Phytochemical Screening, Antioxidant, Anti-Inflammatory and Antibacterial Studies on the Stem Bark Extracts of *Ximenia Americana* Linn.

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ABSTRACT

Medicinal plants have been used in traditional medicine practices since pre-historic times. Any part of the plant could be used as medicine. A survey of traditional medicine men and women in Damaturu, Nigeria indicated that *Ximenia americana* is a plant used in the treatment of inflammation, wounds and cancer. The fresh stem barks of the plant were collected and successively extracted with n-hexane, ethyl acetate, methanol and water. Phytochemical constituents of the extracts revealed the presence of cardiac glycosides, flavonoids, saponins, phenols, tannins, alkaloids, sterols and terpenoids. The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, whereas membrane stabilization method was used to evaluate the anti-inflammatory activities of the extracts. The antibacterial activity was studied against four (4) wound pathogens (*Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumoniae),* using disc diffusion technique. The results of the IC_{50} for antioxidant studies indicated that the methanol (22.4μg/ml) and ethyl acetate (28.4 μg/ml) extracts of *X. americana* exhibited higher antioxidant activities than the standard ascorbic acid (38,9μg/ml). The extracts were found effective in inhibiting the heat induced hemolysis in a concentration dependent manner. The n-hexane extract showed the highest protection level (84.41 – 87.98%, $p<0.05$), followed by water (46.73 – 82.38%, $p<0.05$) and ethyl acetate (71.3o – 77,39%, $p<0.05$) extracts. 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. The hypotonic solution induced study indicated that the extracts exhibited more protection from hemolysis (n-hexane = 75.22 – 85,23%, $p<0.05$; ethyl acetate = 4.09 – 71,40%, $p<0,05$; water = 59.98 – 67.08%, $p<0.05$, and methanol = 48.15 – 58.75%, $p<0.05$) than the standard Diclofenac sodium salt (57.52%, $p<0.05$). 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%). *X. americana* samples showed that all extracts inhibited the growth of *S. aureus, S. pneumoniae and E. coli.* The antioxidant, anti-inflammatory and anti-bacterial activities carried out in this study can be used as scientific explanation for the traditional claim about the wound healing property of the stem bark of *X. americana*.

Keywords: Phytochemical, Antioxidant, Anti-inflammatory, Antibacterial

INTRODUCTION

Medicinal plants are 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 have been 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 (Shoeb, 2006). Since then, plants have been used to treat diseases such as headache, toothaches, stomach aches, diarrhea, wounds, tumors and sexually transmitted diseases (van Wyk and Gericke, 2000; Khaleeliah, 2001; Von Koenem, 2001, Wuyang, 2008).

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 (Mdlolo; 2009, Fawole 2009).

Ximenia americana of the family Olacaceae, is commonly known as Tallow wood in English and 'Tsaada' in Hausa. A survey of medicinal plants within and around Damaturu town, Nigeria indicated that the plant is used in the treatment of 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 part of the plant in order to provide explanation for 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 ailments. Some of these plants are used as food and/or medicine. The extracts from plant have been 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. (Okwu, Ekeke 2003 and Okwu; 2004). For this reason, medicinal plants are considered important to the health of the individuals and communities.

MATERIALS AND METHODS

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 Potiskum Road, Nigeria), empty bottles purchased at Bayan Tasha market Damaturu, Nigeria. 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 Chun Hong, Shanghai, China and other laboratory materials.

Chemicals

n-hexane, ethyl acetate and methanol were 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, Dragendorff's reagent, choloroform and all other Chemicals used are of highest analytical grade and purchased from BDH Chemicals, Poole, England.

Collection and Preparation of Plant Sample

The fresh sample of *X. americana* (Voucher number 1973) (stem bark) was collected at the campus of Yobe State University Damaturu, 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 was collected two (2) meters above the ground. The sample was sorted to ensure no foreign bodies were present, it 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 pulverized into fine powder and sieved with a sieve and weighed. The finely powdered sample was then weighed and stored in sealed containers until required for further analysis.

Extraction of Phytochemicals

About 550g of the powdered plant material of (*X. americana*) 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 further extraction. The procedure was repeated for the remaining solvents namely; ethyl acetate, methanol and water in that order.

Phytochemical Screening

Phytochemical screening of the extracts was performed to detect the presence of phytochemicals using the procedures outlined by Tiwari *et al.* (2011); Sabri *et al.* (2012) and Solomons *et al.* (2013).

Test for alkaloids

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

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, indicated the presence of flavonoids.

Test for saponins

Frothing test: The extract (0.5g) was added to distilled water to a final volume of 20ml and this was shaken vigorously in a graduated cylinder for 15 minutes over a vortex mixer. Formation of 1cm layer of foam indicated the presence of saponins.

Test for cardiac glycosides (Keller-Kelliani test)

To 5ml of the extract (0.5g dissolved in 5ml methanol) was treated with 2ml of glacial acetic acid in a test tube followed by a drop of 2% ferric chloride solution. This was carefully underlaid 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 which indicated the presence of cardiac glycosides.

Test for oxalate

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

Test for quinones

A small portion of the extract (0.2g in 2ml of methanol) was treated with concentrated hydrochloric acid. The formation of yellow precipitate/colouration indicated the presence of quinones.

Test for terpenoids (Salkowski's test)

One ml of chloroform was added to 2ml of the extract (0.2g in 2ml of methanol) followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoids.

Test for tannins (Braymer's test)

Two ml of the extract (0.2g in 2ml of methanol) was treated with 10% alcoholic ferric chloride solution. Formation of blue/greenish colouration indicated the presence of tannins.

Test for sterols (Libermann-Burchard test)

About 1ml of the extract (0.1g in 1ml of methanol) was treated with few drops each of chloroform, acetic anhydride and concentrated sulphuric acid. The formation of dark pink or red colour indicated the presence of sterols.

