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Phytochemical Screening, Antioxidant, Anti-Inflammatory and Antibacterial Studies on the Stem Bark Extracts of *Lannea microcarpa* Engl. & K. Krause

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Abstract: Medicinal Plants are Plants used for traditional medicines. Any part of the plant could be used as medicine. Medicinal plants have been discovered and used in traditional medicine practices since pre-historical times. A survey of traditional medicine man in Damaturu indicated that Lannia microcarpa is a plants used in the treatment of inflammation, wounds and cancer. The fresh samples (stem bark) of the plant was collected and successively extracted with n-hexane, ethyl acetate, methanol and water. Phytochemical constituents of Lannea microcarpa extracts revealed the presence of cardiac glycosides, flavonoids, saponins, phenols, tannins, alkaloids, sterols and terpenoids. The antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, whereas membrane stabilization method was used to evaluate the anti-inflammatory potential of the extracts. The antibacterial potential of the plant extracts against four (4) wound pathogens: Salmonella typhi, Staphylococcus aureus, Escherichia coli and Strephtococcus pneumoneae, was evaluated using disc diffusion technician. The IC_{50} values obtained for the Lannea microcarpa extracts indicated that the methanol and water extracts exhibited higher antioxidant activities than the standard ascorbic acid. The extracts were found effective in inhibiting the heat induced haemolysis. Diclofenac Sodium salt used as standard drug at 100µg/ml offered 90.66% protection a significant ($p<0.05$) protection against damaging effect of heat solution. The results showed that all extracts from the plant offered a significant ($p<0.05$) protection against the damaging effect of hypotonic solution, Diclofenac sodium (100µg/ml), the standard drug offered a significant ($p<0.05$) protection (57.52%). Lannea microcarpa extracts showed zone of inhibition only against Salmonella typhi and Strephtococcus pneumonea.

Keywords: Phytochemical, Antioxidant, Antibacterial, Anti-Inflammatory

1. Introduction

Medicinal Plants are Plants used for making home remedies and traditional medicines or what is well known as herbal medicine. Any part of the plant could be used as medicine, the leaves, roots, seeds, stems, etc. Medicinal plants or the medicinal herbs have been discovered and used in traditional medicine practices since pre-historical times. Early written reports on the use of plants as medicine appeared about 2600 BC when plants were used as medicine by Sumerians and Akkadians [21]. Since then, plants have been used to treat diseases such as headache, toothaches, stomach aches, diarrhea, wounds, tumors and sexually transmitted diseases [11, 22-24].

A plant is considered medicinal if it produces compounds which are therapeutically effective. Plants produce a wide range of secondary metabolites, and the medicinal properties are attributed to the presence of these metabolites such as terpenoids, steroids, saponins, tannins, flavonoids, alkaloids and phenolic compounds [6, 12].

Lannea microcarpa of the family Anacardiaceae, commonly known as African grape or Faaru in Hausa, a survey of medicinal plants within and around Damaturu environs indicated that the plant is used in the treatment hepatitis, inflammation, malaria, wounds and cancer. However, there's no scientifically backed evidence to support

this claim. This poses an important challenge to seek for more scientific studies to be carried out on each of the four plants in order to ascertain these claims.

Herbal medicine in Nigeria is gaining more recognition and this is seen in how much inquiries people make concerning home remedies and traditional medicine. Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases and heal other injuries. Some of these plants are used as food and/or medicine. The extracts from this plant has shown to exhibit a wide range of biological and pharmacological activities such as anticancer, anti-inflammatory, diuretic, laxative, antispasmodic, antihypertensive, antidiabetic, antimicrobial activities, etc. It is generally assumed that the active medicinal constituents contributing to the protective effects are phytochemicals, vitamins and minerals [14, 15]. For this reason, medicinal plants are considered be important to the health of the individuals and communities.

2. Materials and Methods

2.1. Apparatus and Materials

Ultrasonicator (Model/AS3120) was purchased from Tianjin Automatic Science Instrument Co., Ltd. China, analytical weighing balance (Ohaus Corp. Pine Brook, NJ USA), pestle and mortar (wooden) purchased in Damaturu Sunday market, empty bottles purchased at Bayan Tasha market Damaturu, sieve, fume cupboard, drying cabinet (model/FSM140) from 2 Building, Majialong Industrial Zone, Nanshen District, Shenzhen Jinly Technology Co., Ltd. China, UV/VIS spectrophotometer (model/UV752) from Changsha, Hunan, Wincon Company Ltd. China, Autoclave (Model/DWB-280B) and Water bath (Model/DWT-420) from Shanghai Drawell Scientific Instrument Co., Ltd. Room 211 Building 7, sheng Yu Industrial Park No. 365 ChunHong, Shanghai, China and other laboratory materials.

2.2. Chemicals

n-hexane, ethyl acetate and methanol were purchased from BDH Chemicals Ltd., Poole, United Kingdom. 2,2-diphenyl-1-picryl hydrazyl (DPPH) was purchased from SIGMA-ALDRICH Company Ltd., 3050 Spruce street St. Louis, MO63103 USA. Muller Hinton Agar was purchased from TITAN BIOTECH Ltd., A-904A, RIICO Industrial Area, Phase-III, Rajasthan, India. Dimethyl Sulphur Oxide (DMSO) was purchased from Guangdong Guanghua Sci-Tech Company Ltd., Add 6, Jiangyan South Road, Guangzhou, Guangdong, China. ethanol, ascorbic acid, Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), Magnesium metal, Ferric chloride, Dragendroffs reagent, choloroform and all other Chemicals used are of highest analytical grade and purchased from BDH Chemicals, Poole, England.

2.3. Collection and Preparation of Plant Sample

The fresh sample of *Lannea microcarp*a (Voucher number 110395) (stem bark) was collected at Baadaawa/Daura a village in Fune Local Government Area, Yobe State, Nigeria. The herbarium specimen was identified by Mallam Salihu Abdullahi a Taxonomist at the Department of Biological Sciences, Yobe State University, Damaturu. The stem bark of the plant was collected two (2) meters above the ground. The sample was sorted to ensure no foreign bodies were present. This sample was then dried under shade in the laboratory at ambient temperature. The dried sample was then crushed into coarse particles using local pestle and mortar. It was further crushed into fine powder and sieved with a sieve and weighed. The fine powdered sample was then weighed and stored in sealed containers until required for further analysis.

