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Antibacterial Activities and Time-killing Kinetics of *Lannea acida* Extracts Against Selected Microbes

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Abstract

The speedy increase of antimicrobial resistance has become a worldwide problem. This has compelled the need to search for novel antimicrobial molecules. *Lannea acida* extracts are rich sources of potential antimicrobial components base on the ethnopharmacological survey. This study investigates the antimicrobial properties of methanolic extract of *Lannea acida*, flavonoid-rich and alkaloid-rich extracts of *Lannea acida*. Agar well diffusion and time-kill kinetic against the bacterial assays were used to determine the antimicrobial activity of the extracts against selected test organisms. The effect of methanolic extract and flavonoid-rich extract on the growth of *B. subtilis, S. aureus, E. coli, K. pneumoniae, E. aerogenes* were investigated and experienced slow growth in the presence of methanolic crude extract and flavonoid-rich extracts in comparison to organism growing in medium alone. Preliminary secondary metabolite screening revealed the presence of tannins, flavonoids, triterpenoids, quinones, and alkaloids in the methanolic extracts of *Lannea acida,* for the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one hour, the growth rate then rose exponentially for bacterial organisms growing in media alone but remained suppressed for organism growing in the presence of extracts for the next 7 hours with percent OD values. The extracts demonstrated activities against the Grams-positive and Grams-negative bacterial, which showed a bacteriostatic effect in killing rate kinetics. *Lannea acida* extracts exhibited antimicrobial activity and may contain bioactive compounds which may serve as potential antibacterial and antifungal agents.

Keywords

Lannea acida, Antibacterial, Microorganism

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1. Introduction

Infectious diseases pose serious threats to the human existence, health, and survival of mankind [1]. The World Health Organization (WHO) survey in 2019 showed that infectious diseases caused 32% of deaths worldwide with 68% of the deaths occurring in Africa [2]. Infectious diseases still account for a great percentage of death worldwide and in some regions remain the most important cause of diseases [2]. Apart from affecting the health of individuals directly, infectious diseases have substantial effect on whole societies and economies [3]. The discovery of penicillin and subsequent development and synthesis of other antibiotics had been a milestone in the history of medication. However, this medical breakthrough is being lost to the development and rapid spread of bacterial resistance to antimicrobial agents [4]. Globally, the occurrence of antimicrobial resistant bacterial strains [5] is increasingly limiting the potency of current drugs and significantly causing failure of treating infections [6]. This situation shows that the potencies of

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prevalent antibiotics are decreasing gradually [7]. Therefore, there is a great need to develop novel drugs to combat this pathogenic microorganism that have developed widespread microbial resistance to antibiotics [8]. Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in search for new antimicrobial agents is the need of the time [9]. The use of natural products has been extremely successful in the discovery of new medicine, and *Lannea species extracts* could be a source of natural antimicrobials [10]. Ethno survey indicate that *Lannea species* contain many biologically active components that offer health benefits and protection against diseases [11] and are responsible for their immunomodulation [12], anti-inflammatory [13], antioxidant [14], and antimicrobial activities [15]. *Lannea species* have been reported in several studies to be one of the most promising sources for obtaining natural compounds that can act as anti-infective agents. However, the antimicrobial properties of *Lannea acida* have not been studied, this has necessitated the need for the continuous screening of *Lannea acida* for their antimicrobial activities. *Lannea acida plants* belong to the family (anacardiaceae) commonly called, awere kogun in Akoko area of ondo state, akogun in ondo town and are used in traditional medicine in the management of infectious diseases majorly malaria. *Lannea acida* is one of the most widely distributed of the *Lannea* species found in the hot and dry savannahs of sub-Saharan Africa. It has a rich history of ethnobotanical and ethnopharmacological usage in the treatment of a wide range of illnesses including malaria, rheumatism, dysentery and haemorrhoids, Barks of *L. acida* are traditionally used in Nigeria as antiabortifacient, vermifuge and to treat anal haemorrhoids, diarrhoea, dysentery, malnutrition, and debility while the leaves is used to treat rheumatism. Information provided by the traditional healer in Akoko area of ondo state revealed that the bark aqueous or alcoholic extract is used in treatment of many infectious diseases. Even though *Lannea acida* demonstrated biological activity that validate their medicinal roles, no phytochemical studies were performed to isolate the chemical constituents responsible for the observed activity. With this view, the present study was to evaluate the antioxidant and antibacterial activities of the methanolic extract of *Lannea acida* in order to provide scientific evidence for its continuous usage in ethno therapeutic management of infectious diseases.