Test for phenols

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

Measurement of Antioxidant Activities

The antioxidant activities of *X. americana* 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 was 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 Hatano *et al.* (1988). Then the % inhibition was calculated by the following equation;

% Radical scavenging = (absorbance of blank – absorbance of sample) x 100%

    Activity   (absorbance of blank)

A blank was prepared by adding 3 ml of 0.004% ethanolic DPPH to 1 ml of the ethanol and treated in a similar manner to test samples.

Anti-Inflammatory Activity

Membrane stabilization method

The Human Red Blood Cell (HRBC) membrane stabilization was adopted as a method to study the *in vitro* anti-inflammatory activity since the erythrocyte membrane is comparable to the lysosomal membrane (Gandasan *et al*., 1991, Shenoy *et al*., 2010).

Preparation of red blood cells (RBC's) suspension

The blood sample was collected from healthy human volunteers of the Department of Biochemistry of Bayero University Kano, Kano State who had not taken Non-steroidal Anti-inflammatory Drugs (NSAIDs) for the previous 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 to remove plasma. The packed RBCs were washed three (3) times with an equal volume of the normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline.

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. A test tube containing only saline was used as the control. Diclofenac sodium salt was used as a standard drug and was weighed and dissolved in water (0.0025g in 5ml of water). All the centrifuge tubes containing reaction mixture were incubated in a water bath for 30 min at 56ºC. 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 Leelaprakash and Mohan (2010):

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

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 pH 7.4, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Diclofenac sodium salt was used as a standard drug. All the reaction mixtures for the assay were incubated at 37ºC 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 Leelaprakash and Mohan (2010):

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

Measurement of Antibacterial Activity

Media preparation

About 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 medium in an autoclave while undergoing sterilization. The dissolved medium 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 Petri dishes. The media plates were allowed to solidify (Cheesbrought, 2002).

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 the microbiology laboratory at Yobe state University Damaturu. Gram's staining and biochemical tests (Disc diffuse method) were carried out to confirm the organisms.

Sensitivity discs preparation

Whatman 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 sterilized in hot air oven at 140°C for 1 hour. The discs were allowed to cool until use.

Preparation of stock solution

The stock solution of the plant extract was prepared in screw capped test tubes using dimethyl sulfoxide (DMSO) as a standard solvent for antimicrobial stock solution preparation. 1g of each fraction was weighed and dissolved in 10 ml DMSO to give 100,000 µg/ml of stock solution. From this 0.1ml, 0.2ml, 0.3ml, 0.4ml, and 0.5ml were respectively 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. One (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 on the Mueller Hinton agar (MHA) 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 (Okafor, 2002), the test was carried out using (Gentamycin, Ofloxacin, Ciprofloxacin, Augmentin, Amoxicillin, Ceftriaxone, and Cefuroxime 100 µg/disc) as a control (Rubina, 2011).

RESULTS AND DISCUSSION

Extraction Yield

The powdered stem bark of *X. americana* was successively extracted 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 *X. americana.*

	Solvents used	Weight of plant part used (g) (*X. americana*)	Weight of extract(g)	Percentage yield (%)
1	*n*-Hexane	550	17.38	3.16
2	Ethyl Acetate	550	21.94	3.99
3	Methanol	550	9.08	1.65
4	Water	550	6.43	1.17

The results above revealed that in the 550g of *X. americana* extracted with four solvents of increasing polarities revealed that the total phytochemical yield of 54.83g (or 9.97%). Out of this ethyl acetate extracted the highest 21.94g (3.99%), followed by n-hexane with yield of 17.38g (or 3.16%), while methanol (9.08g or 1.65%) and water (6.43 or 1.17%) extracts showed the lower yields.

Phytochemical Screening

Plant secondary metabolites have become areas of great research interests due to their wide-ranging applications for the improvement human health better human life (Baris *et al.,* 2006). For this reason, medicinal plants are regarded as the richest sources of drugs for traditional medical systems, modern medicines, nutraceuticals, food supplements, pharmaceuticals, intermediates and precursors for the synthesis of drugs (Hammer *et al.,* 1999). The traditional medicinal value of plants is mainly attributed to the bioactive phytochemical constituents of the plants that exhibit various physiological effects, this fact has a long history (Sofowora, 1980; 1993). 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.

In the present study the phytochemical constituents of the *X. americana* extracted with *n*-Hexane, Ethyl acetate, methanol and Water revealed the presence of cardiac glycosides, flavonoids, saponins, phenols and tannins. Alkaloids were present in *n*-hexane, ethyl acetate, methanol extracts, terpenoids were present only in ethyl acetate and water extracts while sterols, quinones and oxalates were detected in all the extracts screened as presented in the Table 2 below.

Table 2 The Results of the Phytochemical Screening of *X. americana 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

These results further suggested the presence of both nonpolar, semi-polar and polar compounds of alkaloids, flavonoids, saponins, tannins, phenols and cardiac glycosides in the stem bark of *X. americana*. Terpenoids were found in the ethyl acetate and water extracts indicating the presence of semi-polar and polar moieties of this phytochemical in the stem bark.