2.4. Extraction of Phytochemicals

About 550g of the powdered plant material of *Lannea microcarpa* was separately extracted successively with 2.5 L portions of n-hexane, ethyl acetate, methanol and water in that order using ultrasonicator for two hours at room temperature. The solvent containing the extracts was allowed to settle after the extraction, then the mixture was separated from the residue by filtering with Whatmann No. 1 filter paper and then kept in a clearly labelled container ready for solvent recovery. The residue of the sample was then mixed with the next solvent for the further extraction. The procedure was repeated for the remaining solvents namely; ethyl acetate, methanol and water in that order.

2.5. Phytochemical Screening

Phytochemical screening to detect the presence of phytochemicals from the samples was carried out using the procedures outlined [20, 17].

2.5.1. Test for Alkaloids

The extract (0.5g) was dissolved in 5ml of 2N HCl and filtered. The filtrate was treated with Dragendroff"s reagent (Solution of potassium Iodide and bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2.5.2. Test for Flavonoids

The extract (0.5g dissolved in 2ml of methanol) was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.5.3 Test for Saponins

Frothing test: The extracts (0.5g) were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes over a vortex mixer. Formation of 1cm layer of foam indicates the presence of saponins.

2.5.4. Test for Cardiac Glycosides (Keller Kelliani's Test)

To 5ml of each extract (0.5g dissolved in 5ml methanol) was treated with 2ml of glacial acetic acid in a test tube and a drop of 2%ferric chloride solution was added to it. This was carefully underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may

appear below the ring while in the acetic acid layer, a greenish ring may form.

2.5.5. Test for Oxalate

To 3ml portion of the extract (0.2g in 3ml of methanol) were added a few drops of glacial acetic acid. A greenish black colouration indicates the presence of oxalates.

2.5.6. Test for Quinones

A small portion of the extract was treated with concentrated hydrochloric acid. The formation of yellow precipitate/colouration indicates the presence of quinones.

2.5.7. Test for Terpenoids (Salkowski's Test)

To 1ml of chloroform was added to 2ml of the extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicates the presence of terpenoids.

2.5.8. Test for Tannins (Braymer's Test)

To 2ml of the extract was treated with 10% alcoholic ferric chloride solution. Formation of blue/greenish colouration indicates the presence of tannins.

2.5.9. Test for Sterols (Libermann-Burchard Test)

To 1ml of the extracts was treated with few drops of chloroform, acetic anhydride and concentrated sulphuric acid. The formation dark pink or red colour indicates the presence of sterols.

2.5.10. Test for Phenols

A fraction of the extract was treated with aqueous 5% ferric chloride solution. The formation of deep blue or black colour indicates the presence of sterols.

2.6. Measurement of Antioxidant Activities

The antioxidant activities of *Lannea microcarpa* extracts were determined on the basis of their scavenging activity of stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as follows; to 1 ml of each solution of different concentrations (10, 25, 50. 100, 125, 250, 300, 500µg/ml) of the extracts, 3 ml of 0.004% ethanolic DPPH free radical solution was added. After 30 minutes, the absorbance of the preparations were taken at 517nm by UV spectrophotometer. This was then compared with the corresponding absorbance of standard ascorbic acid concentrations (10, 25, 50. 100, 125, 250, 300, 500 µg/ml) as described by [10], with some modifications. Then the% inhibition was calculated by the following equation;

$$\%\ \text{Radical scavenging Activity} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{(\text{absorbance of blank})} \times 100\%$$

The blank was prepared by adding 3 ml of 0.004% ethanolic DPPH to 1 ml of the ethanol.

Procedure

1) Eight 8 test tubes were taken to prepare the different concentration (10, 25, 50. 100, 125, 250, 300, 500µg/ml) plant extracts and ascorbic acid standard.
2) Extracts and ascorbic acid were accurately weighed and dissolved in ethanol to make the required concentrations by dilution technique.
3) First 0.004g of DPPH was weighed and dissolved in 100ml of ethanol to make 0.004% (w/v) homogenous solution using a vortex mixer.
4) 3ml of 0.004% DPPH solution was added to each of the 8 test tubes by means of a auto pipette, after preparing the desired concentrations.
5) The room temperature was recorded and the test tubes were kept for 30 minutes to complete the reactions.
6) DPPH was also added to the blank test tube at the same time where only ethanol was taken as blank.
7) The absorbance of each test tube was measured using a UV spectrophotometer.
8) $IC_{50's}$ were measured from% Inhibition vs. Concentration graph using Microsoft excel.

2.7. Anti-Inflammatory Activity

2.7.1. Membrane Stabilization Method

The Human Red Blood Cell (HRBC) membrane stabilization has been adopted as a method to study *in vitro* anti-inflammatory activity since the erythrocyte membrane is comparable to the lysosomal membrane [7], [19] and it is believed that its stabilization indicates that the extract may capably stabilize lysosomal membranes. The stabilization of lysosomal membrane is important as it helps in limiting the inflammatory response by stopping the release of lysosomal constituents of activated neutrophils, such as enzymes like proteases and bacterial products which can be the cause of further tissue inflammation and harm upon extra cellular release. The lysosomal enzymes released during inflammation generate various disorders. The extra cellular activity of these enzymes are believed to be connected to acute or chronic inflammation. The Non-steroidal Anti-inflammatory Drugs (NSAIDs) produce their effects either by inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes [16].

2.7.2. Preparation of Red Blood Cells (RBC's) Suspension

The blood sample was collected from healthy human volunteers of postgraduate students of the Department of Biochemistry of Bayero University Kano, Kano State who have not taken Non-steroidal Anti-inflammatory Drugs (NSAIDs) for the past two weeks preceding the experiment and the samples were transferred to centrifuge tubes. The tubes containing the blood were centrifuged at 3,000 rpm for 10 min and were (blood) washed three (3) times with an equal volume of the normal saline. The volume of blood was weighed and re-constituted as 10% v/v suspension with normal saline.