2. Materials and Methods

2.1. Experimental Plant Material

The bark of *Lannea acida* was collected from Ugbe town from a location ($7^0$15′42.9″N $5^0$15′01.9″E), in Ikare Akoko area of Ondo state and was authenticated at the Plant Science Department of Adekunle Ajasin University Botanic Garden Herbarium, and a sample specimen deposited at the herbarium for future reference.

2.2. Extraction and Preparation of the Methanolic Back Extract of *L. acida*

The back of *L. acida* was allowed to dry at room temperature. They were pulverized in mechanized laboratory grinder (Manesty, England) to fine powder. The dried back weighing 1.6 kg were soaked in 5.5 L of absolute methanol. The mixture was thoroughly mixed and filtered after 72 hr using a Buchner vacuum filter. The filtered supernatant was evaporated to dryness with a Rotary evaporator. The percentage yield of the extract was determined according to the expression provided [16].

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \text{ X } 100$$

2.3. Extraction of Flavonoid - rich Fraction

A portion of the methanolic extract was dissolved in 100ml (1:4) of 1% H_2SO_4 in a small flask and was hydrolysed by heating on a water bath until the mixture was half of its volume (30minutes). The mixture was placed on ice for 15minutes, so as to allow flavonoids precipitated. The cooled solution was filtered. The filtrate (flavonoids aglycone mixture) was dissolved 50mls of warm 95% ethanol (50°C), the resulting solution was again filtered and the filtrate was concentrated to dryness using rotary evaporator [17].

2.4. Extraction of Alkaloid - rich Fraction

The fraction was prepared by weighing 20g of the methanolic extract into a beaker containing 300ml of warm distilled water (37°C). The crude extract was allowed to dissolve before transferring it into a separatory funnel. Few drops of conc. H_2SO_4 were then added to make the solution acidic (the solution was tested with litmus paper). After this, it was decanted, and the residue was dissolved in water (test for acidity) and decant the solution, repeat this step severally until u are sure all the alkaloids have been extracted. To the filtrate add NH_3 until alkali (test with litmus paper then add chloroform until complete extraction of alkaloids was obtained. After each extraction with chloroform, the test for alkaloids was carried out. [17].

2.5. Preliminary Pharmacological Screening of the Crude Extracts

The methanolic extract was screened for the presence of some secondary metabolite such as saponin, tannin, alkaloids, terpenoids, steroid, Quinone, flavonoids and cardiac glycosides as directed by sofowora [18]. Phytochemical screening

involves performing simple chemical tests on the sample for the purpose of detecting different phytochemicals present.

2.6. Antimicrobial Assay

2.6.1. Microorganisms and Culture Conditions

The bacterial strains used are Grams positive (*Staphylococcus aureu, and Bacillus substilis)* and Gram negative (, *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter aerogenes),* was obtained from microbiology department, Federal university of Technology Akure, Nigeria and was cultured aerobically at 37°C in nutrient agar medium. Before experimental use, cultures from solid medium will be sub-cultivated in liquid media, incubated for 24hr and was used as source of inoculums for each experiment. Antimicrobial activity was measured using agar-well diffusion method.

2.6.2. Antibiotics

Ciprofloxacin, Erythromycin and tetracycline were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands) and linezolid was obtained from Pfizer BV (Capella an den Ijssel, the Netherlands) as the 2 mg/mL infusion. Water was used as a solvent for preparing the stock solutions. Stock solutions were stored at -80 ^{o}C; prior to each experiment, one aliquot was thawed to prepare the different concentrations to be tested.

