In-Vitro Antioxidant and Anti-Inflammatory Profile of the Stem Bark Extracts of *Guiera Senegalensis* J. F. Gmel

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ABSTRACT

Medicinal Plants are Plants used for traditional medicines to treat ailments. 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 *Guiera senegalensis* 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 *Guiera senegalensis* 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 IC50 values obtained for the *Guiera senegalensis* 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%).

Keywords: Phytochemical, Antioxidant, Anti-inflammatory

INTRODUCTION

Medicinal plants are plants used for 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 (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

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metabolites such as terpenoids, steroids, saponins, tannins, flavonoids, alkaloids and phenolic compounds (Mdlolo; 2009, Fawole 2009).

Guiera senegalensis JF Gmel, of the family Combretaceae, commonly known as Moshi medicine (English) or Saabara in Hausa. *Guiera senegalensis* is a shrubby plant and can grow to a height of 3 to 5 m depending on the habitat (Silva *et al*., 2008). The leaves which are 3 to 5 cm long and 1.5 to 3.0 cm broad are arranged opposite or sub opposite on the stem (Hutchinson and Dalziel, 1972).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. (Okwu, Ekeke 2003 and Okwu; 2004). For this reason, medicinal plants are considered be 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, 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.

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.

Collection and Preparation of Plant Sample

The fresh sample of *Gueira senegalensis* (Voucher number 1973) (stem bark) was collected at Damaturu 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.

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Extraction of Phytochemicals

About 550g of the powdered plant material of *Guiera senegalensis* 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.

Phytochemical Screening

Phytochemical screening to detect the presence of phytochemicals from the samples was carried out 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 Dragendroff's reagent (Solution of potassium Iodide and bismuth Iodide). Formation of red precipitate indicates 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, indicates the presence of flavonoids.

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.

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.

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.

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.

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.

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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.

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.

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.

Measurement of Antioxidant Activities

The antioxidant activities of *Guiera senegalensis* 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 Hatano et al. (1988), with some modifications.

Then the % inhibition was calculated by the following equation;

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

Activity (absorbance of blank)

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

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Anti-Inflammatory Activity

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 (Gandasan et al., 1991, Shenoy et al., 2010) 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 (Rajendran et al., 2008).

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.

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 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:

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

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 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:

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

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RESULTS AND DISCUSSION

Extraction Yield

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

Table 1. Percentage yield of crude stem bark extracts of *Guiera senegalensis*

S/No.	Solvents used	Weight of plant part used (g)	Weight of extract(g)	Percentage yield (%)
1	*n*-Hexane	550 *(Guiera senegalensis)*	8.65	1.57
2	Ethyl Acetate	550 (*Guiera senegalensis*)	10.73	1.95
3	Methanol	550 (*Guiera senegalensis*)	8.19	1.49
4	Water	550 (*Guiera senegalensis*)	8.30	1.51

The results above revealed that in the 550g of *Guiera senegalesis* extracted, all the four extracts of *Guiera senegalesis* exhibited similar pattern of phytochemical yield with ethyl acetate found to be the highest 10.73g (1.95%), followed by n-hexane 8.65g (1.57%), water 8.30g (1.51%) and methanol 8.19g (1.49%). 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.

Phytochemical Screening

Guiera senegalensis

Phytochemical constituents of the *Gueira senegalensis n*-Hexane, Ethyl acetate, methanol and Water extracts were drtermined. The results (Table 2) revealed the presence of alkaloids, flavonoids, saponins, tannins and cardiac glycosides in all the extracts. However, terpenoids were detected only in the *n*-Hexane and water extracts while phenols were present in n-hexane, ethyl acetate and methanol extracts. Quinones, Sterols and Oxalate were not detected in all the extracts.

Table 2. The Results of the Phytochemical Screening of *Gueira senegalensis 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	+	+	+	+

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Sterols	-	-	-	-
Phenols	+	+	+	-

Key: ++ = Highly present, + = Present, - = Absent

The results also suggested the presence of more flavonoids in ethyl acetate and methanol extracts and saponins in the n-hexane and ethyl acetate extracts. It also showed that only non-polar and extremely polar terpenoids were present in *Gueira senegalensis.* The presence of these secondary metabolites may be very essential for the biological and pharmacological activities of this plant.

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 (Vitamin 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 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 *Guiera senegalensis* 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 517nm as reported by Gupta *et al*., (2003). The absorbance and percentage inhibition of the standard and the extracts were shown in Tables 3, respectively.