2.7.3. Heat Induced Haemolysis

The reaction mixture (2 ml) consisting of 1ml of the test extracts of different concentrations (100, 300 and 500 µg/ml) and 1ml of 10% suspension, instead of test sample only the saline was added to the control test tube. Dichlofenac sodium

salt was used as a standard drug and was weighed (0.0025g in 5ml of water). All the centrifuge tubes containing reaction mixture were incubated in a water bath for 30 min at 56°C. At the end of the incubation the tubes were cooled under a running tap water. The reaction mixtures were further centrifuged at 2500 rpm for 5 min and the absorbance of each supernatant was taken at 560nm using UV/VIS spectrophotometer. The experiment was performed in triplicates for all the test samples. The percentage inhibition of haemolysis was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

2.7.4. Hypotonic Solution Induced Haemolysis

Different concentrations of the plant extracts were prepared (i.e. 100, 300 and 500 µg/ml), reference sample and control were both separately mixed with 1 ml of phosphate buffer pH7.4, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Dichlofenac sodium salt was used as a standard drug. All the reaction mixtures for the assay were incubated at 37°C for 30min and thereafter centrifuged at 3000rpm for 10/min. The supernatant liquid was decanted and haemoglobin content was determined by spectrophotometer at 560nm. The percentage of Red Blood Cell membrane stabilization of defensive was calculated by the following equations:

$$\text{\% protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \text{X } 100$$

2.8. Measurement of Antibacterial Activity

2.8.1. Media Preparation

30g of Mueller Hinton agar was weighed using analytical weighing balance. It was dissolved in 1000ml distilled water in a conical flask, the mouth of the flask was plugged with cotton wool wrapped in aluminum foil paper tightened with masking tape. This was to avoid spillage of the media in an autoclave while undergoing sterilization. The dissolved media was sterilized in an autoclave set at 121°C for 15 minutes. It was removed and allowed to cool to 45°C and was gently and aseptically poured into 32 sets of disposable sterilized petridishes. The media plates were allowed to solidify [4].

2.8.2. Test Organisms

The test organism used for the antibacterial activity studies were *Salmonella typhi, Escherichia coli, Streptococcus pneumonea* and *Staphylococcus aureus*. These were obtained from microbiology laboratory in Yobe state University Damaturu. Gram's staining and biochemical tests were carried out to confirm the organisms.

2.8.3. Sensitivity Discs Preparation

Whatmann No. 1 filter paper was punched with paper puncher and the discs of 6.0 mm/dm were obtained. The discs were placed in a screw capped bottle and sterilised in hot air oven at 140°C for 1 hour. The discs were allowed to cool until use.

2.8.4. Preparation of Stock Solution

The stock solution of the plant extract was prepared in a screw capped test tubes using dimethyl sulfoxide (DMSO) as a standard solvent for antimicrobial stock solution preparation. 1g of each fraction was weighed on analytical weighing balance and dissolved in 10 ml DMSO which arrived at 100,000 µg/ml concentration of stock solution. From the stock solution 0.1ml, 0.2ml, 0.3ml, 0.4ml, and 0.5ml was added to 0.9 ml, 0.8 ml, 0.7 ml, 0.6 ml, and 0.5 ml of DMSO in a sterile test tube, which made 1 ml of each fraction respectively. 1 disc was placed in each concentration which arrived at disc potency of 100 µg/disc, 200 µg/disc, 300 µg/disc, 400 µg/disc, and 500 µg/disc respectively.

The test organisms were inoculated unto the Mueller Hinton agar with a sterilized inoculating loop, and the plates were appropriately labelled with the name of the test organism as well as the plant extract for easy identification. The sensitivity disc from concentration of each fraction was placed on the MHA. The plates were incubated for 72 hours and zones of inhibition were detected in mm/dm [13].

3. Results and Discussion

3.1. Extraction Yield

The powdered stem bark of *Lannea microcarpa* was extracted successively with *n*-hexane, ethyl acetate, methanol and water using ultrasonicator. The results of the extraction yield are shown in Table 1 below:

Table 1. *Percentage yield of crude stem bark extracts of Lannea microcarpa.*

	Solvents used	Weight of plant part used (g)	Weight of extract (g)	Percentage yield (%)
1	*n*-Hexane	550 (*Lannea microcarpa*)	13.55	2.46
2	Ethyl Acetate	550 (*Lannea microcarpa*)	16.84	3.06
3	Methanol	550 (*Lannea microcarpa*)	9.08	1.65
4	Water	550 (*Lannea microcarpa*)	6.94	1.26

The results above revealed that in the 550g of *Lannea microcarpa,* the ethyl acetate extracted the highest phytochemical yield of 16.84g (3.06%), followed by n-hexane 13.55g (1.26%). The lowest yield was shown by water (6.94g or 1.236%). These results suggested that crude phytochemicals for each accounted for less than 10% of the plant sample and that ethyl acetate extracted more than the other solvents.

3.2. Phytochemical Screening

Lannea microcarpa

Phytochemical constituents of the *Lannea microcarpa n-*

Hexane, Ethyl acetate, methanol and Water extracts reveal the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins, phenol and Sterols while, terpenoids quinones and oxalates were absent in all the extract as presented in the Table 2 below.

Table 2. *The Results of the Phytochemical Screening of Lannea microcarpa n-Hexane, Ethyl acetate, Methanol and Water Extracts.*

Phytochemicals	*n*-Hexane extract	Ethyl acetate Extract	Methanol Extract	Water Extract
Alkaloid	+	+	+	+
Flavonoid	+	+	++	+
Saponins	++	+	++	+
Cardiac glycoside	+	+	+	+
Oxalate	-	-	-	-
Quinones	-	-	-	-
Terpenoids	-	-	-	-
Tannins	+	+	+	+
Sterols	+	+	+	+
Phenols	+	+	+	+

Key: + = Present, - = Absent

The phytochemical screening of *Lannea microcarpa* stem bark showed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, sterols and phenols. On the other hand oxalates, quinones and terpenoids were not detected. The distribution of these secondary metabolites into solvents of increasing polarities is shown in Table 2. The results showed the presence of non-poloar, semi polar and polar alkaloids, flavonoids, saponins, cardiac glycosides, tannins, sterols and phenols. Flavonoids and saponins seem to be present in large amount in the methanol extracts, and more non-polar saponins were detected in the n-hexane extract.

3.3. Antioxidant Studies

The antioxidants are mainly derived from food and medicinal plants such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs [3]. Natural antioxidants from plant materials are mainly Polyphenols (comprising mainly of Phenolic acids, Flavonoids, Tannins, Anthocyanins, Lignans and Stilbenes), Carotenoids (Xanthophylls and Carotenes) and Vitamins (Vitamin C and E), [1]. These natural antioxidants, especially the Polyphenols and Carotenoids are reported to exhibit a wide range of biological effects, such as anticancer, antibacterial, anti-inflammatory, antiviral and anti-aging [5].

