Bioscience and Bioengineering
Vol. 6, No. 1, 2021, pp. 1-9
http://www.aiscience.org/journal/bio
ISSN: 2381-7690 (Print); ISSN: 2381-7704 (Online)

Anti-infective Properties and Time-Killing Assay of *Lannea acida* Extracts and Its Constituents

Ogunsina Olabode Isaiah[1, *], Olusola Augustine Olusegun[1], Otitolaiye Catherine Adesola[2], Ayedogbon Oluremi Samson[1]

[1]Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Nigeria
[2]Department of Biochemistry, Faculty of Science, Sokoto State University, Sokoto, Nigeria

Abstract

Microbial resistance to currently available antimicrobial medicines continues to be a global problem. There has been a tremendous increase in the hunt for additional antibacterial agents from nature in recent years, with plants becoming the primary focus in most regions of the world due to the huge availability of plants that have not been screened for antimicrobial activity. Antibacterial activities of *Lannea acida* extract and its primary components are thus investigated in this work. The antimicrobial activity of *L. acida* and its fractions against *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Klebsiella pnuemoniae*, and *Enterobacter aerogenes,* as well as *Aspergillus favus, Candida albicans, Candida tropicalis, Rhyzopus stolonifera,* and *Fusarium solani,* was determined using agar well diffusion methods. Phytochemical screening of *L. acida* showed the presence of alkaloids, saponins, tannins, flavonoids and terpenoids. The mean zones of growth inhibition for *L. acida* and fractions were in the range of 26.33±0.19 to 11.33±0.19mm and 25.33±0.50 to 10± 0.57 while Erythromycin 15µg is 37.66±0.50 to 9.66±0.50 mm respectively. The antifungi activities showed the range of 28.33 ± 0.19 to 9.66±0.39. MIC of both extract and its fraction ranged from 26.33±0.19 to 15.33±0.19 and 22.66±0.38 to 10.33±0.19 respectively. The time-killing kinetics study showed that *L. acida* and its fractions act as bacteriostatic agents. The observed antimicrobial activity of the extract and its fraction, may be due in large proportion to its major constituent, flavonoids.

Keywords

Lannea acida, Antibiotic Resistance, Antibacterial, Antifungal

Received: August 3, 2021 / Accepted: August 24, 2021 / Published online: August 30, 2021

1. Introduction

Antibacterial drugs are one of the most essential tools in the battle against bacterial infections, and they have significantly improved the quality of health care since their inception in the fight against infectious disease, they have substantially enhanced the quality of health care. However, these health benefits have been jeopardized in recent decades since many routinely used antibacterial agents have been less effective against known susceptible germs due to the advent of resistance strains. [1-4]. Infectious diseases, along with wars and famines, are among the most important elements threatening humanity's survival worldwide, with emerging countries bearing the brunt of the threat. [4-6]. Despite enormous improvements in our understanding of microorganisms and their control, epidemics caused by drug-resistant microbes and the introduction of new disease–causing microorganisms continue to be major public health problems in developed countries. [3]. Furthermore, due to the rise of multidrug resistant bacteria, treatment options for some illnesses have become limited. [7, 8]. In addition to bacterial infections, fungal infections are a leading source of morbidity and mortality, despite improvements in medicine, and novel antifungal medicines are becoming increasingly scarce. [9, 10].

* Corresponding author
E-mail address: metabolitebode@yahoo.com (O. O. Isaiah)

The hunt for novel antimicrobial compounds from nature to address the microbial threat has accelerated in recent years. Plants have been demonstrated to be one of the most promising sources of natural chemicals that can act as anti-infective agents in various studies. [2, 11, 12]. According to WHO estimates, more than 80 percent of the population in Africa uses traditional medicine to address their healthcare needs. This is due to chemical toxicity, the expensive expense of chemical medications, and the removal or inadequacy of health facilities, particularly in rural regions, which hinder appropriate treatment of public health issues. Furthermore, the control of virus, bacterial, and fungal infections has grown more difficult due to the advent of virus, bacteria, and fungi that are resistant to many traditional antibiotics. Many multidrug-resistant bacteria cases have been documented in African countries. [13] Despite this, bacterial, viral, and fungal infections are among the most hazardous and opportunistic diseases for susceptible persons like children, the elderly, and those who are immunocompromised.

With these public health issues, medicinal plants could provide therapeutic responses tailored to a population's financial means and socio-cultural setting, making them a possible avenue for the creation of enhanced traditional medicines. Plant extracts containing phenolic and flavonoid chemicals have been found to have antioxidant, antibacterial, and anti-infective properties. [14-16].

Lannea acida is a deciduous shrub or tree with a dense, rounded crown that grows to a height of 1.5 to 10 meters, while specimens as large as 18 meters have been documented. The diameter of the bole can range from 50 to 70cm. A vital multi-purpose tree that provides food, medicine, and other necessities to the locals. When the bush is cleared for farming, it is frequently not cut down, and it is occasionally planted, but not farmed on a systematic basis. In Senegal, where it is sold in local markets, it is particularly valued as a medicinal plant. [17]. It is a member of the anacardiaceae family, which is generally known as awere kogun and is utilized ethnomedicinally in the treatment of infectious disorders, including malaria.