Table 3. UV Absorbance measured at 517nm of antioxidant activity of Standard Ascorbic Acid, *n*-hexane extract, ethyl acetate extract, methanol extract and water extract of *Guiera senegalensis*.

			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	0.526	0.513	0.494	0.542
3	25	0.354	0.365	0.322	0.296	0.388
4	50	0.249	0.253	0.206	0.189	0.281
5	100	0.122	0.174	0.128	0.095	0.193
6	125	0.093	0.124	0.097	0.086	0.138
7	250	0.084	0.096	0.088	0.073	0.097
8	300	0.075	0.088	0.073	0.062	0.094
9	500	0.073	0.086	0.073	0.059	0.091

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The Table 3 above was used to plot a graph of Absorbance of standard ascorbic acid and n-Hexane, Ethyl Acetate, Methanol and Water extracts of *Guiera senegalensis* against concentration using MS. Excel.

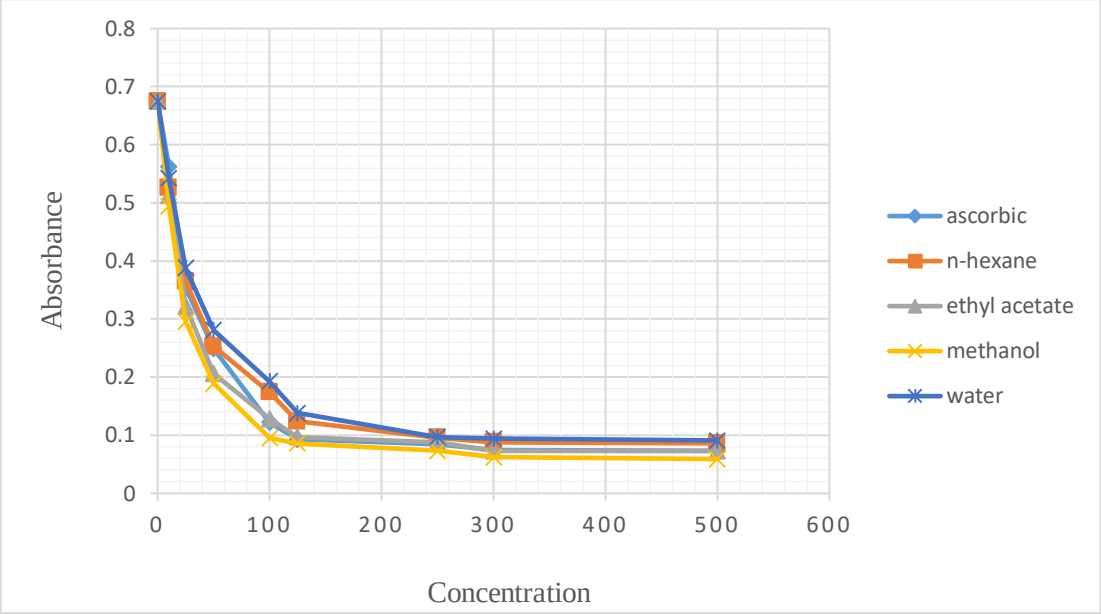

Figure 1. Graph of Absorbance at 517nm Against Concentration of Standard Ascorbic Acid, n-Hexane extract, Ethyl Acetate extract, Methanol extract and Water extract of *Guiera senegalensis.*

The above graph in Fig. 1 is showing the DPPH scavenging activity of n-Hexane, Ethyl Acetate, Methanol and Water extracts of *Guiera senegalensis* compared to that of the standard ascorbic acid. Because of difficulties of interpretation Table 3 was converted to % inhibition as shown in Table 4.

Table 4. Percentage Inhibitions of Ascorbic acid, *n*-Hexane, Ethyl Acetate, Methanol and Water extracts of *Guiera senegalensis.*

S/No.	Concentration (µg/ml)	Ascorbic acid	*n*-Hexane	Ethyl Acetate	Methanol	Water
1	0	0	0	0	0	0
2	10	35.9	22.1	24.0	26.8	19.7
3	25	47.5	45.9	52.3	56.1	42.5
4	50	63.1	62.5	69.5	72.0	58.3
5	100	81.9	74.2	81.0	85.9	71.4
6	125	86.2	81.6	85.6	87.3	79.6
7	250	87.5	85.8	86.9	89.2	85.6
8	300	88.9	86.9	89.2	90.8	86.1
9	500	89.2	87.3	89.2	91.3	86.5

From this table, a graph of % inhibition of n-hexane, ethyl acetate, methanol and water extracts of *Guiera senegalensis* against concentration were plotted separately.