The antioxidant studies in the present work were carried out on the n-hexane, ethyl acetate, methanol and water extracts of *Lannea microcarpa* using the free radical scavenging activities of the samples on 2,2-diphenyl-1-picryldihydrazyl (DPPH) radical and ascorbic acid as standard. From the caliberation curves obtained, the 50% inhibitory concentration (IC_{50}) values were determined. IC_{50} value denotes the concentration of the sample required to scavenge 50% of the DPPH free radicals measured at 517 nm as reported by [8]. The absorbance and percentage inhibition of the standard and the extracts were shown in Tables below:

Table 3. *The UV Absorbance measured at 517nm to determine antioxidant activities of Standard Ascorbic Acid, n-hexane extract, ethyl acetate extract, methanol extract and water extract of Lannea microcarpa against DPPH radical.*

S/No.	Concentration (µg/ml)	Ascorbic Acid	Extract absorbance			
			n-Hexane	Ethyl Acetate	Methanol	Water
1	0	0.675	0.675	0.675	0.675	0.675
2	10	0.433	0.533	0.502	0.489	0.496
3	25	0.354	0.342	0.311	0.286	0.299
4	50	0.249	0.281	0.223	0.175	0.191
5	100	0.122	0.122	0.121	0.083	0.095
6	125	0.093	0.097	0.098	0.072	0.083
7	250	0.084	0.089	0.085	0.063	0.077
8	300	0.075	0.075	0.072	0.054	0.064
9	500	0.073	0.072	0.068	0.051	0.062

The above table was used to plot a graph of Absorbance of standard ascorbic acid and n-Hexane, Ethyl Acetate, Methanol and Water extracts of *Lannea microcarpa* against concentration using MS. Excel.

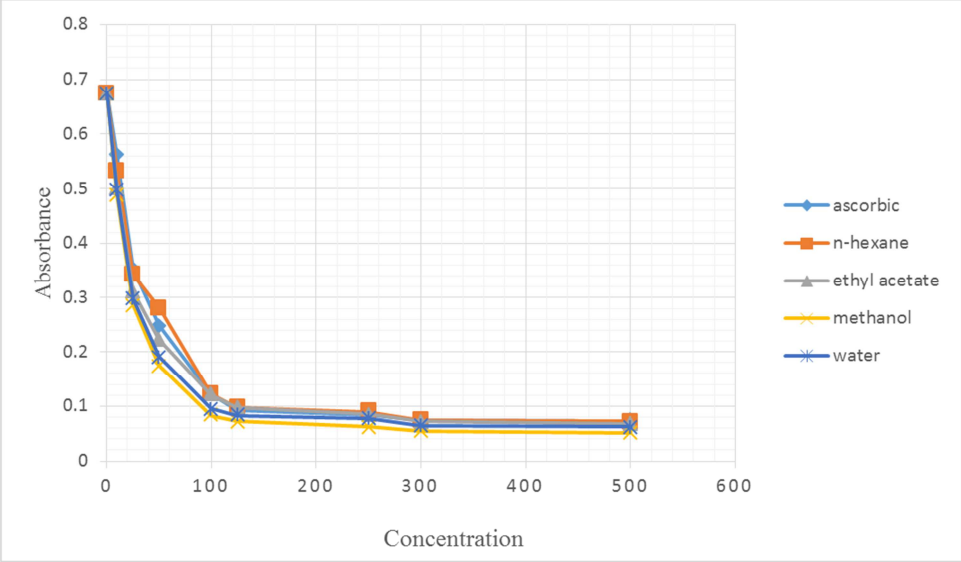

Figure 1. *Graph of Absorbance at 517nm Against Concentration of Standard Ascorbic Acid, n-hexane extract, ethyl acetate extract, methanol extract and water extract of Lannea microcarpa.*

The above graph (Figure 1) shows the DPPH scavenging activity of n-Hexane, Ethyl Acetate, Methanol and Water extracts of *Lannea microcarpa* compared to that of the standard ascorbic acid. The activities were very close and very difficult to draw conclusions. In order to overcome this, a graph of% inhibition Vs concentration was sought following the conversion of absorbances to that of% inhibition as shown in Table 4.

Table 4. *The Percentage Inhibitions of Ascorbic acid, n-Hexane, Ethyl acetate, Methanol and Water extracts of Lannea microcarpa on DPPH Radical.*

S/No.	Concentration (µg/ml)	Ascorbic acid	*n*-Hexane	Ethyl Acetate	Methanol	Water
1	0	0	0	0	0	0
2	10	35.9	21.0	25.6	27.6	26.5
3	25	47.5	49.3	53.9	57.6	55.7
4	50	63.1	58.4	66.9	74.1	71.7
5	100	81.9	81.9	82.1	87.7	85.9
6	125	86.2	85.3	85.5	89.3	87.7
7	250	87.5	86.8	87.4	90.7	88.6
8	300	88.9	88.9	89.3	92.0	90.5
9	500	89.2	89.3	89.9	92.4	90.8

From the Table 4, a graph of% inhibition of n-hexane, ethyl acetate, methanol and water extracts of *Lannea microcarpa* against concentration were plotted separately.

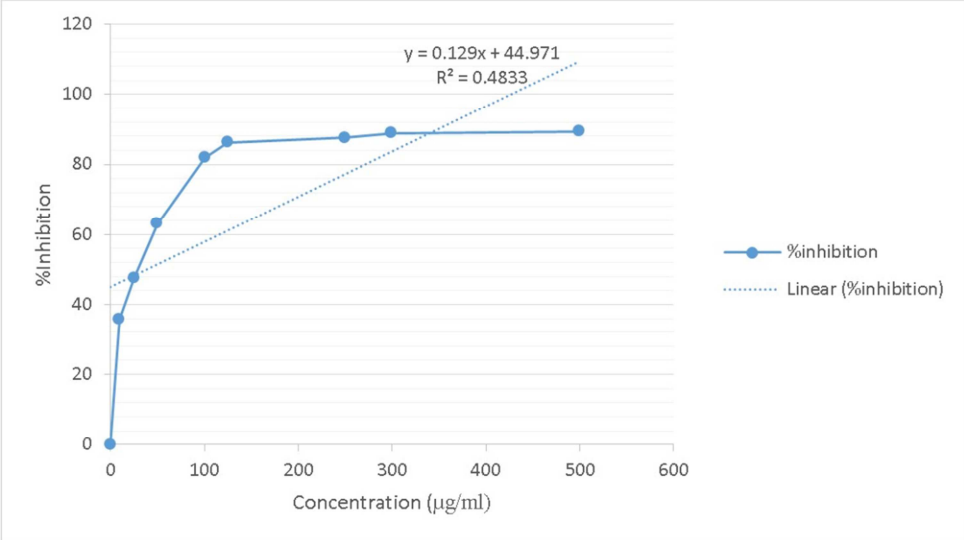

Figure 2. *% inhibition of ascorbic acid for the Calculation Value.*

The IC_{50} value of the standard ascorbic acid was calculated from the above graph by using the equation y = 0.129x + 44.971, where y is 50 and x represents the IC_{50} value. From this IC_{50} value of Standard Ascorbic Acid was calculated to be 38.9µg/ml. The IC_{50} values of all the other test extracts were calculated in a similar manner.