Secondary metabolites such as alkaloids, flavonoids, phenols, tannins, terpenoids, etc. have been reported to be responsible for the therapeutic benefits of medicinal plants Nandagoapalan *et al*., (2016) because of their pharmacological activities. Such activities as antimicrobial, anti-inflammatory, antioxidant, anticancer,

among others, may be principal indicators of a plant's value in medicine. A number reports are available in literatures that lend support to this assumption. For instance, several flavonoids, saponins, alkaloids, tannins and terpenes isolated from medicinal plants have been shown to exhibit significant antinociceptive and anti-inflammatory activities (Suleiman *et al*., 2009). Tanko *et al.* (2007) attributed the analgesic activity of ethanol extract of *Nigeula sativa* to the presence of tannins and flavonoids in the extract. According to Mamta *et al*. (2013) flavonoids have been reported to exert multiple biological effects including anti-inflammatory, antimicrobial as well as antitumor activities. Phenolic compounds found from plants are also reported to be responsible for antioxidant, anti-inflammatory, and other biological properties (Park *et al*., 2011). Flavonoids isolated from medicinal plants have been reported to possess significant anti-inflammatory activity and, in some cases, analgesic activity (Kim *et al.,* 2000; Amin *et al*., 2012, Santosh *et al.,* 2008 and Amin *et al.,* 2012). The flavonoids were reported to exert their anti-inflammatory effect by interfering with inflammatory process by inhibiting enzymes involved in prostaglandin synthesis (Duke, 1992; Kamil, 1993; Kim *et al.,* 2000). Hence, the presence of secondary metabolites in the extracts of *Ximenia americana* may be assumed to exhibit similar physiological activities, especially the presence of flavonoids and phenols which are known to exhibit analgesic and anti-inflammatory activities. Based on this information the present research work studied the antioxidant, antibacterial and anti-inflammatory activities of all the extracts of *Ximenia americana,* an indigenous plant of Yobe State, Nigeria. The findings are reported in subheadings below.

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 (Cai *et al*., 2004). 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 (vitamins C and E), Baiano *et al.,* (2015). 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 as reported by Fang, *et al.,* (2014).

The antioxidant studies in the present work were carried out on the n-hexane, ethyl acetate, methanol and water extracts of *Ximenia americana* stem bark using the free radical scavenging activities of the samples on 2,2-diphenyl-1-picryldihydrazyl (DPPH) radical and ascorbic acid as standard. The activities of different extracts and ascorbic acid standard were measured at 517nm and presented in Table 3. A graph of absorbance against concentration was drawn (Fig. 1) in order to have a glance of the performance of the extracts as compared to standard ascorbic acid. The results indicated that the methanol and the ethyl acetate extracts appeared to show higher free radical scavenging activities than the other extracts and the standard ascorbic acid. To obtain the actual free radical scavenging activities of the extracts and ascorbic acid the data in Table 3 was further converted to percentage (%) inhibition with the aid of Microsoft Excel software and presented in Table 4, from which graph of % inhibition against concentration was drawn for each extract and ascorbic acid (Figs 2-6) from which straight-line equation for the calculation of the IC_{50} values for each sample. The 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 Gupta *et al*., (2003) with slight modification.

Table 3 The UV Absorbances measured at 517nm of Standard Ascorbic Acid, *n*-hexane, ethyl acetate, methanol and water extracts of *Ximenia americana* stem bark.

Extract absorbance					
S/No.	Concentration (µg/ml)	Ascorbic Acid	n-Hexane	Ethyl Acetate	Methanol	Water
1	0	0.675	0.675	0.675	0.675	0.675
2	10	0.433	0.512	0.495	0.489	0.529

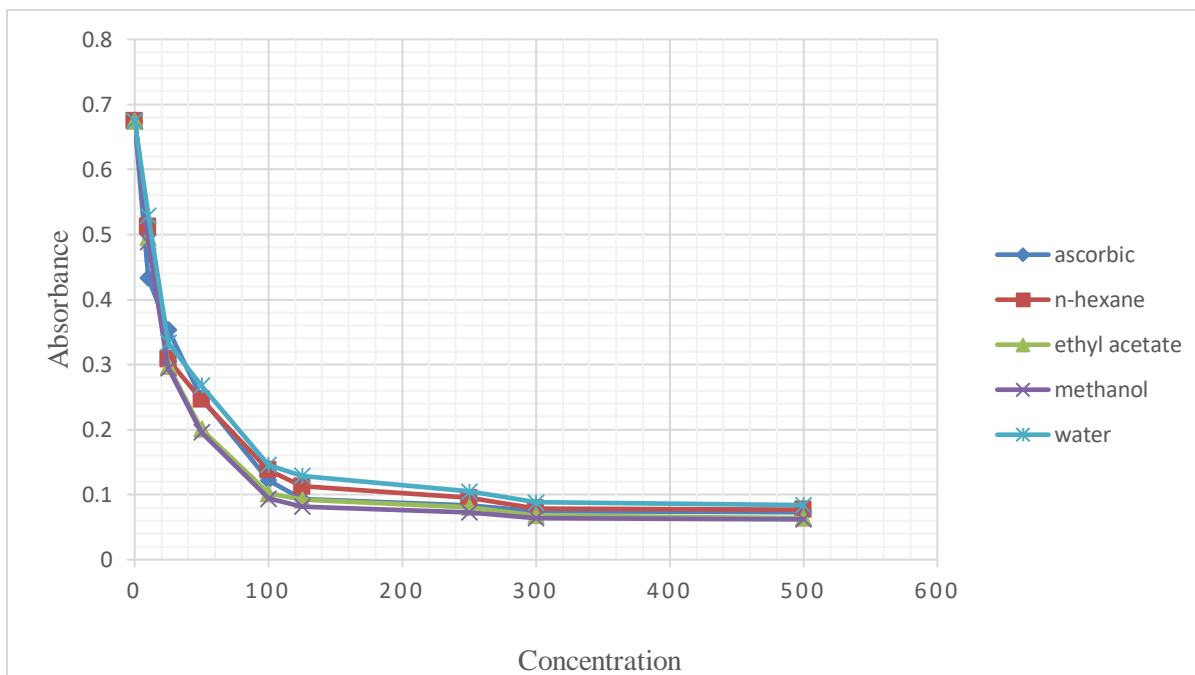| 3 | 25 | 0.354 | 0.308 | 0.298 | 0.295 | 0.334 |
4	50	0.249	0.246	0.201	0.196	0.268
5	100	0.122	0.138	0.102	0.094	0.146
6	125	0.093	0.113	0.092	0.082	0.129
7	250	0.084	0.095	0.081	0.073	0.105
8	300	0.075	0.079	0.068	0.064	0.088
9	500	0.073	0.077	0.064	0.062	0.084

