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Original Research Article

# *In vitro* and *in vivo* antioxidant evaluation of *Guiera senegalensis* methanol leaves extract

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**Abstract**

This study evaluated the *in vitro* and *in vivo* antioxidant effect of methanol extract of leaves of *Guiera senegalensis*. *In vitro* antioxidant assay of *Guiera senegalensis* was carried out using standard protocol. Acute toxicity of *Guiera senegalensis* using Wistar rats were investigated for adverse effect and mortality rate using Lorke method. Twenty five albino rats were apportioned into groups of five (n=5). Untreated control received 40% ethanol to induced-oxidative stress for 14 days. Normal control and graded doses at 50, 100 and 200 mg/kg/day of *Guiera senegalensis* extract were pretreated for 14 days. After being pre-administered with 40% ethanol given at 1 ml/kg/day to induced-oxidative stress for 14 days. Animals were sacrificed in a mild chloroform anaesthetic, and blood samples and organs were isolated for analysis. The *in vitro* antioxidant study showed a significant increase in the level of scavenging free radicals effect of methanol extracts on DPPH and hydroxyl free radical elicited scavenging property of the extract when compared with ascorbic acid, showed a better scavenging effect at 86.77% and 88.93%. The radical scavenging effect of ferric reducing antioxidant power, exhibited scavenging property of the extract via decreasing antioxidant power at 41%. No observable behavioral change with absent adverse effect and lethality at 10 to 5000 mg/kg of *Guiera senegalensis*. *In vivo* antioxidant study of extract indicated significant increase in SOD, catalase, reduced glutathione and glutathione peroxidase ($p < 0.05$) with significant decreased in MDA of antioxidant indexes in plasma, liver and kidney samples showed scavenging effect when compared with untreated control. In conclusion, *Guiera senegalensis* exhibited bio-protective and therapeutic benefits.

**Keywords**: *in vitro* antioxidant, *in vivo* antioxidant, *Guiera senegalensis*, methanol

## 1 Introduction

Traditional medicine serves as the background to knowledge, skills and practices established on several theories, principles and indigenous understandings associated with diverse cultures, whether justifiable or not, involved in health care service also in preventing, diagnosing and treating of mental and physical disorder [1]. Natural products with its derivative compounds obtained from plant materials possess ethnomedicinal uses in the treatment of numerous diseases worldwide. From ancient times, humans depend solely on plants-based medicine as therapeutic mediators [2]. Currently, orthodox medicine play a wide role in modern medicine with supporting evidences obtained from phytochemical reports [3, 4]. Drug discovery includes; yohimbine, artemisinin, quinine, vincristine, ergotamine, emetine etc. are obtained from medicinal plants and aid in the discovery and synthesis of novel and synthetic pharmaceuticals from natural materials [5]. WHO improved herbal mixture in the treatment of limited health associated problems since there are readily cheap and previously integrated into the people's way of life [6, 7].

*Guiera senegalensis* is a known shrub commonly originated from the tropical region in Nigeria, and it belongs to the family Combratacea. It is locally called Sabara by the Hausas [8]. Its stem possesses some loops that impel branches. It is widely distributed in savannah region of West and central Africa including Nigeria, Senegal, Gambia, Mali, Niger, Ghana and Burkina Faso [9, 10, 11]. It is active against respirational congestion, pyresis and tussives [12, 13] and given as anti-tussive, anti-hypotension, anti-hypertension and venereal diseases [12], to discharge and manage bronchial, lung and breathing diseases. Moreover, it is used for treating malaria fever [14].

Free radical are chemical substances proficient to free existence with one or several unpaired electrons found in an atomic orbital [15]. Free radicals are frequently synthesised during metabolism [16]. Cells utilizes oxygen to produce energy in the mitochondria as a byproducts produced during the process. These byproducts are typically reactive oxygen species (ROS) also with reactive nitrogen species (RNS) resulting to cellular redox progression. Free radicals possess