2.6.3. Antimicrobial Screening of *L. acida* Crude Extracts on Selected Pathogens

Antibacterial assay of the plant extracts of *L. acida* and fractions were tested by disc-diffusion method. The minimal inhibition concentrations (MIC) of the extracts were determined. Five bacterial strains, *Staphylococcus aureu, Bacillus substilis, Escherichia coli*, *Klebsiella pneumoniae, and Enterobacter aerogenes* were used in this study. The bacteria were tested for purity by culturing on nutrient agar and maintained on nutrient agar slants.

2.6.4. Antibacterial Activity of the Extracts

Susceptibility of bacteria isolate to plant extract was determined following the BSAC Diffusion Method for Antimicrobial Susceptibility Testing Version 9.1 [19]. This test was carried out to determine the antimicrobial ability of the plant extract to inhibit the growth of the bacteria isolate. The plate diffusion technique [20] was used for the antibiotic sensitivity test. Overnight cultures of the organisms were swabbed on sterile Muller Hilton solidified Agar plates using sterile swab sticks. 8mm sized cork borer was used to bore hole on the agar surface at equidistance the well was filled with the diluted plant extract, a known antibiotic was used as positive control while distilled water was used as negative control. All the plates were incubated at 37°C to 24 hours. The zones of inhibition generated by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive (S), Intermediate (I) and resistant (R). The zones of inhibition were measured and interpreted according to [20]. The zone of inhibition was compared with that of Ciprofloxacin, Erythromycin and tetracycline.

2.6.5. Determination of the Minimum Inhibitory Concentration (MIC)

Four concentrations of each extract (10, 20, 30 and 50 mg/ml) was prepared. The antimicrobial effect of each concentration was measured. The various concentrations were loaded onto 6 mm disks which was then pressed onto already prepared Mueller-Hinton agar plates and SDA plates. The inoculated plates were incubated at 37°C for 48h. MICs were determined after 24h for the bacteria and after 48h for *L. acida* and its fractions extract zones of inhibition were measured at the end of the incubation period. The MICs was determined as the lowest concentrations of extracts inhibiting the visible growth of each organism on the agar plate.

2.6.6. Effects of Selected Plant Extracts on the Killing Rate of Susceptible Bacteria

In order to determine the bacteriostatic effect of the plant extracts on the growth of bacteria, 1/10 of MICs of the active extracts was incorporated into the broth tubes containing selected susceptible bacteria culture adjusted with McFarland standard to give an OD reading of 0.05. The bacterial culture growing in presence or absence of extracts was incubated at 37◦C. OD readings was taken at 0 minute, 30 minutes and then after each hour till 8 hours. Graph was plotted between OD percent values versus time.

3. Results

3.1. Preliminary Extraction

The percentage yields from the methanolic extracts was 18.26% dry weight. The yield (% w/w) was calculated with respect to the dry weight of the starting material.

3.2. Preliminary Phytochemical Screening

The phytochemical screening of the crude aqueous methanolic extract showed that alkaloids, terpenoids (triterpenoids), flavonoids, tannins, and quinone were present. Flavonoid, Quinone, alkaloids and saponin occurred in high amounts while Terpenoids, Tannin, phenol, Vitmins A and Vitamin C were present in moderate and low concentrations. (Table 1).

Table 1. Qualitative and Quantitative of phytoconstituents in Lannea acida extracts.

Samples	Saponin	Flavonoid	Alkaloid	Tannin	Terpenoids	Phenol	Quinone
Qualitative Analysis	+++	+++	+++	++	+	+	+++
Quantitative Analysis	5.05%	3.30%	0.66%	3.57mg/100g	0.35mg/100g	0.18mg/100g	5.99%

3.3. Antimicrobial Effects of the Methanolic Extract of *Lannea acida*, Flavonoid and Alkaloid Fractions of the Plant

The results of the antimicrobial effects of the three different parts of the plant extracts against the five microorganisms tested are shown in table 2. All microbial strains tested were found to be affected by the bark extracts of *L. acida* as assessed by inhibition zones that ranged from 26.33 mm to 7.66 mm. The extract showed antibacterial activity against both Gram positive and negative bacteria. According to the data presented in table 3, all the tested microbial strains were found to be affected by the three standard antibiotics. (Ciprofloxacin, erythromycin and tetracycline).