L. acida is mostly used for gastrointestinal issues, injuries, inflammation, and discomfort, Fever and malaria, gynaecological and pregnancy issues, ethnoveterinary medicine, haemorrhoids, skin diseases, and infections are only a few of the topics covered, according to literature reviews. In the hot and arid savannahs of Sub-Saharan Africa, it is one of the most extensively distributed *Lannea* species. It has a long history of ethnobotanical and ethnopharmacological use for a variety of ailments, including malaria, rheumatism, diarrhea, and hemorrhoids. *L. acida* bark is used as an anti-abortifacient, vermifuge, and to cure anal haemorrhoids, diarrhea, dysentery, malnutrition, and debility in Nigeria, while the leaves are used to treat rheumatism. The bark aqueous or alcoholic extract is utilized in the treatment of numerous infectious disorders, according to an ethnomedicinal survey conducted by a traditional healer in Akoko, Ondo state. Despite the fact that Lannea acida has exhibited biological activity, the mechanism of its action is yet unknown. To confirm its ethnobotanical uses in the treatment of infections, we investigated the antimicrobial properties of aqueous stem bark extract of *L. acida* and its major constituent flavonoid [18].

2. Methodology

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. Experimental Animals

Wistar albino rats of (100-130g) weight respectively were used, bred at the animal house of the Institute of Advanced Medical Research and Training (IAMRATS) of university Teaching Hospital, Ibadan Oyo state Nigeria.

2.3. Extraction of Lannea acida Stem Bark Extract

At room temperature, the back of *L. acida* was allowed to dry. They were ground to a fine powder in a mechanized laboratory grinder (Manesty, England). The 1.6 kg dried back was steeped in 5.5 L of pure methanol. After 72 hours, the mixture was properly mixed and filtered through a Buchner vacuum filter. A Rotary evaporator was used to evaporate the filtered supernatant to dryness. The extract's percentage yield was calculated using the equation provided by [19]

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

2.4. Extraction of Flavonoid- Rich Fraction

In a tiny flask, a part of the methanolic extract was mixed in 100ml (1:4) of 1% H_2SO_4 and hydrolysed by heating over a water bath until the liquid was half its original volume (30minutes). To allow flavonoids to precipitate, the mixture was placed on ice for 15 minutes. Filtration was done on the cooled solution. The filtrate (flavonoids aglycone combination) was mixed in 50mL warm 95 percent ethanol (50°C), filtered again, and the filtrate condensed to dryness using a rotary evaporator. [20]

2.5. Antimicrobial Assay

2.5.1. Microorganisms and Culture Conditions

Gram positive (Bacillus substilis and Staphylococcus aureus)

and Gram negative (Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes) bacteria were obtained from the microbiology department of the Federal University of Technology in Akure, Nigeria, and cultured aerobically at 37°C in nutrient agar medium. Before being used in experiments, solid medium cultures will be sub-cultured in liquid media, incubated for 24 hours, and used as a source of inoculums. The agar-well diffusion method was used to determine antimicrobial activity [21]

2.5.2. Antimicrobial Screening of the Extracts on Selected Pathogens

The disc-diffusion method was used to investigate the antibacterial activity of *L. acida* plant extracts and fractions. The extracts' minimum inhibitory concentrations (MIC) were determined. In this investigation, five bacterial strains were used: Staphylococcus aureus, Bacillus substilis, Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes. The bacteria were cultured on nutrition agar and kept on nutrient agar slants to ensure purity. The BSAC Diffusion Method for Antimicrobial Susceptibility Testing Version 9.1 was used to test the susceptibility of the bacterium isolate on the extract. [22]. The purpose of this test was to see if the plant extract has the ability to limit the growth of the bacteria isolate. The antibiotic sensitivity test was performed using the plate diffusion technique of [23]. Using sterile swab sticks, overnight cultures of the organisms were swabbed on sterile Muller Hilton solidified Agar plates. A cork borer with an 8mm diameter was used to drill a hole on the agar surface at equidistance, and the well was filled with diluted plant extract, a recognized antibiotic as a positive control, and distilled water as a negative control. All of the plates were incubated for 24 hours at 37°C. The drugs' inhibition zones were measured to the closest millimeters (mm) and classified as sensitive (S), intermediate (I), or resistant (R). According to [24], The zones of inhibition were measured and interpreted according to [24]. The zone of inhibition was compared with that of Ciprofloxacin.

2.5.3. Determination of the Minimum Inhibitory Concentration (MIC)

The extracts' minimum inhibitory concentrations were generated using four concentrations of each extract (10, 20, 30, and 50 mg/ml). Each concentration's antibacterial properties were assessed. The varied concentrations were put onto 6 mm disks, which were subsequently pressed onto Mueller-Hinton agar plates and SDA plates that had already been prepared. The inoculation plates were incubated for 48 hours at 37°C. At the end of the incubation period, zones of inhibition were measured and MICs were determined for the bacteria after 24 hours and for the extracts after 48 hours. The MICs were calculated as the lowest concentrations of extracts that prevented each organism from growing visible on the agar plate. [21]

2.5.4. Acute Toxicity Study (LD50)

To calculate the lethal dose, the [25] approach was used (LD_{50}). The experiment was split into two parts:

Stage I: Nine rats were employed in this stage. They were placed into three groups, each with three rodents. The rats in group A were given a 10 mg/kg body weight dosage of methanolic extract of Lannea acida. Group B was given 100 milligrams per kilogram of body weight. Group C was given 1000 mg per kilogram of body weight. For 24 hours, the animals were observed and monitored. The number of people who died in each category was recorded.