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distinct affinity for carbohydrates, nucleic acids, lipids and proteins [17]. The term antioxidant is explained as any constituent responsible for deferment, prevention or elimination of oxidative impairment on target molecule [18]. Humans have developed extremely complex antioxidant structures (enzymatic and non-enzymatic) working synergistically with combination to one another, protecting cells, organ and systems of the body against free radical injury. Antioxidants are classified as endogenous and exogenous substances e.g., part of diet or dietary supplements. Selected dietary compounds cannot neutralize free radicals, rather improve endogenous action can be categorised as antioxidants [19]. Endogenous antioxidants display essential role in sustaining optimal cellular effect, thus systemic health and wellbeing. Nonetheless, under certain conditions, endogenous antioxidants stimulate oxidative stress, which may not be adequate to dietary antioxidants needed to sustain optimal cellular action. The maximum proficient enzymatic antioxidants comprise of catalase, superoxide dismutase and glutathione peroxidase [20]. Nonenzymatic antioxidants involve Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), natural flavonoids, melatonin, carotenoids and others [21].

## 2 Materials and methods

### *2.1 Plant collection*

Fresh leaves of *Guiera senegalensis* was purchased from the Northern part of Nigeria. The plant was identified and authenticated by Dr Akinibosun, in the herbarium unit of the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin with the voucher number UBK-D917. The leaves were rinsed with distilled water and shade dried. It was pulverized using a mechanical grinder and stored in an airtight container.

### *2.2 Preparation of extracts*

The powdered materials were subjected to successive extraction by cold maceration method using methanol solvent with ratio 1:1 for 72 hr. The various extracts were evaporated at 45 °C to get a dried extract. The percentage yield of the extract was 10.97 % w/w and stored for further studies [22].

### *2.3 DPPH free radical scavenging assay*

The antioxidant capacity of the extract was studied through the evaluation of the free radical scavenging effect on the 1, 1-diphenly-2-picrylhydrazyl (DPPH) radical [23]. An aliquot (10 µl) of extract was mixed with 90 µl of distilled water and 3.9 ml of 25 mM DPPH methanolic solution. The mixture was thoroughly mixed and kept in the dark for 30 min, the absorbance was measured at 515 nm, against a blank of methanol without DPPH. Results were expressed as percentage on inhibition of DPPH radical. Percentage of inhibition of DPPH radical was calculated according to the following equation;

$$\%\ Inhibition\ of\ DPPH' = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Absorbance of control is the absorbance of DPPH radical solution without extracts.

### *2.4 Determination of total antioxidant capacity*

Total antioxidant activity was estimated by phosphomolybdenum assay [24]. The method is based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acid pH. 1 ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were mixed together. That is 1:1:1. One milliliter of the extracts (1 mg/ml) were added to 3 ml of molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as the standard.

### *2.5 Ferric reducing antioxidant power (FRAP) assay*

The ferric reducing antioxidant power (FRAP) assay was carried out using a modified method of Ruch *et al.* [25]. The assay is based on the ability of antioxidant to reduce $Fe^{3+}$ to $Fe^{2+}$ in the presence of 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue $Fe^{2+}$- TPTZ complex with an absorption maximum at 593 nm. To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM 2,4,6-tripyridyls- triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3.6H_2O$) solution) was added to 1 mL of the extracts (1 mg/ml) and standard at concentrations of 100-600 µM. The reaction mixtures were incubated at 37 °C for 30 min and the increase in absorbance at 593 nm was measured. $FeSO_4$ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

### *2.6 Experimental Animals*

Male matured albino rats weighing 180-200 g was gotten from the Department of Animals and Environmental Biology animal house, Faculty of Life Sciences, University of Benin. They were housed in conducive cages with free access to pelleted grower marsh and water; the animals were acclimatized to laboratory conditions for 2 weeks. The animals were handled according to standard protocols for the use of laboratory animals. The protocol for the use of animals was reviewed by the ethical committee of Life Sciences, University of Benin, with the ethical number LS100320.

*2.7 Acute toxicity study*

Methanol root bark extract of *Guiera senegalensis* was used for pharmacological screening on male Wistar rats. Acute toxicity test was carried out according to Lorke's method [26]. This method has two phases, 1 and 2 respectively. Phase I: The rats' were allotted into three groups (three rats each). The rats were administered orally doses of 10, 100 and 1000 mg/kg of the extract. The rats were kept under close observation for 24 hr to monitor their behaviour as well as occurrence of mortality. Phase II: Three rats were allotted into three groups (one animal each). The rats were administered with higher doses (1600, 2900 and 5000 mg/kg) of the extract and were observed for 24 hr (special attention given to the first 4 hr) and once daily for a period of 14 days for signs of toxicity which include paw/licking, change in skin colour, changes in fur, eye lacrimation, nostril discharge, salivation, diarrhoea, tremor, convulsion and death.

