	15.383	0.59	3-Azabicyclo[3.2.2]nonane, 3-nitroso-	125.21
38	15.533	0.24	1-Allyl-3-phenyl-2-thiourea	192.28
39	15.787	0.34	4-Acetonylcycloheptanone	154.21
40	16.008	9.41	Pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxypropyl)-	116.11
41	16.375	0.25	1,13-Tetradecadien-3-one	208.34
42	16.449	0.25	Hexadecanoic acid, methyl ester	270.45
43	17.145	0.29	Cholestan-3-ol, 2-methylene-	400.70
44	17.667	0.31	5-Cholestene-3.beta.,26-diol-22-one diacetate	482.70
45	17.801	0.41	Bufa-20,22-dienolide, 14,15-epoxy-3-hydroxy-19-	442.50
46	18.517	0.46	24-Norchol-22-ene-3,7-diol, diacetate	158.15
47	18.615	1.71	Ergost-5-en-3-ol, acetate, (3.beta.,24R)-	442.71
48	19.999	1.71	Stigmasta-5,22-dien-3-ol, acetate,	454.72
49	20.744	0.31	Stigmastan-3,5-diene	396.69
50	21.071	1.47	Stigmasta-5,22-dien-3-ol, acetate	454.72
51	21.342	0.24	A'-Neogammacer-22(29)-en-3-one	424.70
52	21.583	4.38	Stigmastan-3,5-diene	396.69
53	21.902	0.37	Anthraegostatrine	380.64
54	22.127	0.32	dl-.alpha.-Tocopherol	416.68
55	22.311	1.34	3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-	410.70
56	23.484	0.79	7-Dehydrodiosgenin	412.60
57	23.841	0.88	Anthiaergostan-5,7,9,22-tetraen-3-one	394.60
58	24.266	0.31	3-Phenoxybenzyl 2,3,4,5,6-pentafluorobenzoate	212.07
59	24.724	0.71	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester-	470.70

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Table 4: Antibacterial Activity of Aqueous Leaf Extracts of *Lasianthera africana*

		Mean Zones of Inhibition (x̄ ± S.D)					
Bacterial Isolates	**Code**	**12.5 µg/mL**	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**ACA**	**DMSO**
S. aureus	SA01	10.7 ± 0.5[a]	12.3 ± 1.1[a]	15.0 ± 1.0[b]	18.1 ± 1.0[c]	16.7 ± 0.5[b]	NZ
	SA10	9.0 ± 0.0[a]	10.5 ± 0.5[a]	12.6 ± 0.1[a]	13.2 ± 0.1[b]	15.0 ± 0.0[b]	NZ
	SA23	NZ	NZ	NZ	9.8 ± 0.2[a]	10.5 ± 0.5[a]	NZ
S. gallinarium	SG34	11.6 ± 0.2[a]	12.0 ± 0.5[a]	15.3 ± 0.2[b]	17.7 ± 1.2[c]	19.1 ± 1.1[c]	NZ
S. pneumoniae	SP06	12.0 ± 0.0[a]	13.1 ± 1.0[b]	14.5 ± 0.5[b]	17.4 ± 1.0[c]	12.0 ± 1.0[a]	NZ
	SP20	7.5 ± 0.5[a]	11.9 ± 0.4[a]	14.0 ± 0.0[b]	15.8 ± 0.5[b]	12.5 ± 0.0[a]	NZ
H. influenzae	HI02	NZ	NZ	11.1 ± 0.1[a]	14.0 ± 0.0[b]	16.8 ± 0.8[b]	NZ
	HI11	8.4 ± 0.1[a]	10.0 ± 0.0[a]	12.6 ± 0.4[a]	15.5 ± 0.5[b]	16.0 ± 0.0[b]	NZ
	HI15	9.0 ± 0.0[a]	11.8 ± 0.2[a]	15.0 ± 0.2[a]	16.7 ± 1.0[b]	18.1 ± 1.3[c]	NZ
C. freundii	CF13	8.7 ± 0.2[a]	10.0 ± 1.0[a]	13.3 ± 0.3[b]	16.0 ± 1.0[b]	15.5± 0.5[b]	NZ
	CF21	NZ	9.1± 0.1[a]	11.5 ± 0.0[a]	14.5 ± 0.3[b]	17.0 ± 0.0[c]	NZ
S. flexneri	SF08	9.4 ± 0.1[a]	12.5 ± 0.1[a]	13.9 ± 0.4[b]	15.1 ± 0.5[b]	17.7 ± 0.7[c]	NZ
	SF13	NZ	10.5 ± 0.2[a]	11.0 ± 0.2[a]	14.3 ± 0.1[b]	17.5 ± 1.0[c]	NZ
E. vulneris	EV12	8.0 ± 0.0[a]	10.6 ± 0.3[a]	11.5 ± 0.5[a]	13.0 ± 0.0[b]	16.0 ± 1.0[b]	NZ
E. coli	EC28	NZ	NZ	NZ	13.3 ± 0.3[a]	13.4 ± 0.1[a]	NZ
	EC33	11.9 ± 0.1[a]	13.5 ± 0.5[b]	15.0 ± 1.0[b]	17.6 ± 1.1[c]	15.2 ± 0.7[b]	NZ
S. marcescens	SL25	NZ	11.1 ± 0.1[a]	12.9 ± 0.4[a]	14.0 ± 0.5[b]	NZ	NZ
S. typhi	ST07	10.1 ± 0.2[a]	13.8 ± 1.2[b]	16.0 ± 0.5[b]	18.9 ± 1.0[c]	16.0 ± 1.0[b]	NZ
	ST19	10.6 ± 0.1[a]	13.3 ± 0.5[b]	15.7 ± 0.3[b]	18.2 ± 1.2[c]	17.6 ± 1.2[c]	NZ
	ST33	12.3 ± 0.1[a]	14.0 ± 0.0[b]	16.5 ± 0.5[b]	19.5 ± 1.0[c]	17.3 ± 1.0[c]	NZ