Stage II: The outcomes of the prior stage were used to guide this stage. Another three groups of one rat each were employed in this experiment. The extract was given to Group A animals at a dose of 1600 mg/kg body weight. The extract was given to the animals in Groups B and C at doses of 2900 mg/kg body weight and 5000 mg/kg body weight, respectively. The animals were kept under observation for another 24 hours, during which time the number of deaths and odd reactions/behaviour were recorded.

2.5.5. Bactericidal Activities Against Bacterial Organism Exposed to Rats Following Treatment with the Extracts

Methanolic extract and flavonoid-rich fraction were tested *in vivo* to examine if they could protect rats from Gram positive *(Staphylococcus aureus and Bacillus substilis)* and Gram negative bacteria *(Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes)*. The animals were given 400mg/kg bwt of methanolic and flavonoid-rich fraction orally once daily for three days. Another group of animals was given gentamicin at a dose of 5 mg/kg orally. The rats were given a bacterial organism orally on the third day, and the rate of mortality was observed for a month. [26].

2.5.6. Statistical Analysis

Statistical analysis of the data was performed using Graph Pad Prism Software version 7. The one - way ANOVA follow by Tukey's test was used to analyze and compare the results at a 95% confidence level. Differences between means of treated and control group Values of $p<0.05$ were considered significant. Results were expressed as mean ± standard error of mean (SEM).

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

Alkaloids, terpenoids (triterpenoids), flavonoids, tannins, and quinone were found in the crude aqueous methanolic extract during phytochemical screening. Terpenoids, Tannin, Phenol, Vitamin A, and Vitamin C were found in moderate and low concentrations, but Flavonoid, Quinone, alkaloids, and saponin were found in significant numbers. (Table 1).

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

Samples	Saponin	Flavonoid	Alkaloid	Tannin	Terpenoids	Phenol	Quinone
Qualitative Analysis	+++	+++	+++	++	+	+	+++

3.3. Acute Toxicity Study

Using Lorke's modified approach, acute toxicity studies of the crude aqueous methanolic extract following oral administration were performed, the LD_{50} was calculated to be 5000 mg/kg (5 g/kg). The apparently high LD_{50} obtained from this study implies that the extracts are very safe in mice and rats when administered orally.

The results of the antimicrobial effects of the methanolic extract and flavonoid-rich fraction against the five microorganisms tested are shown in table 2. All microbial strains tested were found to be affected by the extracts as assessed by inhibition zones that ranged from 26.33 mm to 9.66 mm. The extracts showed antibacterial activity against both Gram positive and Grams negative bacteria. According to the data presented in table 2, all the tested microbial strains were found to be sensitive by the tested antibiotics. (Erythromycin).

Table 2. Antimicrobial sensitivity activities of the methanolic extract and flavonoid-rich fraction of *L acida*. All values are expressed as mean inhibition zones (mm) ± SEM of three replicates. (mm)

Bacterial Strain	*Methanolic extract* 20mg/ml	*Methanolic extract* 30mg/ml	*Methanolic extract* 50mg/ml	*Flavonoid Rich* 20mg/ml	*Flavonoid Rich* 30mg/ml	*Flavonoid Rich* 50mg/ml	Erythromycin 15µg
Gram positive							
Bacillus subtilis	0.00 ±0.00	17.33±0.19	17.33±0.19	0.00 ±0.00	16.66± 0.19	18.66±0.50	26.66±0.50
Staphylococcus aureus	10.66±0.19	22 ±0.33	26.33±0.19	12.33±0.19	20.33 ±0.50	22.33±0.69	37.66±0.50
Gram negative							
Escherichia coil	0.00±0.00	16 ± 0.33	16.66 ±0.38	0.00±0.00	15.66 ± 0.50	23.0 ±0.33	36.66±0.88
Klebsiella pneumoniae	11.33±0.19	19.33± 0.50	23.66±0.19	13 ±0.57	22.66± 0.69	25.33±0.50	29.66±1.01
Enterobacter aerogenes	0.00±0.00	9.66± 0.50	13.33±0.19	0.00±0.00	10± 0.57	22±0.33	9.66±0.50

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

The results of the antifungi effects of the methanolic extract against the five fungi organisms tested are shown in table 3. All fungi strains tested were found to be affected by the extracts as assessed by inhibition zones that ranged from 26.33 mm to 9.66 mm. The extracts showed antifungi activity against the five fungi organism. According to the data presented in table 3, all the tested fungi strains were found to be sensitive by the tested Nystatin.