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 *X. americana.*

Table 4: Percentage Inhibitions of Ascorbic acid, *n*-Hexane, Ethyl Acetate, Methanol and Water extracts of *X. americana.*

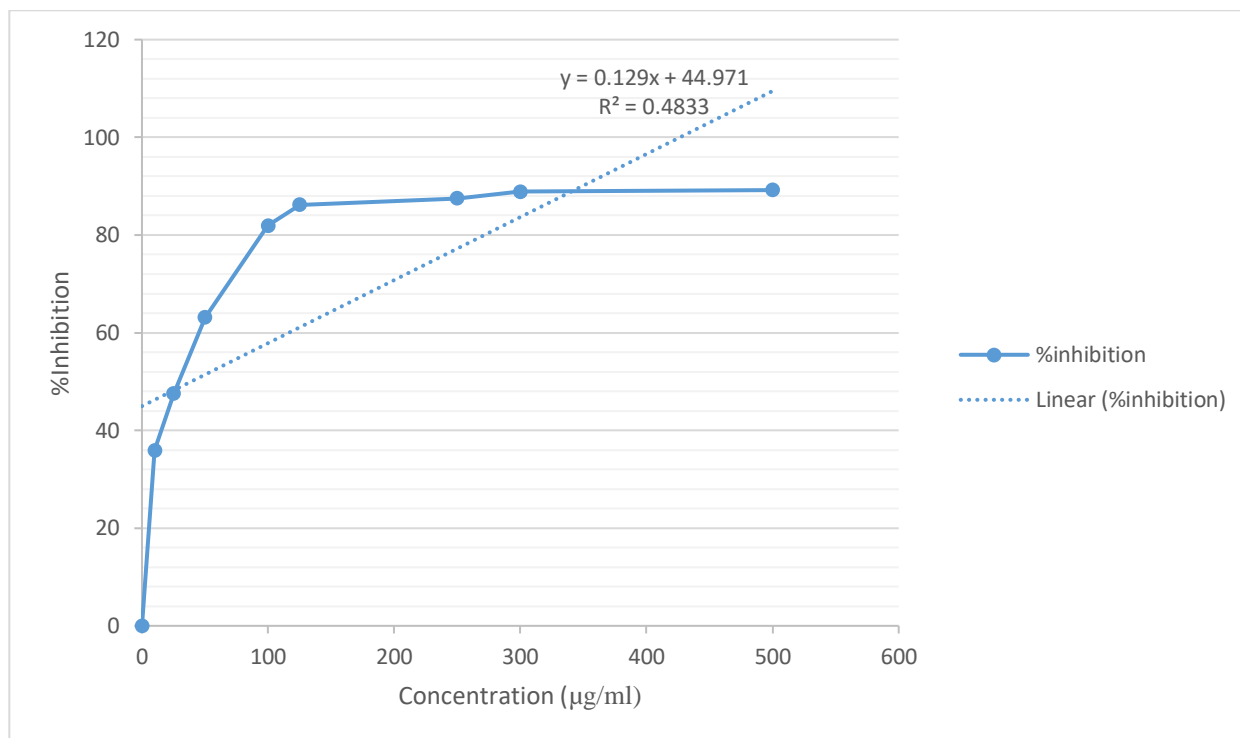| S/No.	Concentration (μg/ml)	Ascorbic acid	*n*-Hexane	Ethyl Acetate	Methanol	Water
1	0	0	0	0	0	0
2	10	35.9	11.6	26.7	27.6	21.6
3	25	47.5	41.3	55.9	56.3	50.5
4	50	63.1	56.9	70.2	70.9	60.3
5	100	81.9	71.3	84.9	86.1	78.4
6	125	86.2	80.4	86.4	87.9	80.9
7	250	87.5	83.3	88.0	89.2	84.4
8	300	88.9	85.8	89.9	90.5	86.9
9	500	89.2	85.8	90.5	90.8	87.6

Figure 2: The % Inhibition of Standard Ascorbic Acid for the Calculation IC_{50} Value