3.4. Minimum Inhibitory Concentration (MIC) of the Extracts of *L. acida* Against Test Microbes

MIC was taken to be the lowest concentration of the extracts that completely inhibited the growth of the microorganism. Data are reported as mean inhibition zones (mm) ± SEM of triplicates. The result of the Minimum Inhibitory Concentration (MIC) in Table 3 were found to be the most effective against methanolic extracts, followed by flavonoid-rich extracts and alkaloid-rich extracts. In order to elucidate the antibacterial effect, the zones of inhibition generated by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive (S), Intermediate (I) and resistant (R). The zones of inhibition were measured and interpreted [21]. From the results presented in Table 3, the methanolic *L. acida* extracts had MIC ranging from 20 to 50 mg/ml with MIC of 20 mg/ml for *S. aureus* and *K. pneumoniae.* 30 and 50 mg/ml for *B. subtilis, S. aureus, E. coli, K. pneumoniae* and *E. coli.* The extract was only active against all the tested organisms at the highest concentration tested (50 mg/ml). The MIC ranged from 20 to 50 mg/ml for the flavonoid rich extract of *L. acida.* The extract had MIC of the ranging concentration of 20 to 50 mg/ml for all the tested microbes. The extract was only active against all the tested organisms at the highest concentration tested (50 mg/ml). Alkaloid rich extracts of *L. acida* had MIC of 50 mg/ml for *S. aureus.* But lacked MIC activity activities against *B. subtilis*, *E. coli*, *K. pneumoniae*, *and E. aerogenes* for 10 to 30 mg/ml concentration.

Table 2. Antimicrobial effects of the methanolic extract of Lannea acida, Flavonoid and Alkaloid fractions of the plant. All values are expressed as mean inhibition zones (mm) ± SEM of three replicates. (mm).

Bacterial Strain	*L. acida* extract	Flavonoid Fraction	Alkaloid Fraction	Ciprofloxacin 5µg	Erythromycin 15 µg	Tetracycline 30 µg
Gram positive						
Bacillus subtilis	17.33±0.19	18.66±0.50	10.66±0.19	34.3±0.19	26.66±0.50	20.33±0.19
Staphylococcus aureus	26.33±0.19	15.66±0.69	7.66±0.50	36.33±0.19	37.66±0.50	21.33±0.19
Gram negative						
Escherichia coil	15.33 ±0.19	23.0 ±0.33	8.0 ±0.33	37.33±0.50	36.66±0.88	30±0.33
Klebsiella pneumoniae	23.66±0.19	18.33±0.50	8.33±0.38	32.33±0.50	29.66±1.01	23.66±0.19
Enterobacter aerogenes	13.33±0.19	22±0.33	7.66 ±0.19	12.66±0.50	9.66±0.50	38.33±0.19

Sensitive (S) ≥ 21, Intermediate (I) 20≤ 15 and resistant (R) ≤ 14

Table 3. Minimum Inhibitory concentration (MIC) of methanolic extract, Flavonoid and Alkaloid Fraction of L. acida against test microbes. (mm).

Plant Extracts	Conc mg/ml	*B. subtilis*	*S. aureus*	*E. coli*	*Pneumoniae*	*E. aerogenes*
Methanolic Extract	50	23.66±0.19	24.33±0.19	25±0.33	20.66±0.19	25.33±0.38
	30	23.33±0.19	24.66±0.19	24.33±0.19	19.66±0.19	25.66±0.38
	20	0.00±0.00	15.33±0.19	0.00±0.00	18.66±0.19	0.00±0.00
	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Flavonoid Fraction	50	17.33±0.19	15.33±0.19	23.33±0.19	21.66±0.19	22.66±0.38
	30	13.33±0.19	13±0.33	16.33±0.19	18.33±0.19	20±0.33
	20	10.33±0.19	10.33±0.19	12.0 ±0.33	11.33±0.19	12.33±0.50
	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Alkaloid Fraction	50	9.66±0.19	9.66±0.50	0.00±0.00	0.00±0.00	0.00 ± 0.00
	30	9.33±0.19	9.66±0.34	0.00±0.00	0.00±0.00	0.00±0.00
	20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Sensitive (S) ≥ 21, Intermediate (I) 20≤ 15 and resistant (R) ≤ 14

Footnote: 0 Sign indicate lack of antimicrobial activity by extract against test organism.