Table 3. Antifungi sensitivity activities of the methanolic extract and flavonoid-rich fraction of *L acida*. All values are expressed as mean inhibition zones (mm) ± SEM of three replicates. (mm)

Fungi organism	*L. acida* 20mg (mm)	*L. acida* 30mg (mm)	*L. acida* 50mg (mm)	Nystatin (mm)
Aspergillus favus	00.0±0.00	0.00± 0.00	17.66±0.19	24.0 ±0.33
Candida tropicalis	00.0 ±0.00	9.66±0.39	18.66 ± 0.19	26.33 ± 0.19
Candida albicans	00.0 ±0.00	16.33±0.19	20.33± 0.50	24.33 ±0.19
Rhyzopus stolonifera	00.0 ±0.00	0.00 ±0.00	24.33±0.19	28.33 ± 0.19
Fusarium solani	00.0 ±0.00	10.33±0.69	17 ±0.33	23.0 ± 0.33

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

3.4. Minimum Inhibitory Concentration of Methanolic Extract and Flavonoid-Rich of *L. acida* Against Test Microbes

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extracts that completely stopped the bacterium from growing. Mean inhibition zones (mm) SEM of triplicates are presented. The crude extract's Minimum Inhibitory Concentration (MIC), followed by flavonoid-rich extracts, was determined to be the most effective against the selected organism. The zones of inhibition induced by the antibiotic were measured to the nearest millimeters (mm) and interpreted as sensitive (S), intermediate (I), and resistant (R) to understand the antibacterial effect (R). According to [24], the zones of inhibition were measured and evaluated. The crude methanolic extracts had MICs ranging from 20 to 50 mg/ml, with MICs of 20 mg/ml for *S. aureus* and *K. pneumoniae,* according to table 4. For *B. subtilis, S. aureus, E. coli, K.*

pneumoniae, and *E. coli*, 30 and 50 mg/ml were used. Only the highest concentration (50 mg/ml) of the extract was effective against all of the species tested. For the flavonoid-rich extract of *L. acida*, the MIC ranged from 20 to 50 mg/ml. For all of the microorganisms tested, the extract had MICs ranging from 20 to 50 mg/ml. The extract was only active against all the tested organisms at the highest concentration tested (50 mg/ml).

Table 4. Minimum Inhibitory concentration (MIC) of methanolic extract, Flavonoid-Rich extract and Alkaloid- rich extract of *L. acida* against test microbes. (mm)

Plant Extracts	Conc mg/ml	*B.subtilis*	*S. aureus*	*E.coli*	*K.pnuemoniae*	*E.aerogenes*
Methanolic Extract	50	25.33±0.19	26.33±0.19	25±0.33	20.66±0.19	26.33±0.19
	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 Rich Extract	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±0.33	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

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

3.5. Effect of Methanolic Extract and Flavonoid-Rich of *L. acida* on the Survival of Rats Exposed to Organisms

The survival rate of the tested rats was dramatically improved after treatment with aqueous methanolic extract and flavonoid-rich extracts. At a dosage of 400mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of L. acida were efficient in extending the survival rate of the tested mice. The rats given the extracts lived for up to 4 weeks, with survival rates ranging from 100% by the end of week 1 to 50% by week 4, before declining until week 4. Up until the end of week 4, the positive control (gentamicin-treated group) had a percent survival rate of over 80%. The negative control group, which was not given extracts, only made it to week two, with a survival rate of 20%. (Figure 1-5).

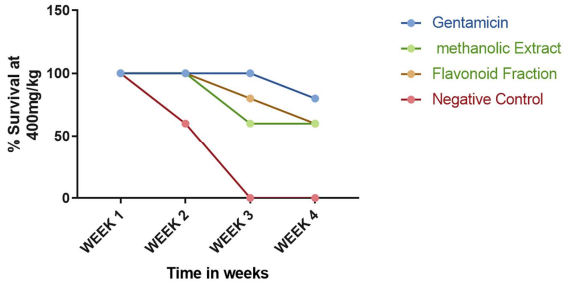

Figure 1. Survival of rats treated with 400mg/kg methanolic extracts of Lannea acida extracts and its flavonoid fraction exposed to B. subtillis.

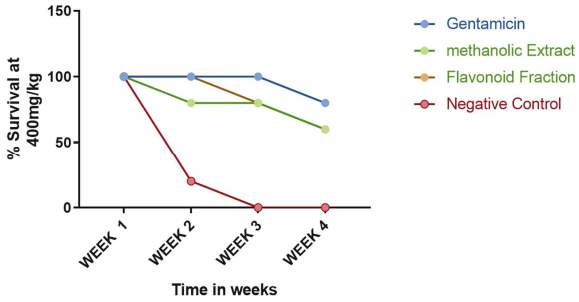

Figure 2. Survival of rats treated with 400mg/kg crude methanolic extracts of Lannea acida extracts and its flavonoid fraction exposed to S. aureus.