x̄: Mean; S.D: Standard Deviation; ACA: Amoxicillin/Clavulanic Acid; NZ: No zone of Inhibition; DMSO: Dimethyl Sulphoxide. Mean within the column followed by the different superscript letters are significant as determined by Duncan multiple range test ($p < 0.05$).

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Table 5 : Minimum Inhibitory and Minimum Bactericidal Concentrations of Aqueous Leaf Extracts of *Lasianthera africana* on NMDR, MDR, and XDR-Bacterial Isolates

			Concentrations of ALELA (µg/mL)						Conc. (µg/mL)		MBC / MIC
Bacterial Isolates	**Code**	**ASP**	**6.25**	**12.5**	**25**	**50**	**100**	**200**	**MIC**	**MBC**	**Ratio**
S. aureus	SA01	NMDR	+	+	-	-	-	-	12.5	25	2
	SA10	NMDR	+	+	-	-	-	-	12.5	100	4
	SA23	MDR	+	+	+	+	+	-	100	200	2
S. gallinarium	SG34	NMDR	+	-	-	-	-	-	6.25	25	4
S. pneumoniae	SP06	NMDR	+	-	-	-	-	-	6.25	25	4
	SP20	MDR	+	+	-	-	-	-	12.5	25	2
H. influenzae	HI02	NMDR	+	+	+	+	-	-	50	100	2
	HI11	NMDR	+	+	-	-	-	-	12.5	50	4
	HI15	MDR	+	+	-	-	-	-	12.5	50	4
C. freundii	CF13	MDR	+	+	-	-	-	-	12.5	25	2
	CF21	MDR	+	+	+	-	-	-	25	25	1
S. flexneri	SF08	NMDR	+	+	-	-	-	-	12.5	25	2
	SF13	NMDR	+	+	+	-	-	-	25	50	2
E. vulneris	EV12	MDR	+	+	-	-	-	-	12.5	50	4
E. coli	EC28	MDR	+	+	+	+	+	-	100	200	2
	EC33	MDR	+	-	-	-	-	-	6.25	6.25	1
S. marcescens	SL25	NMDR	+	+	+	-	-	-	25	100	4
S. typhi	ST07	NMDR	+	+	-	-	-	-	12.5	25	2
	ST19	XDR	+	+	-	-	-	-	12.5	25	2
	ST33	MDR	+	-	-	-	-	-	6.25	25	4

Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; - : No growth; +: Growth; NMDR: Non-Multidrug Resistant, MDR: Multidrug Resistant; XDR: Extensively Drug Resistant; ASP: Antibiotic Susceptibility Profile; ALELA: Aqueous Leaf Extract of *Lasianthera africana*

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Table 6: Median Lethal Dose (LD_{50}) of ALELA on Experimental Wistar Rats

(i) Modified arithmetic method of Reed and Muench

Conc. (mg/kg)	Log conc.	Wistar Rats		Cumulative Total		Mortality	
		Dead	Survived	Dead	Survived	Ratio	Percentage
1500	3.1761	0	7	0	17	0/17	0.0
2000	3.3010	2	5	2	10	2/12	16.7
2500	3.3979	4	3	6	5	6/11	54.5
3000	3.4771	5	2	11	2	11/13	84.6
3500	3.5441	7	0	18	0	18/18	100

$$\text{Proportionate Distance (P.D.)} = \frac{50\% - \text{Next below } 50\%}{\text{Next above } 50\% - \text{Next below } 50\%} = \frac{50 - 16.7}{54.5 - 16.7} = \frac{33.3}{37.8} = 0.881$$

Log 2500 – Log 2000 = 3.3979 - 3.3010 = 0.0969
0.881 x 0.0969 = 0.0854
Log LD_{50} = Log 2000 + 0.0854 = 3.3010 + 0.0854 = 3.3864
LD_{50} = Antilog 3.3864 = 2434 mg/kg.