The IC_{50} value of the standard ascorbic acid was calculated from the above graph using the equation y = 0.129x + 44.971, where y is 50 and x represents the IC_{50} value. From this, the IC_{50} value of the 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 from their respective linear equations and summarized the IC_{50} values in Table 5.
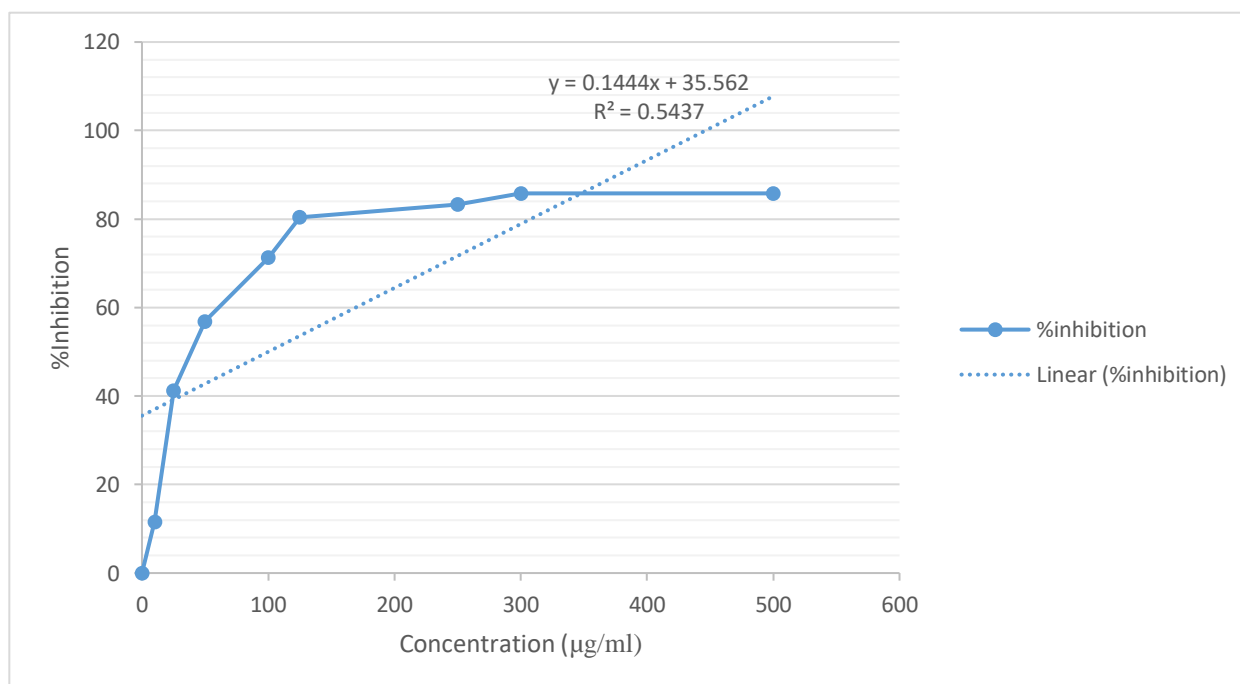

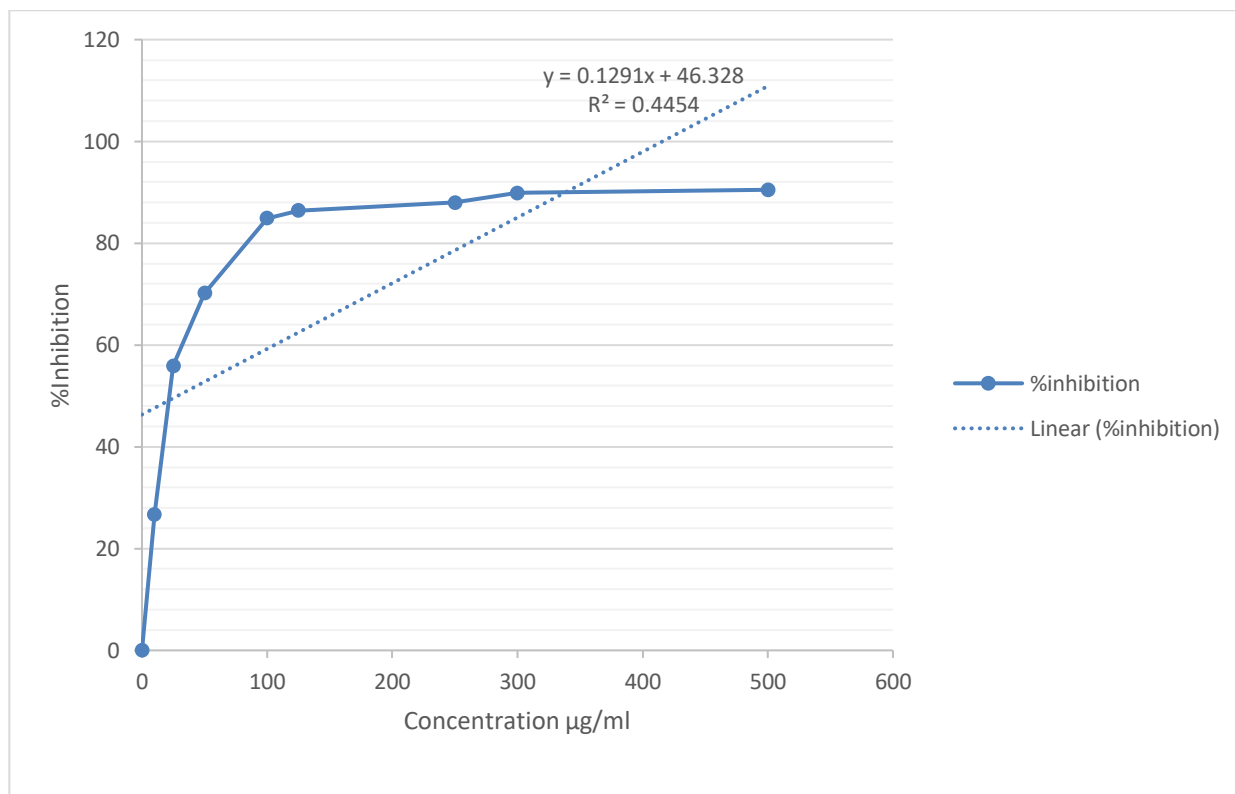Figure 3: The % Inhibition of n-Hexane Extract of *X. americana* Stem Bark