The $LD_{50}$ was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

$D_0$ = Highest dose that gave no mortality,
$D_{100}$ = Lowest dose that produced mortality.

*2.8 In vivo antioxidant evaluation*

Twenty five male albino rats were apportioned into groups of five (n=5). Group A served as untreated control with 40 % ethanol given at (1 ml/kg/day p.o.) for 14 days. Group B served as normal control and the rats were administered with 1 ml/kg distilled water orally. Group C, D and E were administered with oral doses of 50, 100 and 200 mg/kg/day of the extract respectively for 14 days, after being pre-administered with 40% ethanol given at 1 ml/kg/day to induced-oxidative stress for 14 days. Animals were sacrificed in a mild chloroform anaesthesia, and blood samples and organs were isolated for analysis.

*2.9 Determination of antioxidant property of the extract*

Superoxide Dismutase (SOD) effect was read via methods described by Beckman and Koppenol, [27] principle; Autooxidation with hematoxylin (increases the absorbance at 560 nm wavelength) was inhibited by SOD effect assay at pH 7.8; percentage amount of SOD present within a specific range. SOD activity in the sample is determined by measuring the amount of heamatin present. The crucial principle to assay is revealed schematically via the following equation:

$O_2 + HTH_2$ SOD $H_2O_2 + HT$ (Abs560↑)

Procedure: Aliquant mixture of plasma 0.20 ml of the diluted microsome was enclosed using 2.5 ml solution of 0.05 M carbonate buffer. The reactions started by adding 0.3 ml solution of 0.3 mM adrenaline. The standard was combined with 2.5 ml solution of 0.05 M carbonate buffer, 0.3 ml solution of 0.3 mM adrenaline and 0.20 ml distilled water. Absorbance was measured after 30 sec. to 150 sec. using wavelength of 480 nm. Calculations augment absorbance/ minute = % inhibition = 100 - Where As is increase absorbance of substrate and Ab is increase absorbance of blank 1 unit of SOD property is the sum total of SOD required to elicit 50 % inhibition of oxidation via adrenaline to adenochrome per 1 minute. 5 .21 5 A A 100 x AAxs

The catalase activity was assayed using Beckman and Koppenol, [27] method. Test principle: Catalase scavenging hydrogen peroxide, converted into molecular oxygen and water. The action of catalase in this sample was resolute following decreased rate of absorbance using wavelength of 240nm and by monitoring the consumption of $H_2O_2$ substrate at 240 nm spectrophotometrically. 40 $H_2O_2$ Catalase $H_2O + {}_2O_2$ Procedure; Tissue homogenate (10 µL) (100-150 µg of protein) being added to 2.8 ml solution 50 mM potassium phosphate buffer (pH 7.0) in 3 ml cuvette. Reaction was instigated via addition of 0.1 ml solution of freshly prepared 30mM $H_2O_2$ with decomposed rate of $H_2O_2$ measured at 240 nm wavelength for 300 seconds in spectrophotometer. Molar loss coefficient of 0.041 mM-1cm-1 was utilized by calculating catalase effect in $H_2O_2$ mole reduced/min/mg/protein.

Malondialdehyde (MDA) activity was determined using the method Beckman and Koppenol, [27]. Test principle: The MDA assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.

Procedure: following 24 hours incubation, treated blood culture was centrifuged using speed 3000 speed for 20 min, and consequently isolation of the supernatants. 1300 µL of R1 was withdrawn from the microcentrifuge tube. 1 ml supernatant was diluted ten times in Tris HCl and 200 µL further dilute the supernatant in every culture added to 200 µL of distilled water and vortexes. 300 µL of R2 to all the test tube, then vortexes and place under incubation at 45°C for 40 min. following the incubation process, each tube were chilled in ice and centrifuged at 15000 g speed for 10 min at 4 °C. Every sample was measured in spectrophotometer at 586 nm.

Reduced Glutathione activity was measured in terms of the first order rate constant for the decomposition of tetra-butyl hydroperoxide according to Beckman and Koppenol [27].