(ii) Karber's Method

Group	Conc. (mg/kg)	Wistar rats in each group	No of Dead Wistar rats	Dose Difference (a)	Mean Mortality (b)	Probit (a x b)
1	1500	7	0	0	0	0
2	2000	7	2	500	1	500
3	2500	7	4	500	3	1500
4	3000	7	5	500	4.5	2250
5	3500	7	7	500	6	3000

,

N = number of Wistar rats in each group = 7; LD_{100} = 3500
$\Sigma (a \times b) = 0 + 500 + 1500 + 2250 + 3000 = 7250$

$$LD_{50} = LD_{100} - \frac{\Sigma (a \times b)}{N} = 3500 - \frac{7250}{7} = 3500 - 1036 = 2464 \text{ mg/kg.}$$

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The LD_{50} of ALELA on experimental Wistar rats was 2434 mg/kg and 2464 mg/kg using the arithmetic method of Reed and Muench, and Karber's method, respectively (Table 6). The LD_{50} of ALELA on experimental Wistar rats was greater than 2000 mg/kg and according to Hodge and Sterner [34] toxicity scale, this extract is slightly toxic. Table 7 shows a marked reduction in the mean CD4+ T-lymphocyte values of Wistar rats infected with 1.5×10^6 CFU/mL of XDR -*S. typhi* ST19 from 8.9 ± 0.1 to 6.5 ± 0.0 cells/µL after 2 wk. There was a slight increase of 0.3 cells/µL in the mean CD4+ T-lymphocyte values of Wistar rats administered with water. The mean CD4+ T-lymphocyte values of Wistar rats administered with 1000 mg/kg and 2000 mg/kg ALELA ranged from an initial 8.2 ± 0.2 and 8.8 ± 0.5 cells/µL to 10.3 ± 0.3 and 11.5 ± 0.5 cells/µL, respectively. The CD4+ T-lymphocyte values of Wistar rats administered with 1000 mg/kg ALELA and infected with 1.5×10^6 CFU/mL of XDR-*S. typhi* ST19 increased by 0.4 cells/µL in the first week and by 1.0 cells/µL in the second week. The mean CD4+ T-lymphocyte values of Wistar rats administered with 2000 mg/kg ALELA and infected with 1.5×10^6 CFU/mL of XDR-*S. typhi* ST19 increased by 1.7 cells/µL in the second week. An increase of ≤ 1.3 cells/µL in the mean CD4+ T-lymphocyte values in Wistar rats infected with 1.5×10^6 CFU/mL of XDR-*S. typhi* ST19 and given 1000 mg/kg of Ciprofloxacin was obtained (Table 7). A better prognosis and more active immune response to infections are indicated by an increase in CD4+ T-lymphocytes.[35] In our investigation, Wistar rats administered with 1000 mg/kg and 2000 mg/kg of ALELA had increases in $CD4^+$ T-lymphocyte levels within 2 weeks. This findings corroborates the reports of Fitrya et al. [23] on the increase in $CD4^+$ in their study on the immunomodulatory effect of *P. speciosa* pods extract on rat induced by *S. typhimurium.*