3.5. The Effect of the Extracts on Killing Kinetics of B. subtillis, S. aureus, E. coli, K. pneumonie, and E. aerogenes

The effect of selected plant extracts (ME, FR and AR) on the growth of *B. subtilis, S. aureus, E. coli, K. pneumoniae, E. aerogenes* were investigated and experienced slow growth in the presence of methanolic crude extract and flavonoid rich extracts in comparison to organism growing in medium alone. For the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one-hour period, the growth rate then rose exponentially for bacterial organism growing in media alone but remained suppressed for organism growing in the presence of extracts for the next 7 hours with percent OD values. (Figures 1-5).

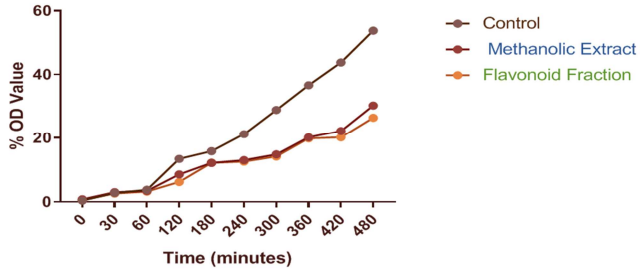

Figure 1. Effect of Lannea acida methanolic extracts and its flavonoid fraction on the growth kinetics of B. subtilis.

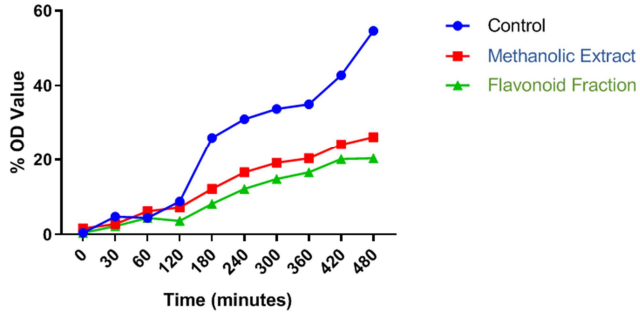

Figure 2. Effect of Lannea acida methanolic extracts and its flavonoid fraction on the growth kinetics of S. aureus.

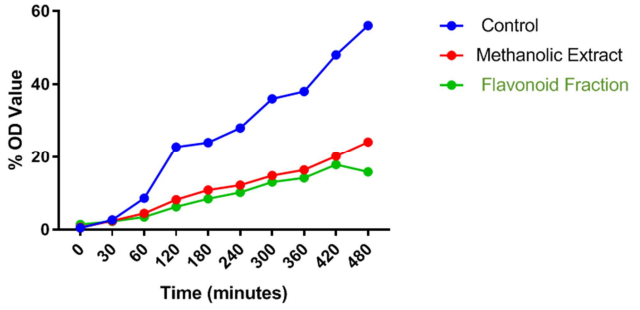

Figure 3. Effect of Lannea acida methanolic extracts and its flavonoid fraction on the growth kinetics of E. coli.

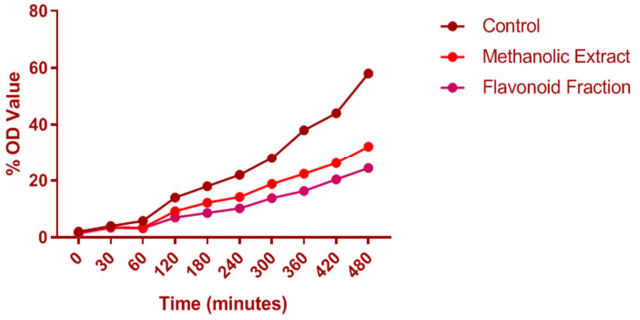

Figure 4. Effect of Lannea acida methanolic extracts and its flavonoid fraction on the growth kinetics of K. pneumonia.