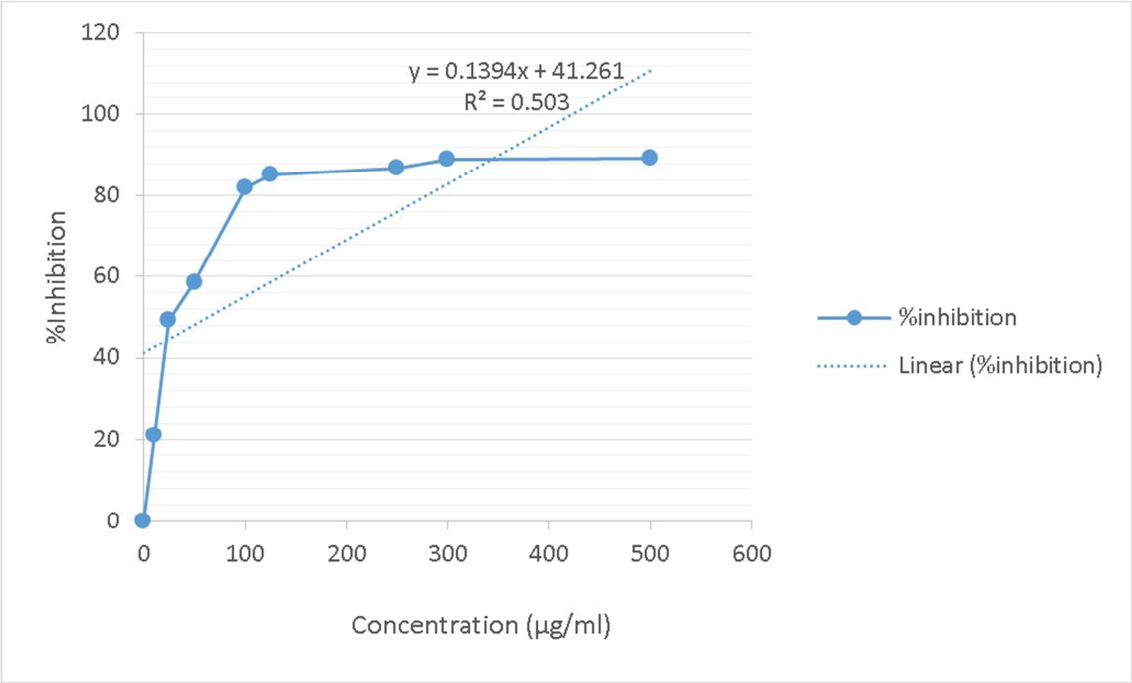

***Figure 3. %** inhibition of n-hexane extract of Lannea microcarpa.*

The IC_{50} value of n-hexane extract of *Lannea microcarpa* was calculated from the y = 0.1394x + 41.261. The IC_{50} value of n-hexane extract of *Lannea microcarpa* was determined as 62.7µg/ml, this was about twice lower quenching activity than standard ascorbic acid. For the ethyl acetate extracts the graph in Figure 3. was used which gave a linear equation y = 0.1315x + 44.638.

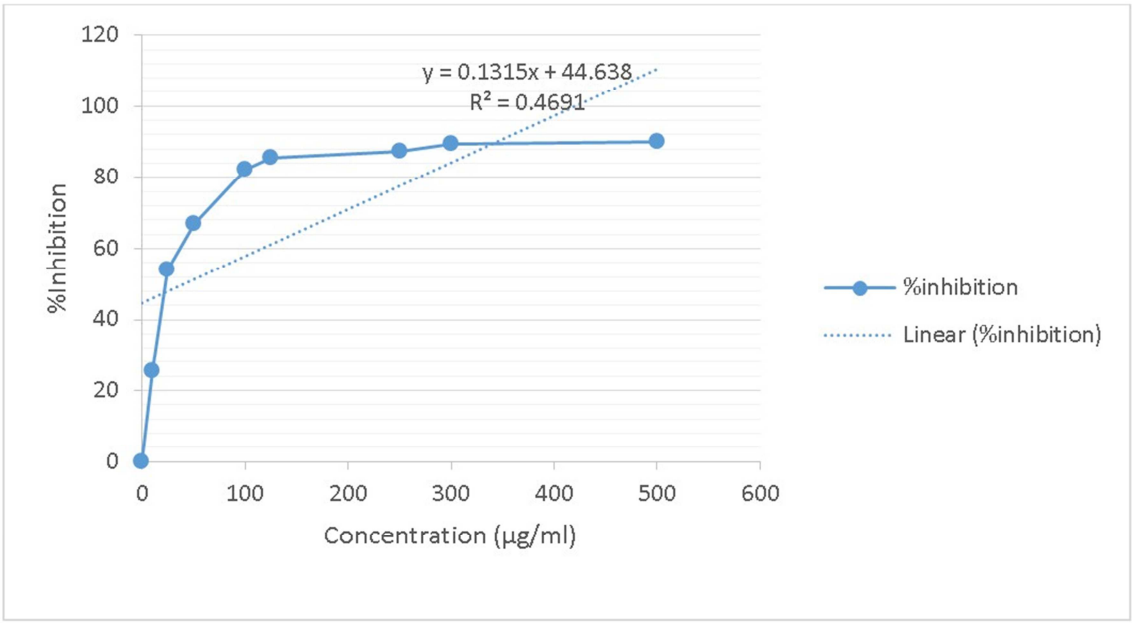

***Figure 4. %** inhibition of ethyl acetate extract of Lannea microcarpa.*

The IC_{50} value of ethyl acetate extract of *Lannea microcarpa* was computed as 40.8 µg/ml, which was found to be higher scavenging activity than the n-hexane extract.

