

**A COMPARATIVE STUDY ON GC-MS PROFILES AND EFFECT OF ETHANOLIC EXTRACTS OF STEM-BARK AND LEAVES OF *Danielliaoliveri* ON CEREBRAL SODIUM PUMP AND THIOLS STATUS IN FENTON REACTION TREATED RAT ORGANS *In-vitro*.**

**ABSTRACT**

*Danielliaoliveri* is one of the most extensively utilized medicinal plants in Nigeria and some West African countries for the treatment of various ailments. In the present study, the GC-MS profiles and effect of ethanolic extracts of stem bark and leaves of *Danielliaoliveri* on lipid peroxidation,  $\text{Na}^+/\text{K}^+$ -ATPase activity and thiols status in  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  (Fenton reaction) assaulted cerebral and hepatic tissue homogenates was investigated. The stem bark and leaves were extracted with absolute ethanol for 72hrs and concentrated in a rotary evaporator. Wistar rats were euthanized and the brains and livers were removed, homogenized, centrifuged and the supernatants were used for lipid peroxidation,  $\text{Na}^+/\text{K}^+$ -ATPase activity, and thiol assays in the presence of prooxidants ( $1\text{mM H}_2\text{O}_2$  or  $10\mu\text{M Fe}^{2+}$  or  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$ ). The results revealed that ethanol extract of both the leaf and stem-bark inhibited lipid peroxidation induced by  $\text{H}_2\text{O}_2$ ,  $\text{Fe}^{2+}$  and combination of  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  and this was evident in the reduction of lipid peroxidation adducts formation in rat liver and brain homogenates with the stem-bark possessing more efficacy. Furthermore, the results of  $\text{Na}^+/\text{K}^+$ -ATPase and thiol assays revealed that  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  inhibited the activity of cerebral  $\text{Na}^+/\text{K}^+$ -ATPase, while  $\text{H}_2\text{O}_2/\text{Fe}^{2+}$  caused a marked reduction in the levels of both protein and non-protein thiols in the tissues homogenates. However, the ethanolic extract of *Danielliaoliveri* extirpated this anomaly by increasing the activity of cerebral  $\text{Na}^+/\text{K}^+$ -ATPase as well as the thiol level in the rat hepatic and cerebral tissues. Finally, GC-MS analysis was carried out on the ethanolic extracts of the stem bark and leaves of *Danielliaoliveri* and results revealed the presence of 21 compounds including 1,2-Benzisothiazol-3-amine, 1H-Indole-2-carboxylic acid, 2-Ethylacridine, Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(3-fluoroanilino) propionate and trans-1-Butyl-2-methylcyclopropane in the stem bark and 10 compounds including Methylthio-acetonitrile, 2-Methyl-Z,Z-3,13-octadecadienol, Desulphosinigrin and 4H-1,2,4-triazole-3,5-diamine in the

leaves, justifying the observed biological activities of *Danielliaoliveri* and the higher potency of the stem bark may be attributed to the presence of more bioactive constituents found in it. This study therefore justifies the medicinal usage of leaves and stem-bark of *Danielliaoliveri* and suggests its consideration in the treatment and management of degeneration diseases with etiology associated with oxidative stress.

**Key words: pro-oxidants, lipid peroxidation, Antioxidant, thiols, Na<sup>+</sup>/K<sup>+</sup>-ATPase, GC-MS profile, *Danielliaoliveri*.**

## 1.0 INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine and about 80% of the rural population relies heavily on them as their sole source of medical care [1]. The use of plants as sources therapies in treating diseases all over the globe especially in Africa has been in practice right from ancient days. Despite the remarkable success achieved from the use of synthetic drugs in the twentieth century, more than 25% of prescribed drugs are derivatives of plants [2]. Conversely, medicinal plants are yet to be fully studied and the price of synthetic drugs are often outrageous and unaffordable in the developing countries, particularly in Africa [3]. Thus, making the larger population depend on herbal therapies [3,4]. According to WHO, traditional medicine is regarded as one of the feasible ways to actualize global health care as about 25% of drugs in developed countries such as United States is from plant sources, while as much as 80% of drugs in fast developing countries is contributed by herbal sources [5].

*Danielliaoliveri* is a plant which grows in the Amazonia, certain part of America, and some countries including Nigeria. Roots of this plant are used in traditional medicine to treat anxiety and schizophrenia [6]. Leaves decoction and stem-bark infusion have been used as diuretic and aphrodisiac and applied as skin lotion. Dry leaves powder is also administered to treat yellow fever, back ache, and headache and for wound healing. Research has shown that *Danielliaoliveri* plant possess anti-diabetic activity, anti-convulsant activity/ anti-diarrheal activity, anti-wrinkle activity, cytotoxic activities, anti-helminthic activities/ anti-spasmodic activity, and anti-oxidant/anti-radical activities [7].