Figure 4: The % Inhibition of Ethyl Acetate extract of *X. americana* Stem Bark for the Calculation of IC_{50} value.
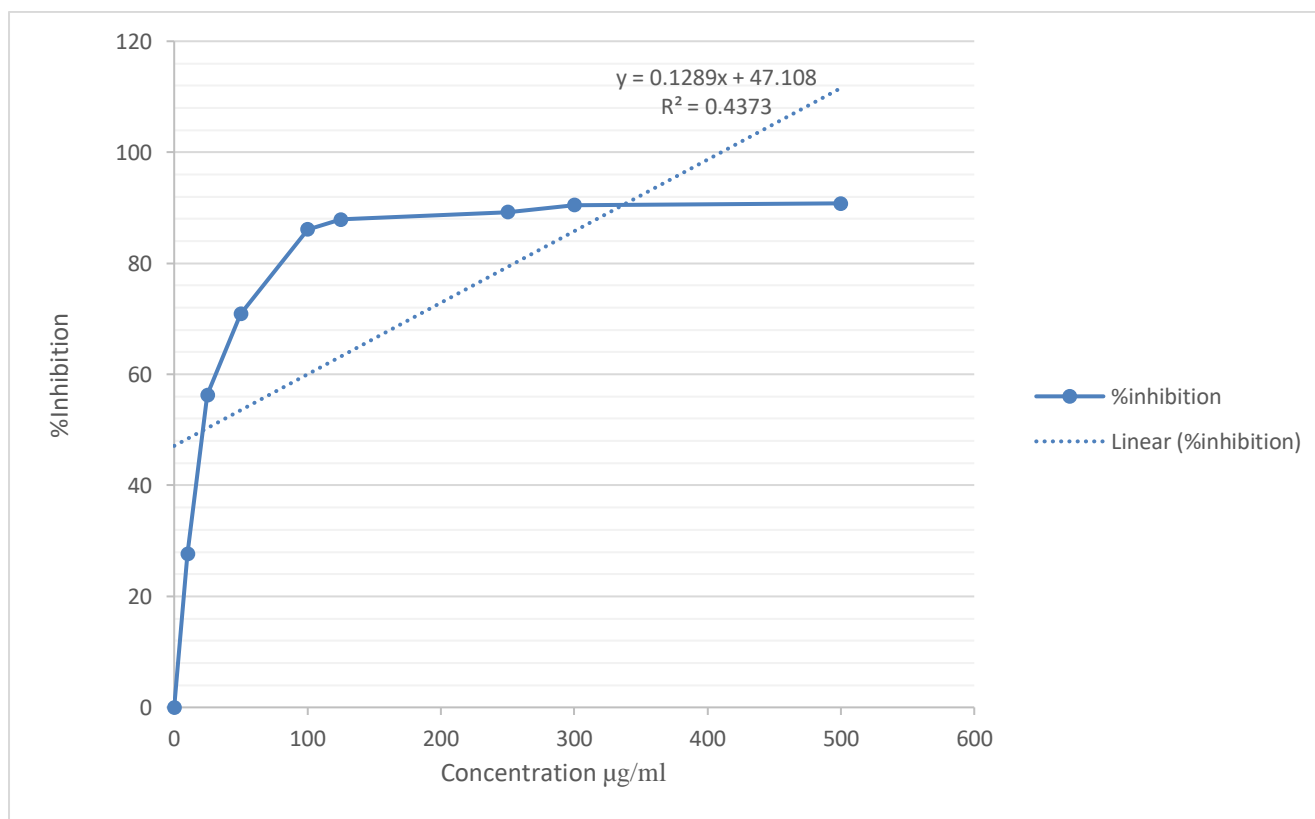

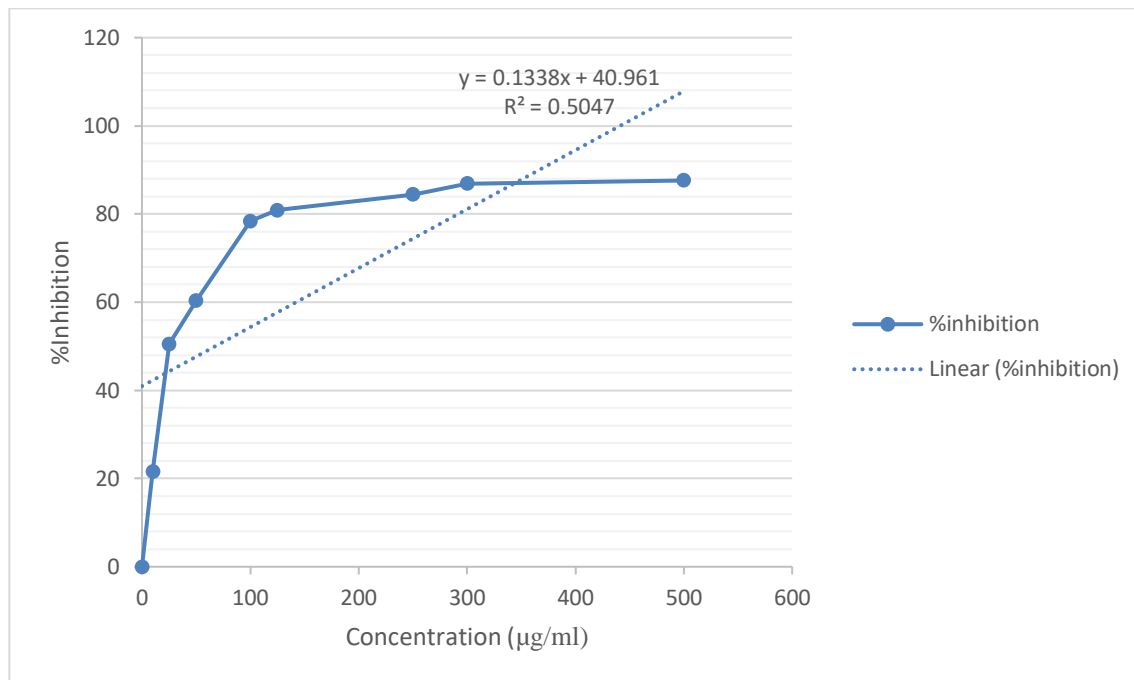Fig. 5: The % Inhibition of Methanol Extract of *X. americana* Stem Bark

Fig. 6: The % Inhibition of Water Extract of *X. americana* Stem Bark

Table 5. The summary of the IC_{50} values of *X. americana* Stem Bark extracts as compared to standard ascorbic acid.