Table 8 shows the effect of ALELA and ciprofloxacin on the microbial loads of Wistar rats with or without XDR-*S. typhi* ST19 (1.5×10^6 CFU/mL). The mean microbial loads of Wistar rats given water increased from $3.1 \pm 0.3 \times 10^9$ to $3.9 \pm 0.8 \times 10^9$ CFU/mL after incubation for 144 h. There was a marked increase in the mean microbial loads of Wistar rats infected with XDR-*S. typhi* ST19 from $8.0 \pm 0.1 \times 10^{10}$ to $5.5 \pm 0.2 \times 10^{12}$ CFU/mL. The mean microbial loads (CFU/mL) of Wistar rats given 1000 mg/kg and 2000 mg/kg ALELA were significantly reduced from $5.3 \pm 0.0 \times 10^9$ and $1.6 \pm 0.1 \times 10^9$ to $2.8 \pm 0.1 \times 10^8$ and $1.3 \pm 0.0 \times 10^6$, respectively. The Wistar rats given 1000 mg/kg ALELA and infected with XDR-*S. typhi* ST19 had a mean microbial load reduction from $4.2 \pm 0.1 \times 10^{10}$ to $9.7 \pm 0.3 \times 10^8$ CFU/mL. The mean microbial loads of Wistar rats given 2000 mg/kg ALELA and infected with XDR-*S. typhi* ST19 decreased from $4.0 \pm 0.5 \times 10^{10}$ to $6.5 \pm 0.2 \times 10^8$ CFU/mL. A marked reduction from $1.0 \pm 0.0 \times 10^{10}$ to $5.5 \pm 0.1 \times 10^8$ CFU/mL in mean microbial loads was obtained in Wistar rats infected with XDR-*S. typhi* ST19 and given 2000 mg/kg of Ciprofloxacin (Table 8). The succession patterns of the gastrointestinal flora of Wistar rats administered with water, ALELA, Ciprofloxacin, and/or infected with XDR-*S. typhi* ST19 at 0, 48, 96, and 144 h of incubation are presented in Table 8. The reduction in microbial load and effects of ALELA on the gastrointestinal flora were similar to the reports by Adebolu et al.[36] and Nyegue et al. [37] in their studies on the effect of *A. sativum* on *S. typhi* and the effect of *P. pinnata* on *S. flexneri* - induced diarrhoea in Wistar rats, respectively. Our study shows that ingestion of raw ALELA might disrupt the equilibrium of microbial flora in the gastrointestinal tracts of consumers and could effectively reduce the population of pathogenic microorganisms.

Table 7: Effect of ALELA and Ciprofloxacin on CD4+ T- Lymphocyte Values of Wistar Rats Infected with XDR-*S.typhi*

Grp	Treatments	Dose	CD4+ Counts (cells/µL)				
			'0' wk	1 wk	Df	2 wk	Df
1	Water	10 mL	9.2 ± 0.1^a	9.5 ± 0.1^a	+ 0.3	9.5 ± 0.5^a	+ 0.3
2	XDR-*S. typhi* ST19	1.5×10^6 CFU/mL	8.9 ± 0.1^a	7.3 ± 0.3^a	- 1.6	6.5 ± 0.0^a	- 2.4
3	ALELA	1000 mg/kg	8.2 ± 0.2^a	10.0 ± 0.0^b	+ 1.8	10.3 ± 0.3^b	+ 2.1
4	ALELA	2000 mg/kg	8.8 ± 0.5^a	10.6 ± 0.5^b	+ 1.8	11.5 ± 0.5^b	+ 2.7
5	ALELA + XDR-*S. typhi* ST19	1000 mg/kg + 1.5×10^6 CFU/mL	8.1 ± 0.2^a	8.5 ± 0.0^a	+ 0.4	9.1 ± 0.2^a	+ 1.0
6	ALELA + XDR-*S. typhi* ST19	2000 mg/kg + 1.5×10^6 CFU/mL	8.5 ± 0.5^a	9.7 ± 0.2^a	+ 1.2	10.2 ± 0.5^b	+ 1.7
7	Ciprofloxacin + XDR-*S. typhi* ST19	1000 mg/kg + 1.5×10^6 CFU/mL	9.0 ± 1.0^a	9.8 ± 1.0^a	+ 0.8	10.3 ± 1.0^b	+ 1.3

ALELA: Aqueous Leaf Extract of *Lasianthera* africana; CFU: Colony Forming Units; XDR: Extensively Drug Resistant; df: Difference.

Table 8: Effect of ALELA and Ciprofloxacin on Microbial Loads and Gastrointestinal Flora of Wistar Rats Infected with XDR-*S. typhi*