Glutathione peroxidase activity was measured in terms of the first order rate constant for the decomposition of tetra-butyl hydroperoxide according to Beckman and Koppenol [27].

*2.10 Statistical analysis and data presentation*

Obtained results were expressed as Mean ± SEM. Data were compared using one-way analysis of variance and Dunnet multiple comparison test. Differences were considered to be significantly different at $p < 0.05$.

## 3 Results

The radical scavenging effect of methanol extracts on DPPH shown in Figure 1, elicited scavenging property of the extract via increasing antioxidant property when compared

with ascorbic acid. At 80 µg/ml, the methanol extract had a better scavenging effect at 86.77%.

The radical scavenging effect of methanol extracts on hydroxyl free radical shown in Figure 2, exhibited scavenging property of the extract via increasing antioxidant property when compared with ascorbic acid. At 80 µg/ml, the methanol extract had a better scavenging effect at 88.93%.

The radical scavenging effect of methanol extracts on ferric reducing antioxidant power shown in Figure 3, exhibited scavenging property of the extract via decreasing antioxidant power when compared with ascorbic acid. At 80 µg/ml, the methanol extract had a better decreasing antioxidant power at 41%.

No mortality was recorded at the maximum dose of 5000 mg/kg after fourteen days of observation. No sign of toxicity was observed during the fourteen days observation period of the methanol extract when compared with the control (Table 1).

The methanol extract slowed down over secretion of *in vivo* free radicals to the systems, since the level of MDA in plasma is sustained at normal range across the treatment groups when compared with the control. The significant reduction in plasma MDA in the treatment groups controlled oxidative stress, possibly due to the natural antioxidant in the plant. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Sub-chronic activity of the extract increases CAT and SOD effect when compared with untreated control (Table 2).

The methanol extract slowed down over production of *in vivo* free radicals to the systems, the level of MDA in liver homogenates is sustained at normal values in the treatment groups when compared with control. The significant reduction in liver homogenates MDA in the treatment groups controlled oxidative stress, possibly due to the natural antioxidant in the plant. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Sub-chronic activity of the extract increases CAT and SOD effect when compared with control (Table 3).

Results from the methanol extract in Table 4 showed the effect of *in vivo* free radicals in the systems, the level of MDA in kidney homogenates is normal across the treated groups when compared with control. The significant reduction in kidney homogenates MDA in the treatment regulate oxidative stress. CAT together with SOD and GPx establish primary enzymatic defence, catalysing ROS decomposition. Sub-chronic activity of the extract increases CAT and SOD effect when compared with control.