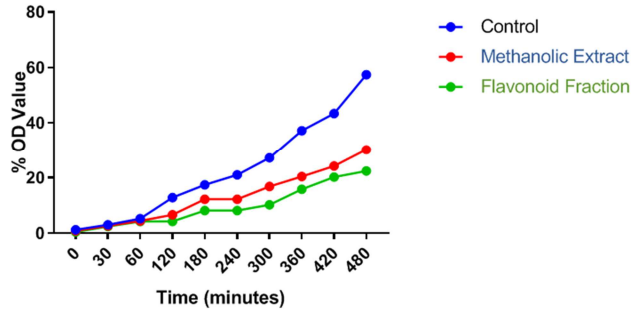

Figure 5. Effect of Lannea acida methanolic extracts and its flavonoid fraction on the growth kinetics of E. aerogenes.

4. Discussion

The pharmacological activities of plant extracts especially their antimicrobial activity has attracted attention recently. The phytoconstituents such as alkaloids, flavonoids and tannins have been reported to possess antibacterial activity and it would therefore suffice to say that the antibacterial activity shown by some of these extracts could be attributed to these compounds. It could be possible that the antibacterial activity seen with the bark extracts of *L. acida* compared to the other *Lannea species* extracts was due to the presence of both flavonoids, alkaloids and other phytoconstituents acting synergistically. Although other extracts exhibited antibacterial activity, it wasn't as great as the bark extracts. This could be explained by the fact that the extracts had only either one kind of phytochemical compound present in them and even so, the phytochemicals may have been present in minute amount to bring about the desired effect. The results of the antimicrobial effects of the different extracts of the plant against the five microorganisms tested compared with the standard antibiotics are shown in Table 2. All microbial strains tested were found to be sensitive by methanolic extract, flavonoid rich and alkaloid rich extracts from *L. acida* bark extracts as assessed by inhibition zones that ranged from ME (13.33 ±0.19 to 26.33 ± 0.19) mm, FR (18.66 ± 0.50 to 25.33± 0.50) mm and AR (9.33± 0.19 to 10.66 ±0.19. The extract showed antibacterial activity against

both Gram positive and negative bacteria. Amongst all the extracts tested in this study ME and FR extracts from *L. acida* bark extracts demonstrated the highest antibacterial activity with an inhibition zone of (26.33±0.19 and 25.33±0.50) mm against *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively, while the lowest inhibition zone was (13.33±0.19 and 18.66±0.50) mm against *Enterobacter aerogenes* and *Bacillus subtilis*. Alkaloid rich extract showed a minimal antibacterial activity with the highest zone of inhibition as 10.66±0.19 mm against *Bacillus subtilis* and *Staphylococcus aureus* respectively, while the lowest inhibition zone was 8.0 ±0.33 against *E. coli*. The antimicrobial effects of standard antibiotic drugs shown that most of the tested microbial strains were sensitive to the effect of ciprofloxacin, erythromycin and tetracycline. The ciprofloxacin and erythromycin exhibit low activity against *Enterobacter aerogenes* in all the three extracts. Tetracycline exhibited its highest antibacterial activity against *Enterobacter aerogenes* for ME, *E. coli* for FR and AR (inhibition zone 38.33±0.19 and 28.66 ±0.19 mm) but had the lowest antibacterial activity against *B. subtilis* (inhibition zone 20.33 ±0.19 and 21.33 ± 0.50 mm) respectively. Ciprofloxacin was observed to be active against all the tested microbes except *Enterobacter aerogenes* showing its highest antibacterial activity against *E. coli* and *S. aureus* for ME, FR and AR (inhibition zone 37.33±0.50 and 34.33 ± 0.50mm) respectively, its lowest antibacterial effect was seen with *Enterobacter aerogenes* which gave a zone of inhibition of (12.66±0.50mm). Erythromycin also displayed better antibacterial activity against all bacterial strain but resistance against *Enterobacter aerogenes*. The highest antibacterial activity of erythromycin was observed against *S. aureus* (inhibition zone 37.66 ±0.50 mm) for ME and (34.33 ±1.67mm) for FR and AR respectively.