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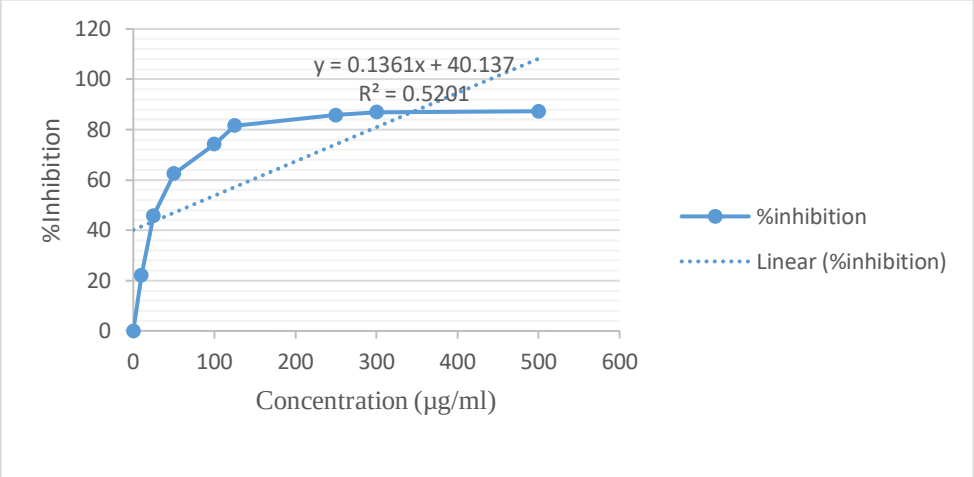

Figure 2. %inhibition of n-hexane extract of *Guiera senegalensis*

The IC_{50} value of n-hexane extract of *Guiera senegalensis* was calculated from the Fig. 2. The Inhibitor Concentration against the percent activity is plotted using the linear equation provided in the graph (y = 0.1361x + 40.137), for y = 50 value becomes IC_{50} value of n-hexane extract of *Guiera senegalensis* which determined to be 72.5 µg/ml, a value much lower than that shown by ascorbic acid.

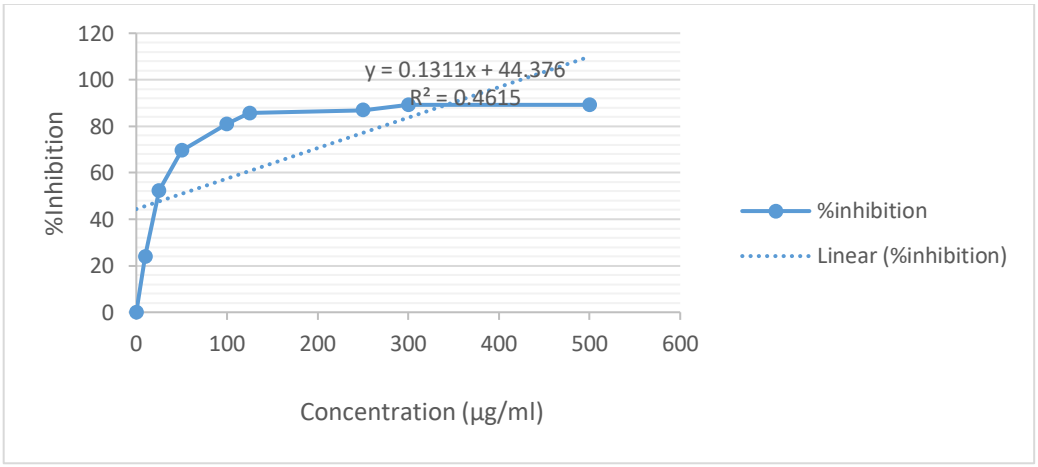

Figure 3. %inhibition of ethyl acetate extract of *Guiera senegalensis*

The IC_{50} value of ethyl acetate extract of *Guiera senegalensis* was calculated from Fig. 3. The IC_{50} value of ethyl acetate extract of *Guiera senegalensis* was calculated to be 42.9µg/ml using the straight line equation (y = 0.1311x + 44.376) obtained from the graph. The value was slightly in activity than ascorbic acid.