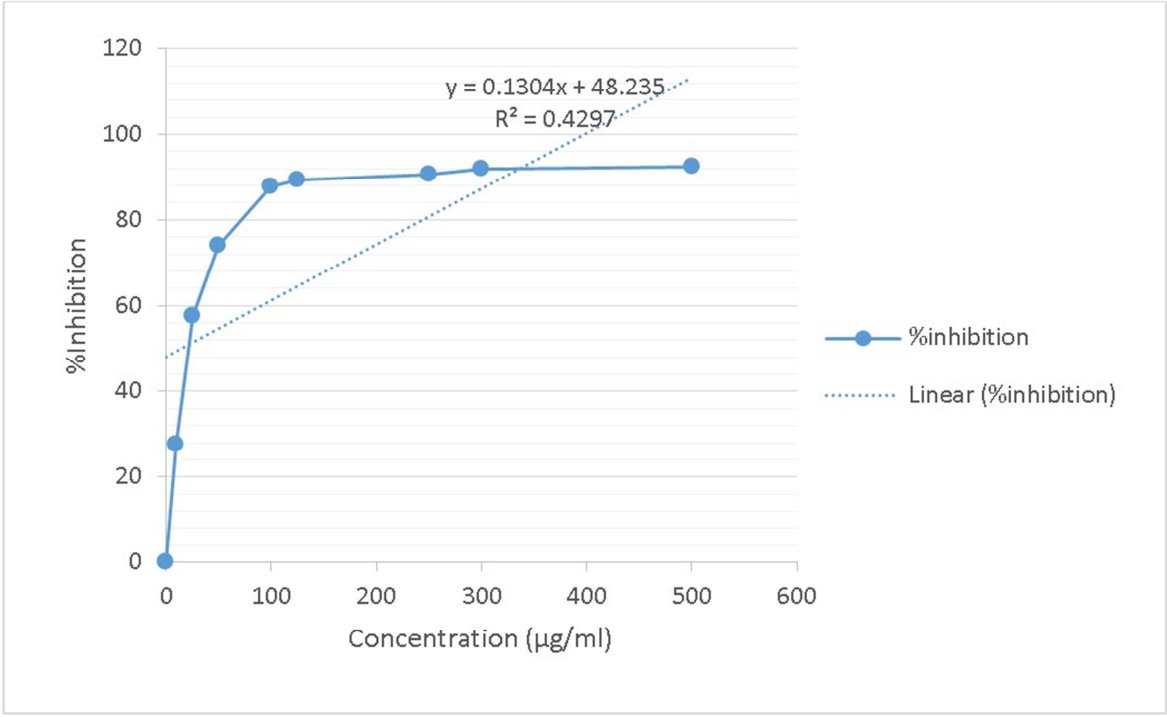

Figure 5. *% inhibition of methanol extract of Lannea microcarpa.*

The IC_{50} value of methanol extract of *Lannea microcarpa* was calculated from the graph in Figure 5. The Inhibitor Concentration against the percent activity is plotted using the linear equation generated from the graph (y = 0.1304x + 48.235), for y = 50 the value of x becomes IC_{50} value. The IC_{50} value of methanol extract of *Lannea microcarpa* calculated to be 13.5µg/ml. this value indicated that the methanol extract of *L. microcarpa* was about three fold more potent scavenger than ascorbic acid.

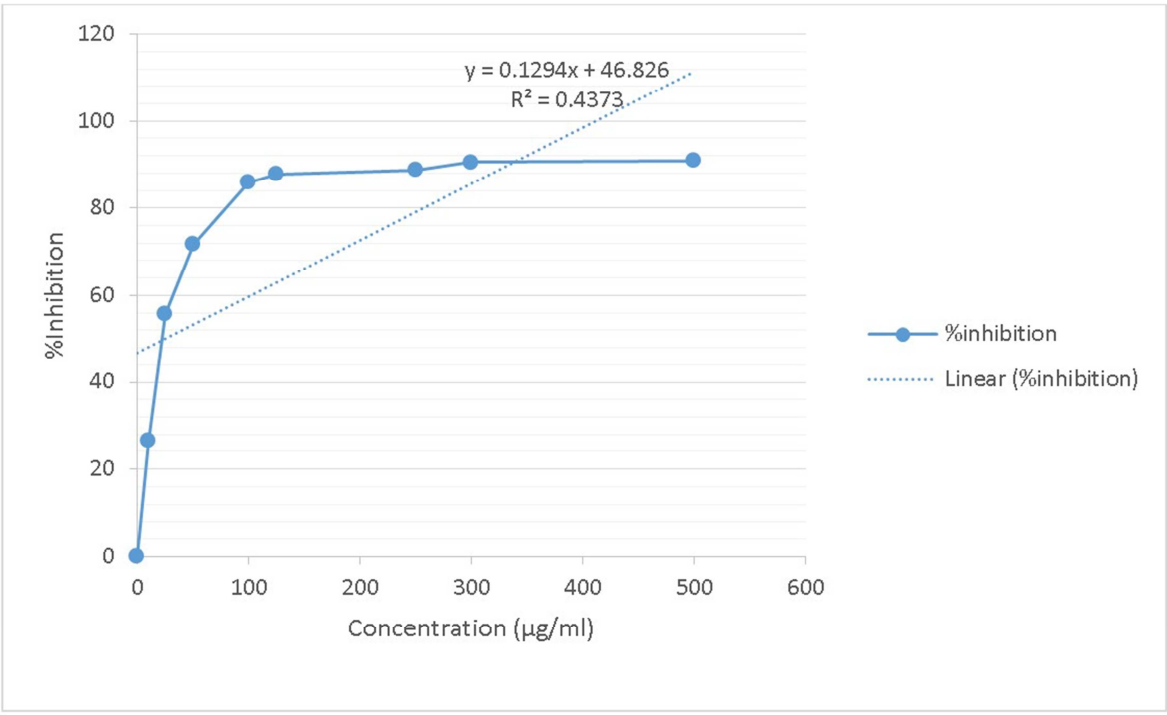

Figure 6. *% inhibition of water extract of Lannea microcarpa.*

The IC_{50} value of water extract of *Lannea microcarpa* was calculated from the graph in Figure 6 The Inhibitor Concentration against the percent activity was plotted and using the linear equation generated was y = 0.1294x +

46.826, for y = 50, the value of x represents the IC_{50} value of the water extract of *Lannea microcarpa* and it came out to be 24.5µg/ml. This also suggests that the water extract of *L. microcarpa* contains compounds that are more potent in quenching free radicals than ascorbic acid standard.