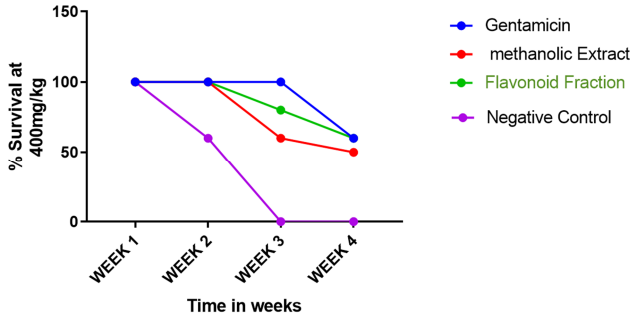

Figure 3. Survival of rats treated with 400mg/kg methanolic extracts of Lannea acida extracts and its flavonoid fraction exposed to E. coli.

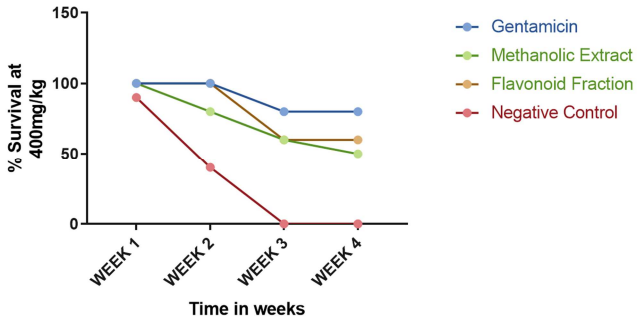

Figure 4. Survival of rats treated with 400mg/kg methanolic extracts of Lannea acida extracts and its flavonoid fraction exposed to K. pneumonia.

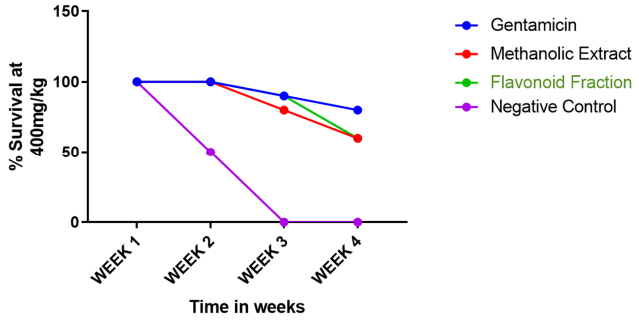

Figure 5. Survival of rats treated with 400mg/kg methanolic extracts of Lannea acida extracts and its flavonoid fraction exposed to E. aerogenes.

4. Discussion

Alkaloids, flavonoids, tannins, glycosides, terpenoids, and steroids are secondary metabolites found in plants that act individually or synergistically to trigger biological action [27-29]. Alkaloids, glycosides, saponins, tannins, flavonoids, and terpenoids were found in aqueous stem bark extract of *L. acida* during phytochemical screening for secondary metabolites (Table 1). The agar diffusion method has been widely used and is recommended as a useful way to determine the relative strength of complex extracts and their antibacterial spectrum [30, 31]. *L. acida* and its constituents had antibacterial and antifungal action against Gram–positive and Gram–negative bacteria (Tables 2). The existence of broad-spectrum antimicrobial agent(s) or compound(s) may be indicated by antibacterial activity against Gram–positive and Gram–negative bacteria [32]. Many microbes, insects, and herbivores use phytochemical elements such tannins, flavonoids, alkaloids, and glycosides as defensive mechanisms against predation. One or more phytochemical compounds present in L. acida may be responsible for the plant's antibacterial action [33, 34].

Recent research has found that tannins [35], alkaloids [36], saponins [37], glycosides [38], flavonoids [39], and terpenoids [40] have antibacterial action and that their effects are mediated through the bacteria's cell membrane integrity [41]. Antimicrobial action has also been discovered for polyphenolic chemicals, which could be owing to their propensity to form complexes with bacterial cell walls, inhibiting microbial growth [42]. Plant extracts with MIC values between 2.5 and 8 mg/mL have been demonstrated to lead to the identification of strong antibacterial chemicals in studies [43-46]. This therefore suggests that *L. acida* can be a source of potentially active antimicrobial compound. Table 2 shows the antibacterial properties of the various *L. acida* extracts against the five microorganisms that were tested. Methanolic extract and flavonoid-rich extracts of *L. acida* stem bark were shown to be sensitive to all microbial strains tested, as measured by inhibition zones ranging from 9.660.50 to 26.33 0.19) mm and (10.00 0.57 to 37.66 0.50) mm, respectively. The extract showed antibacterial activity against both Gram-positive and negative bacteria. Amongst all the organism tested in this study for both ME and FR of *L. acida* stem bark extracts demonstrated the highest antibacterial activity with an inhibition zone of (26.33±0.19 and 37.66±0.50) mm against *Staphylococcus aureus* respectively, while the lowest inhibition zone was (9.60±0.50 and 10±0.57) mm against *Enterobacter aerogenes*. The antimicrobial sensitivities of standard antibiotic drug according to the data presented in table 2, most of the tested microbial strains was sensitive to the effect of Erythromycin was observed to be active against all the tested microbes [26]. Antifungal activity of *L. acida* extract was assayed by agar well diffusion method. The result revealed that the extract of the medicinal plants showed significant reduction in growth of *Aspergillus favus, Candida tropicalis, Candida albicans, Rhyzopus stolonifera and Fusarium solani.* [47]