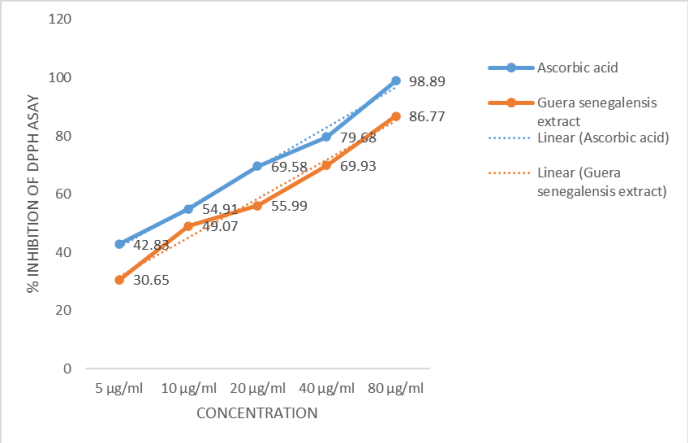


**Figure 1.** Effect of *Guiera senegalensis* methanol extract on DPPH free radical.

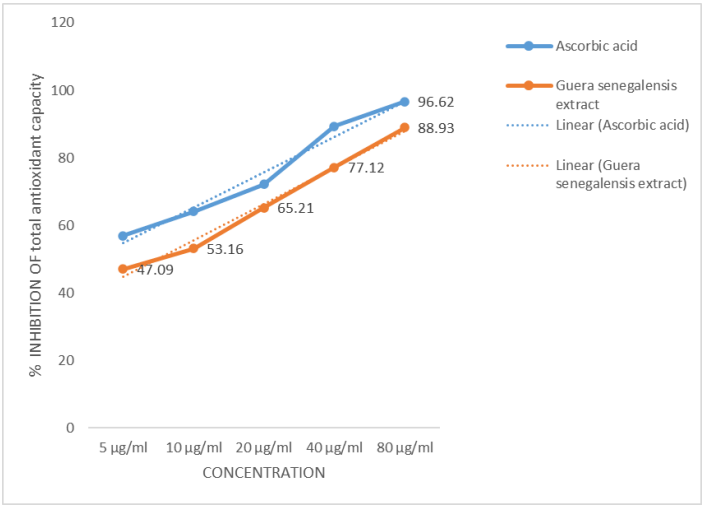


**Figure 2**. Effect of *Guiera senegalensis* methanol extract on hydroxyl free radical

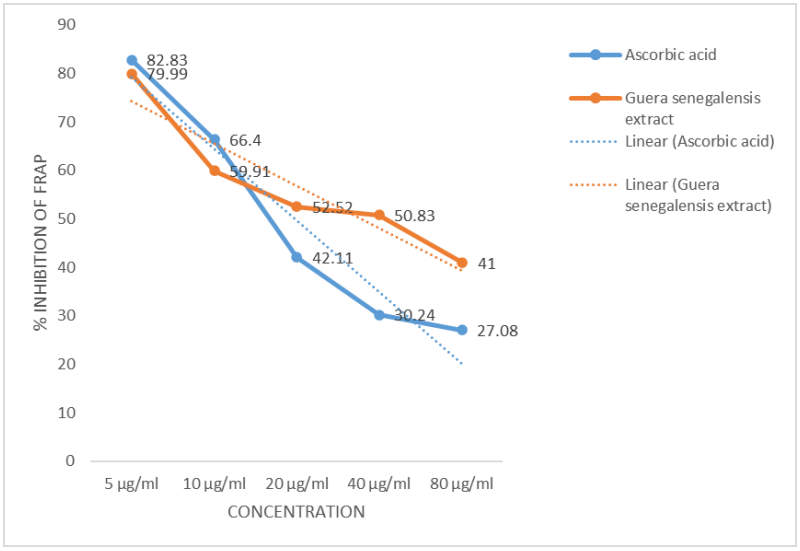


**Figure 3**. Effect of *Guiera senegalensis* methanol extract on ferric reducing antioxidant power

**Table 1.** Acute toxicity effect of methanol leaves extract of *Guiera senegalensis*

| Treatment | Doses (mg/kg) | Number of animals used | Number of deaths | Toxicological observations |
|---|---|---|---|---|
| *G. senegalensis* | 10 | 5 | 0/5 | No adverse effect |
| *G. senegalensis* | 100 | 5 | 0/5 | No adverse effect |
| *G. senegalensis* | 1000 | 5 | 0/5 | No adverse effect |
| *G. senegalensis* | 1600 | 5 | 0/5 | No adverse effect |
| *G. senegalensis* | 2900 | 5 | 0/5 | Hyperactive |
| *G. senegalensis* | 5000 | 5 | 0/5 | Mild restlessness |
| Control | - | 5 | 0/5 | No adverse effect |

**Table 2.** Effect of *Guiera senegalensis* on plasma *in vivo* antioxidant assay in oxidative stress-induced rats

| Group | Dose mg/kg | MDA ($\times10^{-3}$ mmol/ml) | SOD (U/ml) | Catalase (U/ml) | Reduced Glutathione (U/ml) | Glutathione peroxidase (U/ml) |
|---|---|---|---|---|---|---|
| 40% ethanol | - | 39.21±0.28$^{a}$ | 2.16±0.21$^{a}$ | 163.9±2.62$^{a}$ | 1.98±0.10$^{a}$ | 4.89±0.19$^{a}$ |
| Normal control | | 21.33±0.13$^{b}$ | 4.07±0.32$^{c}$ | 256.4±3.81$^{b}$ | 4.59±0.28$^{c}$ | 2.22±0.07$^{c}$ |
| *G. senegalensis* | 50 | 23.00±0.20$^{b}$ | 3.12±0.25$^{b}$ | 248.5±4.43$^{b}$ | 3.52±0.17$^{b}$ | 2.10±0.05$^{c}$ |
| *G. senegalensis* | 100 | 22.15±0.19$^{b}$ | 3.56±0.33$^{b}$ | 250.6±4.27$^{b}$ | 4.23±0.22$^{c}$ | 3.10±0.10$^{b}$ |
| *G. senegalensis* | 200 | 20.54±0.24$^{c}$ | 4.29±0.39$^{c}$ | 259.9±4.82$^{b}$ | 4.68±0.24$^{c}$ | 3.20±0.14$^{b}$ |

Values are expressed as Mean ±SEM n=5. ($p<0.05$).