The result of the Minimum Inhibitory Concentration (MIC) were found to be the most effective against methanolic extracts, followed by flavonoid-rich extracts and alkaloid-rich extracts. In order to elucidate the antibacterial effect, the zones of inhibition generated by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive (S), Intermediate (I) and resistant (R). The zones of inhibition were measured and interpreted according to (NCCLS, 2000). From the results presented in Table 3, the methanolic *L. acida* extracts had MIC ranging from 20 to 50 mg/ml with MIC of 20 mg/ml for *S. aureus* and *K. pneumoniae.* 30 and 50 mg/ml for *B. subtilis, S. aureus, E. coli, K. pneumoniae* and *E. coli.* The extract was only active against all the tested organisms at the highest concentration tested (50 mg/ml). The MIC ranged from 20 to 50 mg/ml for the flavonoid rich extract of *L. acida. The* extract had MIC of the ranging concentration of 20 to 50 mg/ml for all the tested microbes. The extract was only active against all the tested organisms at the highest concentration tested (50 mg/ml). Alkaloid rich extracts of *L. acida* had MIC of 50 mg/ml for *S. aureus.* But lacked MIC activity activities against *B. subtilis*, *E. coli*, *K. pneumoniae*, *and E. aerogenes* for 10 to 30 mg/ml concentration.

Time-kill kinetic studies indicate the effect of extracts (ME, FR and AR) on the growth of all the microbial strains. *S. aureus* experienced slow growth in the presence of methanolic crude extract and flavonoid rich extracts in comparison to *S. aureus* growing in medium alone. For the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one-hour period, the growth rate then rose exponentially for *S. aureus* growing in media alone but remained suppressed for *S. aureus* growing in the presence of extracts for the next 7 hours with percent OD values ranging from 3.3% to 26.2% compared to percent OD value of *S. aureus* growing in media alone that was almost 55% (Figure 1). *Bacillus subtillis* experienced slow growth in the presence of methanolic crude extract and flavonoid rich extracts in comparison to *Bacillus subtillis* growing in medium alone. For the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one-hour period, the growth rate then rose exponentially for *B. subtillis* growing in media alone but remained suppressed for *B. subtillis* growing in the presence of extracts for the next 7 hours with percent OD values ranging from 6.2% to 30.2% compared to percent OD value of *Subtillis* growing in media alone that was almost 54% (Figure 2). *Escherichia coli* experienced slow growth in the presence of methanolic crude extract and flavonoid rich extracts in comparison to *E. coli* growing in medium alone. For the first one hour, the growth rates for the microorganisms remained the same regardless of whether it was growing in the presence or absence of extracts. After the one-hour period, the growth rate then rose exponentially for *E. coil* growing in media alone but remained suppressed for *E. coil* growing in the presence of extracts for the next 7 hours with percent OD values ranging from 3.4% to 24.2% compared to percent OD value of *E. coil* growing in media alone that was almost 56% (Figure 3). The capacity of methanolic crude extract and flavonoid rich extract of *Lannea acida* to affect the growth rate of *Klebsiella pneumoniae and Enterobacter aerogenes* were also tested. For the first one hour, growth rate remained the same for all the groups of microorganisms growing in the presence or absence of the different extracts. After that the growth rate rose exponentially for most of the groups, although growth rates for groups growing in the presence of extracts remained

low when compared with the growth rate of *Klebsiella pneumoniae and Enterobacter aerogenes* growing in medium alone. The growth rate of *Klebsiella pneumoniae and Enterobacter aerogenes* growing in the presence of the extracts were the most suppressed with percent OD values greatly inhibited (Figures 4 and 5).

5. Conclusion

The extracts of *Lannea acida* exhibited antimicrobial activity. The bacteriostatic action of the extracts was also confirmed by the time-kill kinetic studies. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of pathogenic microorganisms and thus require the host immune system to aid in the elimination of the pathogen, hence there is a need to isolate, purify and elucidate the structure of the molecules from the extracts responsible for the antimicrobial properties which may serve as potential antibiotics.

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Availability of Data and Materials

The data sets analysed in this current study are available from the corresponding author on request.

Consent for Publication

Not applicable.

Competing Interests

The author declared that there is no competing interest.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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