Furthermore, plant constituents or agents are frequently identified as possible antimicrobials based on susceptibility testing with MICs ranging from 100 to 1000 g/mL [20]. This could indicate that flavonoids could be used as an antibacterial agent. Antimicrobials are considered bactericidal if the MIC ratio is less than 4, and bacteriostatic if the ratio is greater than 4 [48]. Methanolic extracts were found to be the most effective against the results of the Minimum Inhibitory Concentration (MIC), followed by flavonoid-rich extracts (table 4). The zones of inhibition induced by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive (S), intermediate (I), and resistant (R) to understand the antibacterial impact (R). According to [24], the zones of inhibition were measured and evaluated. The MIC of the methanolic crude extract ranged from 20 to 50 mg/ml, with a MIC of 20 mg/ml for *S. aureus and K. pneumoniae*, according to the data in table 2. For *B. subtilis, S. aureus, E. coli, K. pnuemoniae, and E. coli*, 30 and 50 mg/ml were used. Only the highest concentration (50 mg/ml) of the extract was effective against all of the microorganisms tested. For the flavonoid-rich extract of *L. acida*, the MIC ranged from 20 to 50 mg/ml. The extract's MIC ranged from 20 to 50 mg/ml for all of the microorganisms tested, and it was only active against all of them at the highest concentration tested (50 mg/ml).

The ratios obtained for all of the test organisms were greater than 4, indicating that *L. acida* and Flavonoid were both bacteriostatic and fungistatic against the test organisms (Tables 2 & 3). Preliminary acute toxicity studies established *Lannea acida* extracts and fractions from the different areas as safe judging from the calculated LD_{50}. From the acute toxicity tests of the extracts following oral administration using [25] modified method, the LD_{50} was generally higher than 5000 mg/kg. The apparently high LD_{50} implies that the extracts are very safe in mice and rats when administered orally. There was no death of the mice or rats used in the first stage and the death pattern in the second stage was used to calculate the LD_{50} values. These findings are in agreement with previous reports on the acute toxicities of these species of *Lannea acida* [49]. Time-kill kinetic experiments validated the bacteriostatic activity of *L. acida* and flavonoid (Figures 1-5). Antimicrobial drugs that are bacteriostatic or fungistatic only limit the growth or multiplication of bacteria, giving the host's immune system time to eliminate the microbes from the system [50]. After treatment with aqueous methanolic extract and flavonoid-rich extracts of *L. acida* extract, the survival rate of the treated rat was significantly improved. At a dosage of 400mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of *L. acida* were efficient in prolonging the survival of

rats exposed to *S. aureus*. The rats subjected to the extracts lived for up to four (4) weeks, with survival rates ranging from 100 percent at the end of week one to 80 percent at the end of week two, 80 percent at the end of week three, and 60% at the end of week four. Up until the end of week 4, the positive control (gentamicin-treated group) had a percent survival rate of over 80%. The negative control group, which was not given any treatment, only made it to week two, with a survival rate of 20%. (Figure 1). At 400 mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of *L. acida* were efficient in prolonging the survival of *Bacillus subtillis*-exposed rats. The rats subjected to the extracts lived for up to four weeks, with survival rates ranging from 100 percent by the end of week two to 80 percent to 60 percent by week three, and 60 percent by week four. The positive control (gentamicin-treated group) had a percent survival rate of over 80% until the end of week 4, but the negative control (non-treated group) only lasted until week 2 with a percentage survival rate of 40%. (Figure 2). [26]

At a dose of 400mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of L. acida were efficient in prolonging the survival of rats infected with Klebsiella pnuemoniae. Animals given extracts of both the crude and flavonoid-rich extracts survived for up to 4 weeks, with survival rates ranging from 80 percent to 100 percent by week 2, 50 percent to 60 percent by week 4, and 80 percent to 100 percent by week 4. The positive control (gentamicin-treated group) had a percent survival rate of over 80% until the end of week 4, but the negative control (non-treated group) only lasted until week 2 with a percentage survival rate of 40%. (Figure 3). However, at a dose of 400mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of L. acida were efficient in extending the survival of rats exposed to E. coli. Animals given extracts of both the crude and flavonoid-rich extracts lived for up to four weeks, with survival rates ranging from 100 percent until the end of week 2, 60 percent to 80 percent by the end of week 3, and 50 percent to 60 percent by the end of week four. The positive control (gentamicin-treated group) had a percent survival rate of over 60% until the end of week 4, but the negative control (non-treated group) only lasted until week 2 with a percentage survival rate of 60%. (See Figure 4). In addition, at a dose of 400 mg/kg, the aqueous methanolic extract and flavonoid-rich extracts of L. acida were efficient in prolonging the survival of rats challenged to Enterobacter aerogenes. Animals given extracts of both the crude and flavonoid-rich extracts lived for up to 4 weeks, with survival rates ranging from 100% until the end of week 2, 80 percent to 90 percent by the end of week 3, and 60% by the end of week 4. The positive control (gentamicin-treated group) had a percent survival rate of over 80% until the end of week 4, but the negative control (non-treated group) only lasted until week 2 with a percentage survival rate of 50%. (Figure 5) [26].