**Table 3.** Effect of *Guiera senegalensis* on liver homogenate *in vivo* antioxidant assay in oxidative stress-induced rats Values are expressed as Mean ± SEM n=6. ($p < 0.05$).

| Group | Dose mg/kg | MDA (x10-3 mmole/ml) | SOD (U/ml) | Catalase (U/ml) | Reduced Glutathione (U/ml) | Glutathione peroxidase (U/ml) |
|---|---|---|---|---|---|---|
| 40% ethanol | - | 25.09±0.27$^{a}$ | 2.31±0.12$^{a}$ | 92.16±6.14$^{a}$ | 1.62±0.20$^{a}$ | 1.73±0.17$^{a}$ |
| Normal control | - | 12.33±0.10$^{c}$ | 3.93±0.13$^{b}$ | 117.34±6.72$^{a}$ | 2.41±0.28$^{b}$ | 2.65±0.21$^{b}$ |
| *G. senegalensis* | 50 | 14.11±0.09$^{b}$ | 3.40±0.11$^{b}$ | 115.21±5.54 | 2.05±0.23$^{b}$ | 2.09±0.19$^{b}$ |
| *G. senegalensis* | 100 | 13.32±0.07$^{b}$ | 3.69±0.12$^{b}$ | 116.91±7.11 | 2.37±0.26$^{b}$ | 2.33±0.25$^{b}$ |
| *G. senegalensis* | 200 | 12.03±0.11$^{c}$ | 3.91±0.20$^{b}$ | 118.72±7.09 | 2.48±0.24$^{b}$ | 2.59±0.22$^{b}$ |

**Table 4.** Effect of *Guiera senegalensis* on kidney homogenate *in vivo* antioxidant assay in Sodium chloride-induced hypertensive rats

| Group | Dose mg/kg | MDA (x10-3 mmole/ml) | SOD (U/ml) | Catalase (U/ml) | Reduced Glutathione (U/ml) | Glutathione perioxidase (U/ml) |
|---|---|---|---|---|---|---|
| 40% ethanol | - | 31.22±2.51$^{a}$ | 1.28±0.36$^{a}$ | 79.74±5.36$^{a}$ | 1.74±0.41$^{a}$ | 1.85±0.22$^{a}$ |
| Normal control | - | 18.24±1.32$^{b}$ | 2.23±0.11$^{b}$ | 113.53±6.12$^{b}$ | 2.33±0.62$^{b}$ | 2.58±0.43$^{b}$ |
| *G. senegalensis* | 50 | 23.53±2.23$^{b}$ | 1.99±0.27$^{b}$ | 97.52±4.86$^{b}$ | 2.07±0.55$^{c}$ | 2.14±0.32$^{c}$ |
| *G. senegalensis* | 100 | 19.06±1.77$^{b}$ | 2.25±0.24$^{b}$ | 110.26±5.35$^{b}$ | 2.18±0.37$^{b}$ | 2.40±0.37$^{b}$ |
| *G. senegalensis* | 200 | 17.89±1.45$^{b}$ | 2.57±0.41$^{b}$ | 110.93±6.30$^{a}$ | 2.20±0.77$^{b}$ | 2.67±0.38$^{b}$ |

Values are expressed as Mean ± SEM n=6. ($p < 0.05$).