Grp	Treatment	Time (h)	PC (CFU/mL)	% Redu./ Incr.	Bacterial Isolates
1	Water (10 mL)	0	$3.1 \pm 0.3 \times 10^9$	NA	*S. aureus, E coli, C. freundii, P. substilis, E. faecalis*
		48	$3.2 \pm 0.3 \times 10^9$	*3.13	*S. aureus, E coli, C. freundii, P. substilis, E. faecalis*
		96	$3.5 \pm 0.0 \times 10^9$	*8.57	*S. aureus, E coli, C. freundii, P. substilis, E. faecalis, E. aerogenes*
		144	$3.9 \pm 0.8 \times 10^9$	*10.26	*S. aureus, E coli, C. freundii, P. substilis, E faecalis, E. aerogenes*
2	XDR-*S. typhi* ST19 (1.5×10^6 CFU/mL)	0	$8.0 \pm 0.1 \times 10^{10}$	NA	*S. aureus, E coli, C. freundii, P. aeruginosa,, E faecalis, A. baumannii*
		48	$4.4 \pm 0.5 \times 10^{11}$	*81.82	*S. aureus, E coli, C. freundii, P. aeruginosa, E faecalis, A. baumannii, S. typhi*
		96	$1.7 \pm 0.5 \times 10^{12}$	*74.12	*S. aureus, E coli, C. freundii, P. aeruginosa, E faecalis, A. baumannii, S. typhi*
		144	$5.5 \pm 0.2 \times 10^{12}$	*69.01	*S. aureus, E coli, C. freundii, P. aeruginosa, E faecalis, A. baumannii, S. typhi*
3	ALELA (1000 mg/kg)	0	$5.3 \pm 0.0 \times 10^9$	NA	*K. oxytoca, Shigella* sp., *E coli, C. freundii, P. substilis, E. aerogenes*
		48	$1.3 \pm 0.2 \times 10^9$	75.24	*K. oxytoca, Shigella* sp., *E coli, C. freundii, E. aerogenes*
		96	$5.7 \pm 0.5 \times 10^8$	56.15	*K. oxytoca, Shigella* sp., *C. freundii, E. aerogenes*
		144	$2.8 \pm 0.1 \times 10^8$	50.88	*K. oxytoca, Shigella* sp., *E. aerogenes*
4	ALELA (2000 mg/kg)	0	$1.6 \pm 0.1 \times 10^9$	NA	*S. aureus, E coli, Shigella* sp., *P.mirabilis, A. baumannii E. faecalis*
		48	$5.8 \pm 0.2 \times 10^7$	96.38	*S. aureus, E coli, A. baumannii E. faecalis*
		96	$8.2 \pm 0.2 \times 10^6$	85.90	*S. aureus, A. baumannii E. faecalis*
		144	$1.3 \pm 0.0 \times 10^6$	84.15	*A. baumannii, E. faecalis*
5	ALELA (1000 mg/kg) + XDR-*S. typhi* ST19 (1.5×10^6 CFU/mL)	0	$4.2 \pm 0.1 \times 10^{10}$	NA	*S. aureus, E coli, Shigella* sp., *C. freundii, P. substilis, E. aerogenes, K. oxytoca*
		48	$1.0 \pm 0.5 \times 10^{10}$	76.19	*S. aureus, E coli, Shigella* sp., *E. aerogenes, S typhi*
		96	$3.0 \pm 0.3 \times 10^9$	70.00	*S. aureus, E coli., E. aerogenes, S typhi*
		144	$9.7 \pm 0.3 \times 10^8$	67.66	*S. aureus, E. aerogenes*

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6	ALELA (2000 mg/kg) + XDR-*S. typhi* ST19 (1.5×10^6 CFU/mL)	0	$4.0 \pm 0.5 \times 10^{10}$	NA	*C. freundii, E coli, P. substilis, K. oxytoca , E. aerogenes, E. faecalis, S. aureus*
		48	$8.6 \pm 0.1 \times 10^{9}$	78.50	*C. freundii, P. substilis, E. aerogenes, S. aureus, S typhi*
		96	$2.1 \pm 0.5 \times 10^{9}$	75.58	*P. substilis, E. aerogenes, S. aureus*
		144	$6.5 \pm 0.2 \times 10^{8}$	69.04	*P. substilis, S. aureus*
7	CPF (1000 mg/kg) + XDR-*S. typhi* ST19 (1.5×10^6 CFU/mL)	0	$1.0 \pm 0.0 \times 10^{10}$	NA	*E coli, Shigella* spp., *P. aeruginosa, K. oxytoca , A. baumannii, E. faecalis*
		48	$3.2 \pm 0.2 \times 10^{9}$	68.00	*P. aeruginosa, K. oxytoca , A. baumannii, E. faecalis, S. typhi*
		96	$1.2 \pm 0.5 \times 10^{9}$	62.50	*P. aeruginosa,. faecalis, S. typhi*
		144	$5.5 \pm 0.1 \times 10^{8}$	54.17	*P. aeruginosa, E. faecalis*

Conclusion

The study has provided significant insights into the antibacterial efficacy of ALELA against XDR-*S. typhi* and other gastro-intestinal flora in Wistar rats, suggesting a promising potential for combating bacterial gastro-intestinal tract infections. The observed mild toxicity of ALELA underscores its relative safety, and its positive impact on the CD4+ T-lymphocyte values in Wistar rats infected with XDR-*S. typhi* indicates an immunomodulatory effect.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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