5. Conclusion

The aqueous methanolic stem bark extracts of *Lannea acida* and its flavonoid-rich fractions found to possess antimicrobial activity that may confirms its traditional use as anti-infective agent. The antimicrobial activity of *L. acida* may largely be due to its major constituents and they act by synergistic means.

Authors' Contributions

I declare that this work was done by the authors named in this article.

Conflicts of Interest

No conflicts of interest are associated with this work.

Funding

This research was funded from personal contributions by the authors.

Ethics Approval and Consent to Participate

This study was approved by Adekunle Ajasin university Animal laboratory handling Committee.

References

[1] Howard S. J, Hopwood S, Davies S. C (2014). Antimicrobial resistance: a global challenge. Science Translation Medicine 6: 236ed10.

[2] Infectious Diseases Society of America (2010). The 10 x '20 Initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. *Clinical Infectious Diseases* 50: 1081-1083.

[3] Augustine Olusegun Olusola, Olabode Isaiah Ogunsina, Adesayo Olufunmi Olusola, Akindele Fakoya (2020). *In vitro* Antioxidant and Antimicrobial activities of *Lannea acida (anacardiaceae)* stem bark extracts. *Journal of Global Biosciences*, Volume 9, Number 7, pp. 7709-7724.

[4] World Health Organization (2001) WHO global strategy for containment of antimicrobial resistance.

[5] Morens D. M, Folkers G. K, Fauci A. S (2004) The challenge of emerging and reemerging infectious diseases. *Nature* 430: 242-249.

[6] Khosravi A. D, Behzadi A (2006) Evaluation of the antibacterial activity of the seed hull of Quercus brantii on some gram-negative bacteria. *Pakistan Journal of Medical Science* 22: 429-432.

[7] Wenzel RP, Edmond MB (2000) Managing antibiotic resistance. *New England Journal of Medicine* 2000; 343: 1961-1963.

[8] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, (2012). Multidrug- resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology Infection* 18: 268-281.

[9] MacNeil I. A, Tiong C. L, Minor C, August P. R, Grossman T. H (2001). Expression and isolation of antimicrobial small molecules from soil DNA libraries. *Journal of Molecular Microbiology and Biotechnology* 3: 301-308.

[10] Pfaller MA, Diekema D. J (2004). Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus fumigatus. *Journal of Clinical Microbiology* 42: 4419-4431.

[11] Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, et al. (2006) Biological activities of the essential oil and methanol extract of Achillea Biebersteinii Afan (Asteraceae). *Turkish Journal of Biology* 30: 65-73.

[12] Ahmad M, Wajid M (2013) Plants as potential source of antimicrobial agents. *Journal of Pharmaceutical Alternative Medicine* 2: 18-25.

[13] L. Ouattara, J. Koudou, C. Zongo, N. Barro, A. Savadogo, I. H. N. Bassole, A. S. Ouattara and Alfred S. Traore, 2011. Antioxidant and Antibacterial Activities of Three Species of *Lannea* from Burkina Faso. *Journal of Applied Sciences, 11: 157-162.*

[14] Da Silva, J. F. M., De Souza, M. C., Matta, S. R., Andrade, M. R., & Vidal, F. V. N. (2006). Correlation analysis between phenolic acids of Brazilian propolis extracts and their antimicrobial and antioxidants activities. *Food Chemistry*, 99, 431-435.

[15] Majhenic, L., M. Skerget and Z. Knez, (2007). Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chemistry*., 104: 1258-1268.

[16] Pereira, R. B., Sousa, C., Costa, A., Andrade, P. B. and Valentao, P. (2013). Glutathione and the Antioxidant Potential of Binary Mixtures with Flavonoids: Synergisms and Antagonisms. Molecules. 18: 8858-8872.

[17] Burkill, H. M., (2004). The Useful Plants of West Tropical Africa. Vol. 3, Ithaka Harbors Incorporation, New York, ISBN: 10: 1900347660, pp: 369.

[18] Olusola Augustine O, Ogunsina Olabode I, Olusola Adesayo O, Ubadigha Nchedo N. (2020). *In-Vivo* antioxidant and immunomodulatory activity of flavonoid-rich extract of *Lannea acida* stem bark extracts. *International journal of Pharmacy and Pharmaceutical Research*. Volume 18, Issue 4, pp. 913-931.

[19] Banso, A. and Adeyemo, S. (2006). Phytochemical screening and antimicrobial assessment of *Abutilon mauritanum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistriz, 18*, 39-44.

[20] Olusola A. O., Ogunsina O. I., and Olusola A. O. (2020a). Antimalarial potential of flavonoid-rich extract of *Lannea acida* and chloroquine in mice infected with *Plasmodium berghei. International Journal of Scientific & Engineering Research* Volume 11, Issue 3: 201-206. 6 (1), 27-33.

[21] Olabode Isaiah Ogunsina. (2020d). Antibacterial activities and Time-killing kinetics rate of *Lannea acida* extracts against selected microbes. *Agricultural and Biological Sciences Journal* (AIS). Vol. 6, No. 3, pp. 129-136.

[22] Andrews, S. (2009). Diffusion Method for Antimicrobial Susceptibility Testing Version 9.1. Determining Ed following the BSAC disk diffusion procedure.