Table 5. *The summary of the IC_{50} values of Lannea microcarpa extracts as compared to standard ascorbic acid.*

S/No.	Extract/*Standard	IC_{50} (µg/ml)
		L. M
1	*Ascorbic acid	38.9
2	n-hexane	62.7
3	Ethyl acetate	40.8
4	Methanol	13.5
5	Water	24.3

Key: L. M - Lannea microcarpa

IC_{50} values obtained for the *Lannea microcarpa* extracts indicated a different pattern in that the methanol and water extracts exhibited higher antioxidant activities (13.5 and 24.5 µg/ml respectively) than the standard ascorbic acid, while the ethyl acetate and n-hexane extracts exhibited lower activities (50.8 and 62.7 µg/ml respectively) compared to the standard ascorbic acid. Indeed, the methanol extract showed almost three times more antioxidant activity than the ascorbic acid standard.

3.4. RBC Membrane Stabilization

Membrane stabilization is a process of maintaining the integrity of biological membranes such as the erythrocyte and lysosomal membranes against osmotic and heat-induced lysis as reported [18]. In this study the effects of the various extracts on RBC membrane stabilization against haemolysis induced by heat and hypotonicity were determined. The results of each test were expressed as mean ± SD using Graph Pad prism (version 4), using a one-way analysis of variance (ANOVA). The statistical method applied in each analysis was described in each Table. Results were considered to be significant when *p*-values were less 0.05 ($p<0.05$).

3.4.1. Heat Induced Haemolysis.

The results of the effect of extracts on heat induced haemolysis of RBC Are represented in (Table 6). The extracts were found to be effective in inhibiting the heat induced haemolysis at different concentrations. Diclofenac Sodium salt used as standard drug at 100µg/ml offered 90.66% protection a significant ($p<0.05$) protection against damaging effect of heat solution.

Table 6. *Effect of n-Hexane, Ethyl acetae, Methanol and Water Extracts of Lannea microcarpa on Heat Induced Haemolysis.*

Extract	Treatment (S)	Absorbance at 560nm	% Inhibition
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
n-hexane	100	0.5530 ± 0.0061^{c}	56.25
	300	0.2843 ± 0.0026^{d}	77.50
	500	0.2760 ± 0.0030^{d}	78.16
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
Ethyl acetate	100	0.6333±0.0003c	46.89
	300	0.5653 ± 0.0020^{d}	55.27
	500	0.5543 ± 0.0013^{d}	56.14
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
Methanol	100	0.3970 ± 0.0000^{e}	68.59
	300	0.2690 ± 0.0006^{d}	78.71
	500	0.2267 ± 0.0007^{d}	82.06
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
Water	100	0.4997 ± 0.0003^{c}	60.46
	300	0.4837 ± 0.0007^{c}	61.73
	500	0.3550 ± 0.0042^{d}	71.91

Values are expressed in mean ± SD (n = 3). Data analysed using one way Anova;
Values along same column differently superscripted differ significantly ($P<0.05$)

The results from *Lannea microcarpa* extracts showed the all the four extracts have membrane stabilizing effect. The methanol extract showed the highest values (68.59-82.06%, $p<0.05$). This was followed by n-hexane extract (56.25-78.16%, $p<0.05$). Water extract was the found to be the third highest value (60.46-71.91%, $p<0.05$) and ethyl acetate extract showed the lowest effect but significant (46.89-56.14%, $p<0.05$). All the extracts exhibited concentration dependent increases in RBC membrane stabilization against heat induced haemolysis.

3.4.2. Hypotonicity Induced Haemolysis of RBC

Table 7. *Effect of n-Hexane, Ethyl acetate, Methanol and Water Extracts of Lannea microcarpa on Hypotonicity Induced Haemolysis.*

Extract	Treatment (S)	Absorbance at 560nm	% Inhibition
	Negative Control (Hyposaline)	0.5690±0.0000^{a}	0
	Positive Control (Diclofenac Sodium)	0.2417±0.0003^{b}	57.52
n-hexane	100	0.2553±0.0009^{b}	55.13
	300	0.2090±0.0000^{c}	63.26
	500	0.1247±0.0009^{d}	78.08
	Negative Control (Hyposaline)	0.5690±0.0000^{a}	0
	Positive Control (Diclofenac Sodium)	0.2417±0.0003^{b}	57.52
Ethyl acetae	100	0.5273±0.0007^{a}	7.32
	300	0.4117±0.0007^{c}	27.64
	500	0.2510±0.0010^{b}	55.88
	Negative Control (Hyposaline)	0.5690±0.0000^{a}	0
	Positive Control (Diclofenac Sodium)	0.2417±0.0003^{b}	57.52
Methanol	100	0.2000±0.0000^{c}	64.85
	300	0.1274±0.0546^{d}	78.71
	500	0.0930±0.0000^{e}	83.65
	Negative Control (Hyposaline)	0.5690±0.0000^{a}	0
	Positive Control (Diclofenac Sodium)	0.2417±0.0003^{b}	57.52
Water	100	0.4997±0.0003^{c}	12.17
	300	0.4837±0.0007^{c}	14.99
	500	0.3550±0.0042^{d}	37.60

Values are expressed in mean ± SD (n = 3). Data analysed using one way Anova;
Values along same column differently superscripted differ significantly (P<0.05).

Lannea microcarpa extracts generally showed higher protections (p<0.05) than Diclofenac sodium especially as the concentration increases. The n-hexane and methanol extracts showed significantly higher inhibitions (55.13 – 78.08% and 64.85 – 83.65% respectively) than the standard drug. The ethyl acetate extract showed comparable inhibitions (55.88%) to Diclofenac sodium at higher concentration of 500µg/ml, while the water extract produced lower protection (12.17 – 37.6%) to RBC lysis. These results suggest that *Lannea microcarpa* extracts can protect RBCs from hypotonic induced haemolysis.