		IC_{50} (μg/ml)	
S/No.	Extract/ Standard		
1	Ascorbic acid		38.9
2	n-hexane		51.3
3	Ethyl acetate		28.4
4	Methanol		22.4
5	Water		67.6

The results of the IC_{50} for antioxidant studies indicated that the methanol and ethyl acetate extracts of *X. americana* exhibited higher antioxidant activities (22.4 and 28.4μg/ml respectively) than the standard ascorbic acid (38.9 μg/ml). The methanol extract was almost twice (or 1.7 times) more potent free radical scavenger than the standard ascorbic acid. The n-hexane and water extracts exhibited lower activities (51.3 and 67.6μg/ml respectively). These results were taken to indicate that *X. americana* stem bark can be used as reliable source of antioxidants which are very important components of wound healing. The extent of antioxidant activities recorded for the *X. americana* stem bark is adequate to protect cells and tissues from destruction by free radicals. Furthermore, Lodhi et al (2016) reported that phytochemicals can partake in wound healing by exerting anti-inflammatory and/or analgesic and antioxidant activities.

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 by Sadique *et al.*, (1989). 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 (Rajendran *et al*., 2008). In this study the effects of the various extracts of *X. americana* 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), employing 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)

Heat induced haemolysis.

The results of the effect of *X. americana* extracts on heat induced haemolysis of RBC are presented 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, indicating a significant (p<0.05) protection against damaging effect of heat.

Table 6. The Effect of *n*-Hexane, Ethyl Acetate, Methanol and Water Extracts of *X. americana* on Heat Induced Haemolysis.

Extract	Treatment	Absorbance at 560nm	% Inhibition
	Negative Control (Normal Saline)	1.264±0.0012[a]	0
n-hexane	Positive Control (Diclofenac Sodium)	0.1180±0.0000[b]	90.66
	100	0.1970±0.0006[b]	84.41
	300	0.1833±0.0009[b]	85.49
	500	0.1523±0.0003[b]	87.95
	Negative Control (Normal Saline)	1.264±0.0012[a]	0
Ethyl acetate	Positive Control (Diclofenac Sodium)	0.1180±0.0000[b]	90.66
	100	0.3627±0.0009[c]	71.30
	300	0.3227±0.0003[c]	74.46
	500	0.2857±0.0003[c]	77.39
	Negative Control (Normal Saline)	1.264±0.0012[a]	0
Methanol	Positive Control (Diclofenac Sodium)	0.1180±0.0000[b]	90.66
	100	0.7100±0.0021[c]	43.82
	300	0.6840±0.0026[c]	45.88
	100	0.4737±0.0003[d]	62.52
	Negative Control (Normal Saline)	1.264±0.0012[a]	0
Water	Positive Control (Diclofenac Sodium)	0.1180±0.0000[b]	90.66
	100	0.6733±0.0012[c]	46.73
	300	0.5673±0.0012[c]	55.11
	500	0.2227±0.0041[b]	82.38

Values are expressed in mean ± SD (n = 3). Data analyzed using one way ANOVA;

Values along same column differently superscripted differ significantly (P<0.05)

The results showed that all extracts of *X. americana* exhibited membrane stabilizing effects, but to varying degrees. The n-hexane extract showed the highest values (84.41-87.95%, $p<0.05$). This was followed by the ethyl acetate extract (71.3-77.4%, $p<0.05$). The lowest level of protection was shown by the methanol extract (43.82-62.52%, $p<0.05$). All the extracts exhibited concentration dependent increases in RBC membrane stabilization against heat induced haemolysis. It could be concluded that the n-hexane, ethyl acetate and water (at high concentrations) extracts of *X. americana* may serve as reliable sources of anti-inflammatory drugs/agents. However, further research work is needed to confirm this speculation.

Hypotonicity induced haemolysis of RBC.

The results generally showed that *X. americana* extracts at the concentration range of 100 – 500μg/ml showed significant protection ($p<0.05$) of RBC membrane. These results are presented in (Table 7). The membrane stabilizing effect was higher especially in the n-hexane extract where the % inhibition of RBC lysis ranged from 75.21 to 85.23% as concentration increases. This was followed by the ethyl acetate extracts at concentrations greater than 100 µg/ml (71.3 to 71,40%), Water extract provided more protection (59.98 to 67.08%) at 300 and 500 µg/ml concentrations which were higher than those exhibited by the methanol extract (48.15 to 58.77%). Diclofenac sodium (100 µg/ml), the standard drug used, offered a significant ($p<0.05$) protection (57.52%) against the damaging effect of hypotonic solution. The results showed that all the extracts exhibited higher levels of protection than the standard drug, Diclofenac sodium salt. These findings also suggest that the n-hexane and ethyl acetate extracts contain more potent agents that can protect the RBC from hypotonicity-induced haemolysis. This finding is similar to the conclusion drawn on the results of the heat induced haemolysis above, in both cases these two extracts showed higher RBC haemolysis and hence they could be used as sources of anti-inflammatory agents/drugs.

Table 7. Effect of *n*-Hexane, Ethyl Acetate, Methanol and Water Extracts of *X. americana* on Hypotonicity Induced Haemolysis

Extract	Treatment(S)	Absorbance at 560nm	% Inhibition
	Negative Control (Hyposaline)	0.5690 ± 0.0000^a	0
n-hexane	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^b	57.52
	100	0.1410 ± 0.0130^c	75.21
	300	0.1390 ± 0.0000^c	75.57
	500	0.0840 ± 0.0000^d	85.23
	Negative Control (Hyposaline)	0.5690 ± 0.0000^a	0
Ethyl acetate	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^b	57.52
	100	0.5457 ± 0.0093^a	4.09
	300	0.1633 ± 0.0063^c	71.30
	500	0.1627 ± 0.0044^c	71.40
	Negative Control (Hyposaline)	0.5690 ± 0.0000^a	0
Methanol	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^b	57.52
	100	0.2950 ± 0.0000^c	48.15
	300	0.2593 ± 0.0003^b	54.42
	500	0.2347 ± 0.0003^b	58.75
	Negative Control (Hyposaline)	0.5690 ± 0.0000^a	0
Water	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^b	57.52
	100	0.2277 ± 0.0018^b	59.98