## 4 Discussion

Drug discovery begins with crude evaluation of potent phytochemicals responsible for viable health remedy and further with the isolation of active compounds such as yohimbine, artemisinin, ergotamine, emetine, quinine, vincristine etc. several medicinal plants elicit enormous potential aid in the discovery and production of novel and synthetic pharmaceuticals [5]. WHO enhanced herbal preparations in the treatment of confined health problems where the products are easily affordable and already integrated into the people's culture [6]. Since antiquity, traditional medicines and several formulations from herbal plant are known to be safe and effective [28]. Results from this study showed increased antioxidant activity of *Guiera senegalensis* leaves extracts at against DPPH, hydroxyl free radical and ferric reducing antioxidant power, when compared with ascorbic acid. Antioxidant properties of the extract could possibly be liable for several therapeutic effect exhibited (Figure 1, 2 and 3). These findings are in line with the report of Muanya and Odukoya [29] showing high lipid peroxidation inhibitory effect of *Anthocleista djalonensis* root extract in raw and cooked fish homogenate. Studies from Aitken *et al.* [30] showed that reactive oxygen species (ROS) are associated in the pathogenesis of several diseases. Over secretion of ROS in the system affects blood cells, tissues and organs, hence, antioxidant defensive mechanism inhibits oxidative stress [31]. Antioxidants scavenging free radicals in the system, protected several cells responsible for the occurrence of diseases [32]. Antioxidant property of *G. senegalensis* leaves extract indicated that it possesses scavenging capacity of excess reactive oxygen species (ROS) in cells liable for oxidative impairment in diseases state.

Acute toxicity study of the methanol leaves extract of *G. senegalensis* exhibited comparatively non-toxic effect with $LD_{50}$ greater than 5000 mg/kg as shown in Table 1. No observable treatment-related toxic signs such as respiratory distress, salivation, alteration in fur, weight loss, paw licking, skin colour and mortality in the pre-treated animals during the period of study. Thus, it is suggested that the methanol extract of *G. senegalensis* could possibly be safe at the tested doses. Lorke [26] categorized substances as toxic if the $LD_{50}$ is above 1000 mg/kg body weight. Clarke and Clarke, [33] also reported that substance with toxicity greater than $LD_{50}$ above 1000 mg/kg body weight is declared toxic.

Conventional uses of biomarkers like transaminases aid in the evaluation of redox state of plasma and organs using antioxidant enzymes action with macromolecule oxidation determinant [34, 35]. Oxidation implicated by cellular membranes injury retrieved by thiobarbituric acid reactive substances (TBARS) assay. This assay explains malondialdehyde (MDA) in association with lipoperoxidation (LPO). *G. senegalensis* inhibits free radicals production to regulate normal plasma MDA values across treatment groups when compared with control groups (Table 2). *G. senegalensis* aid in the control of oxidation, perhaps due to the present of antioxidant compounds in the plant. These constituents could be implicated in radical scavenging effect as ancillary deactivated transcription factors responsible for the regulation of expressed genes encrypted by antioxidant enzymes [34]. Tissue sulfhydryl act contrary to reactive oxygen species (ROS) linked to toxic tissues triggered by some oxidative phytochemicals in plant. The major NPSH is made up of approximately 90% of the entire intracellular NPSH reduced in glutathione (GSH) [35]. GSH showed essential function implicate in antioxidant defense mechanisms, possibly due to direct radical-scavenging effects, vital constituent of glutathione peroxidase (GPx) systems, elimination of diverse hydro-peroxides [36]. Poisonous plants evaluated in chronic study deplete GSH materials found in several visceral cells, tissues and organs with a pro-oxidant activity at increased doses to suppress free-radical stress, GSH is useful as initial defense against several diseases [34] as shown in this study. Non-enzymatic defences, showed no significance changes in thiols content (NPSH), due to synergistic antioxidant effect between NPSH and SBSB phytochemicals in visceral tissue. Catalase (CAT) and super oxide dismutase (SOD) antioxidant activities were evaluated across treatment groups. CAT together with SOD and GPx constitute enzymatic defense, catalysing decomposition of ROS against diseases. *G. senegalensis* methanol leaves extract at graded doses elicited increased CAT and SOD effects when compared with control (Figure 2, 3 & 4) [37]. Theoretically, SOD and CAT activity rises as compensatory mechanism to scavenge free-radical stress [18, 37].

## 5 Conclusion

These findings showed that methanol leaves extract of *G. senegalensis* possesses a relative safe dose. It also comprises of bioactive components that triggered the *in vitro* and *in vivo* antioxidant activities possibly responsible for various biological effects. Hence, this study advances credibility for the validation of its folklore report.

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