[23] Willey, J. M., Sherwood, L. M. &Woolverton, C. J. (2008). *Prescott, Harley and Kleins's Microbiology* (7th ed.). USA: McGraw-Hill Higher Education.

[24] National Committee for Clinical Laboratory Standards methods (2000).

[25] Lorke, D. (1993). Approach to acute toxicity testing. *Archieves of Toxicology*, *53,* 275-287.

[26] Ogunsina O. I. (2020). Evaluation of antiplasmodial and Immunomodulatory effect of methanolic stem bark extract of *Lannea acida. Ph. D Thesis.*

[27] Barbosa-Filho J. M, Piuvezam M. R, Moura M. D, Silva M. S, Lima KVB (2006). Anti- inflammatory activity of alkaloids: A twenty-century review. Rev Bras Farmacogn 16: 109-139.

[28] Kubmarawa D, Khan M. E, Punah AM, Hassan M (2008). Phytochemical screening and antimicrobial efficacy of extracts from Khaya senegalensis against human pathogenic bacteria. *African Journal of Biotechnology* 7: 4563-4566.

[29] Maganha E. G, Da Costa Halmenschlager R, Rosa R. M, Henriques JAP, De Paula Ramos (2010). Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. *Food Chemistry* 118: 1-10.

[30] Rios J. L, Recio M. C (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 100: 80-84.

[31] Rios J. L, Recio MC, Villar A (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of Ethnopharmacology* 23: 127-149.

[32] Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology* 74: 217-220.

[33] Cowan M. M (1999). Plant products as antimicrobial agents. Clinical Microbiology Review 12: 564-582.

[34] Nweze E. I, Okafor J. I, Njoku O (2004) Antimicrobial activities of methanolic extracts of *Tremaguineensis* (Schumm and Thorn) and *Morinda Lucida* Benth used in Nigeria herbal medicinal practice. *Journal of Biological Research and Biotechnology* 2: 36-39.

[35] Fiori GML, Fachin A. L, Correa V. S, Bertoni B. W, Giuliatti S, (2013) Antimicrobial activity and rates of tannins in *Stryphnodendron adstringens* Mart accessions collected in the Brazilian Cerrado. *American Journal of Plant Science* 4: 2193.

[36] Singh G, Kumar PADMA (2012) Antibacterial potential of alkaloids of *Withania somnifera* L and *Euphorbia hirta* L. *International Journal of Pharmaceutical Science* 4: 78-81.

[37] Murugan T, Wins J. A, Murugan M (2013) Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of *Cassia auriculata*. *Indian Journal of Pharmaceutical Science* 75: 122-125.

[38] Zearaha S. A, Al-Fartosy AJ, Al-Kanany G. F (2013). Antibacterial activity of the glycosidic extract from Citrus *laurantifoia* L fruits. Pharmaceutical Chemistry 5: 73-78.

[39] Cushnie T. P, Lamb A. J (2005) Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 26: 343-356.

[40] Irfan M, Ahmed S, Sharma M (2014) Antimicrobial activity of terpenoids from *Sphaeranthus indicus* L. *Asian Journal of Plant Science Resources* 4: 1-6.

[41] Trombetta D, Castelli F, Sarpietro M. G, Venuti V, Cristani M (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents Chemotherapy* 49: 2474-2478.

[42] Sivapriya M, Dinesha R, Harsha R, Gowda SST, Srinivas L (2011). Antibacterial activity of different extracts of sundakai *Solanumtorvum* fruit coat. *International Journal of Biological Science* 5: 61-67.

[43] Lozoya X, Navarro V, GarcÃa M, Zurita M (1992). *Solanum chrysotrichum* (Schldl.) a plant used in Mexico for the treatment of skin mycosis. *Journal of Ethnopharmacology* 36: 127-132.

[44] Navarro V, Villarreal ML, Rojas G, Lozoya X (1996). Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *Journal of Ethnopharmacology* 53: 143-147.

[45] Fabry W, Okemo P. O, Ansorg R (1998). Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology* 60: 79-84.

[46] Navarro V, Delgado G (1999). Two antimicrobial alkaloids from *Bocconia arborea*. *Journal of Ethnopharmacology* 66: 223-226.

[47] Ambikapathy, V., Gomathi, S., and Panneerselvam, A. (2011). Effect of antifungal activity of some medicinal plants against Pythium debaryanum (Hesse). *Asian Journal of Plant Science and Research*, 1 (3): 131-134.

[48] Keepers TR, Gomez M, Celeri C, Nichols W. W, Krause K. M (2014) Bactericidal activity, absence of serum effect, and time-kill kinetics of ceftazidime-avibactam against ß-lactamase-producing Enterobacteriaceae and *Pseudomonas aeruginosa. Antimicrobial Agents Chemotherapy* 58: 5297-5305.

[49] Okoth, A. D. (2014). Phytochemistry and Bioactive Natural Products from *Lannea Alata, Lannea Rivae, Lannea Schimperi and Lannea Schweinfurthii* (Anacardiaceae) (Doctoral Thesis, University of Kwazulu-Natal, South Africa).

[50] Pankey G. A, Sabath L. D (2004) Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical Infectious Diseases* 38: 864-870. 7.