300		0.2100 ± 0.0000^c	63.09
500		0.1873 ± 0.0024^c	67.08

Values are expressed in mean ± SD (n = 3). Data analyzed using one way ANOVA;

Values along same column differently superscripted differ significantly ($P<0.05$)

Antibacterial activity Assays

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

Table 8. Antibacterial activity of Standard drugs

Standard Conc.100µg/disc	(Zone of inhibition mm/dm)			
	Gram Positive		Gram Negative	
	S. aureus	*S. pneumoniae*	*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 *Ximenia americana*

Extract	Concentration (µg/ml)	Zones of Inhibition (mm/dm)			
		S. Typhi	*S. aureus*	*E. coli*	*S. pneumoniae*
n-Hexane	100	0	7	8	9
	200	0	1	10	12
	300	0	16	13	15
	400	0	22	16	17
	500	0	25	18	18
Ethyl acetate	100	0	9	12	8
	200	0	13	13	11
	300	0	15	15	14

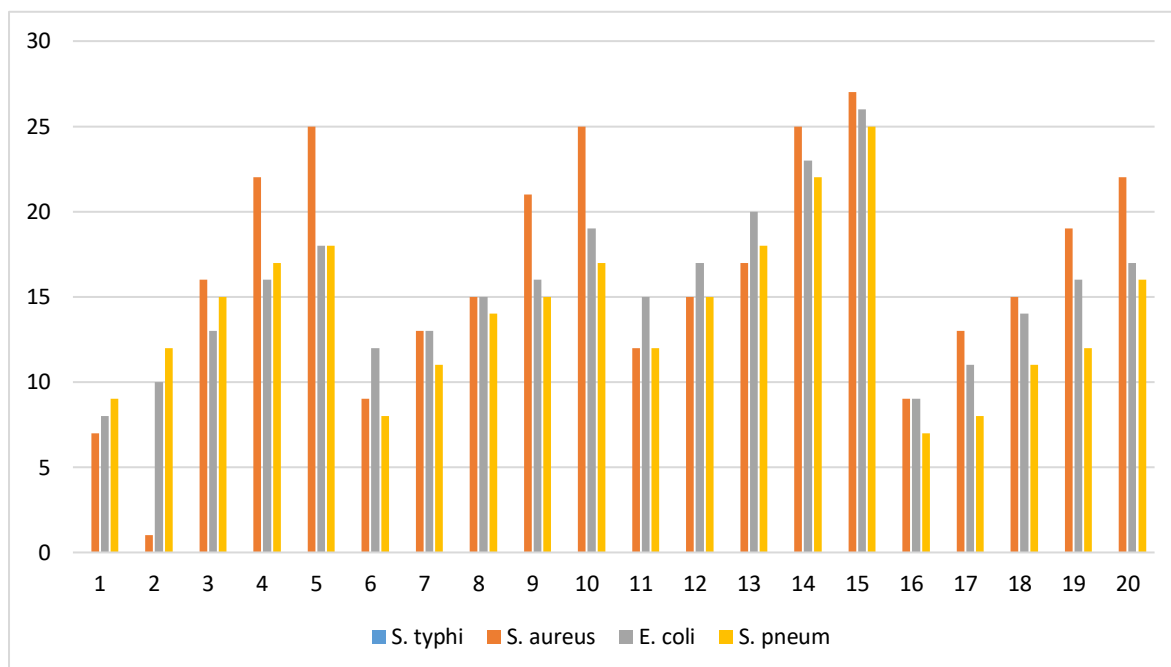| | 400 | 0 | 21 | 16 | 15 |
	500	0	25	19	17
Methanol	100	0	12	15	12
	200	0	15	17	15
	300	0	17	20	18
	400	0	25	23	22
	500	0	27	26	25
Water	100	0	9	9	7
	200	0	13	11	8
	300	0	15	14	11
	400	0	19	16	12
	500	0	22	17	16
Key: 0 = No activity					

Figure 7. Antibacterial activity from *X. americana* extracts against *S. Typhi, S. aureus, E. coli* and *S. pneumoniae.*

The four extracts of *X. americana* were studied for their possible antibacterial activities. Results obtained (Table: 9 and Fig. 7) showed that all extracts inhibited the growth of *S. aureus, S. pneumoniae* and *E. coli* while *S. Typhi* was not inhibited by any of the extracts. The methanol extract showed the highest zones of inhibition against *S. aureus* (12-27 mm)*, S. pneumoniae* (12-25 mm) and *E. coli* (12-26 mm), these results indicate that the extracts of *X. americana,* particularly the methanol extract, can be used to treat infections or ill-health conditions caused by *S. aureus, E. coli* and *S. pneumoniae.*

CONCLUSION

The present research work was carried out on phytochemical screening, antioxidant, antibacterial and anti-inflammatory activities of four extracts of *X. americana* stem bark, an indigenous plant of Yobe State, Nigeria. These extracts showed good antioxidant anti-inflammatory and antibacterial activities. The

therapeutic potential of the stem barks of *X. americana* could be attributed to classes of active components present in the stem barks such as alkaloids, flavonoids, tannins and phenolic compounds 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 bark of *X. americana.*

Secondary metabolites such as alkaloids, flavonoids, phenols, tannins, terpenoids, etc. may be responsible for the positive antioxidant, anti-inflammatory and antibacterial activities reported in this study thus comparing with report of Nandagoapalan *et al*., (2016). Such activities as antimicrobial, anti-inflammatory, antioxidant, anticancer, among others, may be principal indicators of a plant's value in medicine.

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