3.5. Antibacterial Activities

In this study the antibacterial effects of the standard drugs and the n-hexane, ethyl acetate, methanol and water extracts of *Lannea mirocarpa* were studied against isolate of two Gram positive (*Staphylococcus aureus and Strephtococcus pneumonea*) and two Gram negative (*Escherichia coli and Salmonella typhi*) bacteria. The effects the standard drugs (Gentamycin, Ciprofloxacin, Augmentin, Amoxicillin, Ceftriaxone, and Cefuroxime) are presented in (Table 8). The results of the *in vitro* antibiotic sensitivity tests showed that isolates of *Staphylococcus aureus, Strephtococcus pneumonea, Salmonella typhi* and *Escherichia coli* were generally susceptible to Ofloxacin, Ciprofloxacin, Augmentin and Amoxicilin. *Staphylococcus aureus, Strephtococcus pneumonea* and *Escherichia coli* were found to be resistant to Ceftriaxone and Cefuroxime but susceptible to Gentamycin while *Salmonella typhi* was found to be susceptible to the Ceftriaxone and Cefuroxime and resistant to Gentamycin.

Table 8. *Antibacterial activity of Standard drugs.*

Standard	(Zone of inhibition mm/dm)			
	Gram Positive		Gram Negative	
	S. aureus	*S. pneumonea*	*S. typhi*	*E. coli*
Gentamycin	22	25	0	20
Ofloxacin	20	15	13	10
Ciprofloxacin	35	12	15	22
Augmentin	10	25	10	30
Amoxicillin	10	15	12	15
Ceftriaxone	0	0	15	0
Cefuroxime	0	0	10	0

Key: 0 = No Zone of Inhibition Detected

Table 9. *Zone of inhibition (ZI) of extracts of Lannea microcarpa.*

		Zones of Inhibition (mm/dm)			
Extract	**Concentration µg/ml**	***S. typhi***	***S. aureus***	***E. coli***	***S. pneumonea***
	100	0	3	8	0
	200	0	5	10	0
n-Hexane	300	0	7	13	0
	400	0	10	16	0
	500	0	13	18	0
	100	0	5	9	0
	200	0	7	11	0
Ethyl acetate	300	0	10	12	0
	400	0	15	15	0
	500	0	20	18	0
	100	0	8	12	0
	200	0	10	15	0
Methanol	300	0	13	17	0
	400	0	15	22	0
	500	0	22	23	0
	100	0	12	8	0
	200	0	15	10	0
Water	300	0	17	11	0
	400	0	20	12	0
	500	0	21	15	0

Key; 0 = No activity

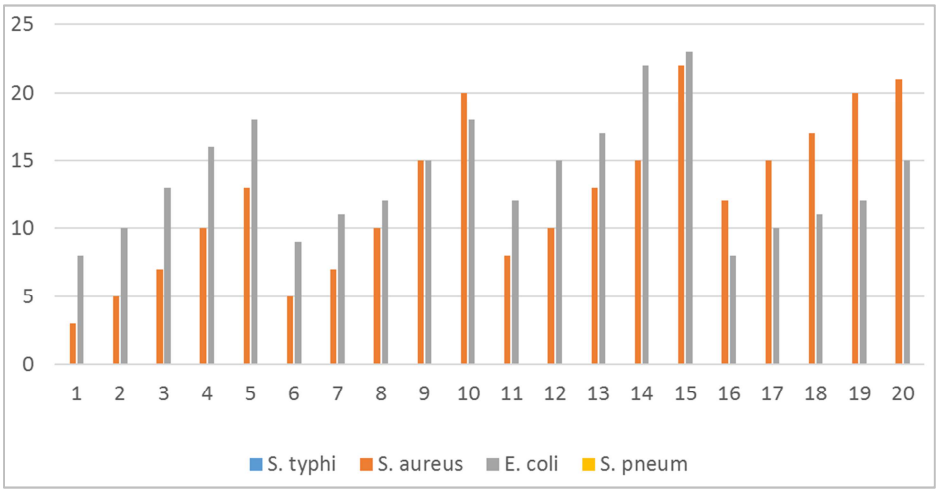

Figure 7. *Antibacterial activity from Lannea microcarpa extracts against Salmonella typhi, Staphylococcus aureus, Escherichia coli and Strephtococcus pneumonea.*

The stem bark extracts of *Lannea microcarpa* also tested for their antibacterial activities and the outcome of the study (Table 9) and Figure 7. The results obtained showed that *Salmonella typhi* and *Strephtococcus pneumonea* were not susceptible to any of the *Lannea microcarpa* extracts at all the different concentrations used. However, the extracts were found to be active against the growth of *Staphylococcus aureus* and *Escherichia coli* at all the varying concentrations. Methanol extract showed the highest values in both the two pathogens with a zone of inhibition ranging from (8 to 22 mm/dm) for *Staphylococcus aureus* and (12 to 23 mm/dm) for *Escherichia coli.*

4. Conclusion

Plant-derived substances have recently become areas of great research interests owing to their versatile applications for the betterment of human life [2]. There is little wonder that medicinal plants are constituting the richest bio-resources of drugs for traditional medical systems, modern medicines, nutraceuticals, food supplements, pharmaceuticals, intermediate and precursors for the synthesis of drugs [9]. The traditional medicinal value of plants is mainly attributed to the bioactive phytochemical constituents of the plants that exhibit various physiological effects. Therefore, through phytochemical screening one could detect the various important compounds which could be used as the base for the development of modern drugs for curing various diseases. Phytochemical constituents of Lannea *microcarpa* extracted with *n*-Hexane, Ethyl acetate, methanol and Water were identified to be alkaloids, flavonoids, cardiac glycosides, saponins, tannins, phenols and Sterols, while terpenoids, quinones and Oxalates were not detected in all the extracts.

These plants extracts showed good antioxidant activity, anti-inflammatory activity and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* The therapeutic potential of the stem barks of *Lannea microcarpa,* could be attributed to classes of active components present in the stem barks such as alkaloid, flavonoids, tannins and phenolic etc. which may be acting in synergy or individually.

The antioxidant, anti-inflammatory and anti-bacterial activities carried out in this study lend credence to the traditional claim about the wound healing property of the stem barks of *Lannea microcarpa*.

5. Recommendations

Traditionally the stem bark of *L. microcarpa* is used in the treatment of oxidation stress, inflammation, bacterial disease and cancer. This research validate the oxidation stress, inflammation, bacterial disease claim. Further studies should be carried out on the anticancer claim and other uses of not only the stem bark of the plant but other parts of which may probably possess some therapeutic activities.

Also further studies should be done to identify the bioactive compounds responsible for the antioxidant, anti-inflammatory and antibacterial activities and acceptable dosage forms that will aid the use of the stem barks as antioxidant, anti-inflammatory and antibacterial agent.

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