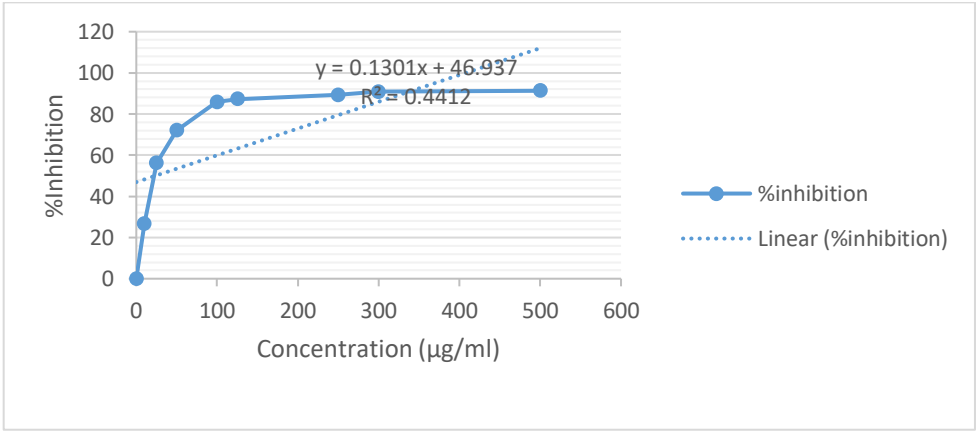

Figure 4. %inhibition of methanol extract of *Guiera senegalensis*

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The IC_{50} value of methanol extract of *Guiera senegalensis* was calculated from the Fig. 4. The IC_{50} value of methanol extract of *Guiera senegalensis* was calculated to be 23.5µg/ml using the straight line equation (y = 0.1301x + 46.937) obtained from the graph. This value was much higher in activity than ascorbic acid. The water extract of *Guiera senegalensis* was subjected to similar analysis as shown in Fig. 5.

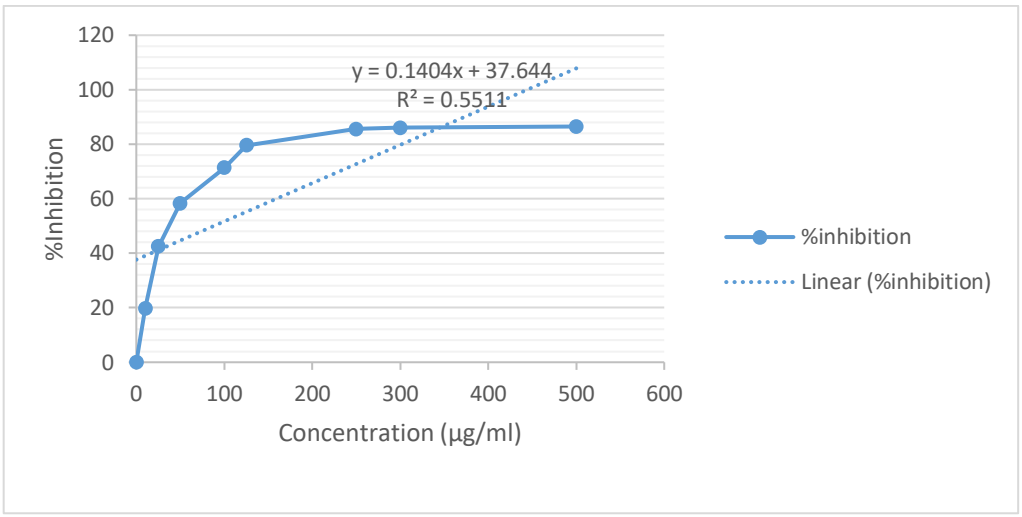

Figure 5. %inhibition of water extract of *Guiera senegalensis* for its IC_{50} Value.

The IC_{50} value of the aqueous extract of *Guiera senegalensis* was then calculated to be 88.0µg/ml using the straight line equation (y = 0.1404x + 37.644) obtained from the graph. This value was more than twice lower activity than ascorbic acid.

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

		IC_{50} (µg/ml)
S/No.	Extract/*Standard	G. S
1	*Ascorbic acid	38.9
2	n-hexane	87.3
3	Ethyl acetate	42.9
4	Methanol	23.5
5	Water	88.0

Key: G. S - *Guiera senegalensis*

The IC_{50} results of *Guiera senegalensis* samples showed that only methanol sample exhibited higher antioxidant activity (23.5 µg/ml) than the standard ascorbic acid. These results were taken to indicate that *Guiera senegalensis* stem bark can be used as the reliable antioxidant which is very important component of wound healing.

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). 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

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(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 ($p<0.05$)

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. The results showed that n-Hexane extract of *Guiera senegalensis* at concentration 500µg/ml protect significantly ($p<0.05$) the erythrocyte membrane against lysis induced by heat Table (4.31). 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 acetate, Methanol and water Extracts of *Gueira senegalensis* on Heat Induced Haemolysis

Extract	**Treatment(S)**	**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	0.2780 ± 0.0020^{c}	78.01
	300	0.2530 ± 0.0015^{c}	79.98
	500	0.2440 ± 0.0006^{c}	80.69
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
Ethyl acetate	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
	100	0.5397 ± 0.0097^{c}	57.30
	300	0.3500 ± 0.0023^{d}	72.31
	500	0.3407 ± 0.0012^{d}	73.04
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
Methanol	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
	100	0.7333 ± 0.0037^{c}	41.98
	300	0.4617 ± 0.0009^{d}	63.47
	500	0.3430 ± 0.0051^{e}	72.86
	Negative Control (Normal Saline)	1.264 ± 0.0012^{a}	0
Water	Positive Control (Diclofenac Sodium)	0.1180 ± 0.0000^{b}	90.66
	100	0.4017 ± 0.0487^{c}	68.21
	300	0.3990 ± 0.0020^{c}	68.43
	500	0.3923 ± 0.0003^{c}	68.96

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

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

The results showed that all extracts of *Guiera senegalensis* have membrane stabilizing effects. The n-hexane extract showed the highest values of % inhibition (78.01 – 80.69%, (P<0.05) and no much differences were observed with increase in concentration. This followed by ethyl acetate extract with the values of (57.30 – 73.04%, (P<0.05). The water extract which also showed very little difference as concentration increases (68.21 – 68.96%, (P<0.05). Methanol extract showed the lowest stabilizing effect (41.98 – 72.86%, (P<0.05).

Hypotonicity induced haemolysis of RBC.

Table 6. Effect of *n*-Hexane Extract of *Gueira senegalensis* 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	0.3843 ± 0.0007^{c}	32.46
	300	0.2238 ± 0.0007^{b}	60.66
	500	0.1897 ± 0.0003^{d}	66.66
	Negative Control (Hyposaline)	0.5690 ± 0.0000^{a}	0
Ethyl acetate	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^{b}	57.52
	100	0.5583 ± 0.0007^{a}	1.88
	300	0.4973 ± 0.0009^{c}	12.60
	500	0.2487 ± 0.0007^{b}	56.29
	Negative Control (Hyposaline)	0.5690 ± 0.0000^{a}	0
Methanol	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^{b}	57.52
	100	0.3653 ± 0.0322^{c}	35.79
	300	0.3350 ± 0.0150^{c}	41.12
	500	0.2877 ± 0.0027^{d}	49.43
	Negative Control (Hyposaline)	0.5690 ± 0.0000^{a}	0
Water	Positive Control (Diclofenac Sodium)	0.2417 ± 0.0003^{b}	57.52
	100	0.2053 ± 0.0003^{c}	63.91
	300	0.2047 ± 0.0003^{c}	64.02
	500	0.1453 ± 0.0003^{d}	74.46

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

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

The results of the study on the extracts of *Guiera senegalensis* (Table 6) have revealed variable levels of membrane stabilizing effects against haemolysis induced by hypotonic solution. The water extract showed the highest significant values of 63.91 – 74.46%, (P<0.05), even at lower concentration it showed a significant value higher than the standard drug. The next extract with the high protective effect was n-hexane which showed a significant values of 32.46 – 66.66%, (P<0.05). The methanol extract follows the n-hexane extract but it showed lower inhibitions (35.79 – 49.43%, (P<0.05)) than the standard drug it was also observed that as the concentration increases the inhition of haemolysis also increases. The lowest anti haemolytic effect of *Guiera senegalensis* came from the ethyl acetate extract with a significant values of 1.88 – 56.29%, (P<0.05), but showed a very little effects at lower concentrations. These results suggest that *Guiera senegalensis* extract, especially the aqueous and hydrophobic, can protect RBC from hypotonic induced haemolysis.

CONCLUSION

These plants extracts showed good antioxidant activity and anti-inflammatory activity. The therapeutic potential of the stem barks of *Guiera senegalensis* 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 and anti-inflammatory activities carried out in this study lend credence to the traditional claim about the wound healing property of the stem barks of *G. senegalensis*.

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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