Lipid peroxidation is a complex process known to occur in both plants and animals. Lipid peroxidation often involves oxidative damage by free radical assault on the membrane lipids. The pathogenesis of several diseases such as diabetes, Parkinson disease and cancer is being mediated by lipid peroxidation. Reactive oxygen species (ROS) attack membrane lipids containing double bonds and abstract hydrogen atom, leading to the destruction of membrane lipids consequent to the formation of toxic hydroperoxides and lipid peroxy radicals [8]. Lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. Lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. Lipid peroxidation has become rampant in many organisms especially humans, causing a great challenge due to generation of reactive oxygen species and nitrogen species. This causes oxidative damage of cells leading to several diseases that may eventually result to cell death [9].

The sodium-potassium pump or ATPase ( $\text{Na}^+/\text{K}^+$  ATPase) is a membrane-embedded protein of living organism particularly animals. The nature of constituents comprising the membrane components is one of the important factor for the determination of the enzyme function [10]. The sodium-potassium pump works by pumping two potassium ions into the cell and three sodium ions outside the cell using the energy from adenosine-triphosphate (ATP) molecule. It is essential for maintaining various cellular functions, its inhibition could result in diverse pathologic states, such as heart failure, neurodegenerative diseases, diabetes, hypertension and cardiovascular diseases [11]. Research also revealed that some heavy metals such as scandium, cadmium, lead, mercury, arsenic, copper, and iron inhibit the activity of  $\text{Na}^+/\text{K}^+$ -ATPase and this may result in neurodegenerative disorders [12]. This work aimed at comparing the GC-MS profiles as well as investigating the effect of the stem bark and leaves of *Daniellia oliveria* thiol status, lipid peroxidation and  $\text{Na}^+/\text{K}^+$ -ATPase activity in rat treated with Iron ( $\text{Fe}^{2+}$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).

## **2.0 MATERIALS AND METHODS**

### **2.1.1 Chemicals**

Thiobarbituric acid (TBA), Sodium dodecyl sulphate (SDS), Trichloro acetic acid (TCA), Adenosine Triphosphate (ATP), 5,5 dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals which were of analytical grade were obtained from standard commercial suppliers.

### **2.1.2 Plant Collection**

The stem-bark and leaves of *Daniellia Oliveri* were collected in Ngawa village of Girei local government area, Adamawa state, Nigeria in September, 2020. The plants were identified by an expert botanist at the department of Biological sciences, Federal University Wukari, Taraba State, Nigeria.

### **2.1.3 Preparation of the Plant Sample**

The collected samples of stem-bark and leaves of *Daniellia Oliveri* were washed thoroughly with clean water and air-dried for two (2) weeks at room temperature. The dried samples were pulverized separately with mechanical blender.

### **2.1.4 Plant Extraction**

100g of the pulverized sample of stem-bark and leaves of *Daniellia Oliveri* were weighed and extracted in 400ml of absolute ethanol by maceration for 72 hours and stirred after every 4 hours. The extracts were filtered using Whatman filter No.1 paper. The resulting extracts were concentrated, using water bath at 45°C temperature and kept in refrigerator for assays.

### **2.1.5 Experimental Animals**

Male adult Wistar rats (weighing between 200-250 g) were used for the experiment. Animals were housed in separate animal cages, on an average of 12 hours light and dark cycle at ambient temperature with free access to food and water. The animals were used according to standard guideline of the Committee on Care and Use of Experimental Animal Resources.

### **2.1.6 Preparation of Tissue Homogenate**

Animals were anaesthetised with mild ether and euthanized by decapitation and the brains and livers were removed, quickly placed on ice and weighed. The brains and livers were rinsed thoroughly with cold 50 mM Tris-HCl buffer to ensure that they are free from blood stain and immediately homogenised in cold at approximately 1,200 rev/min in a Teflon-glass homogenizer with 50 mM Tris-HCl buffer, pH 7.4 (1/10, w/v). The homogenate was centrifuged at 4000 x g for 10 minutes to yield the low speed supernatant (S1) fraction that was used for the assays.

#### **1.2.1 Thiobarbituric Acid Reactive Species (TBARS) Assay**

Lipid peroxidation was carried out by measuring TBARS (thiobarbituric acid reactive species) using the modified method of Ohkawa *et al.* [13]. An aliquot of 100 µl of S1 was incubated for 1 hour at 37°C in the presence of stem-bark and leaves extracts, (final concentrations range of 0-100 mg/ml), in a reaction system containing 50 mM Tris-HCl buffer (pH 7.4), with pro-oxidants [1mMFeSO<sub>4</sub> (10 µM) or 1mMH<sub>2</sub>O<sub>2</sub> (1 µM)] or both. The colour reaction was developed by adding 200 µl of 8.1% SDS (Sodium Dodecyl Sulphate) to the reaction mixture containing S1. This was subsequently followed by the addition of 500 µl of acetate buffer, pH 3.4, and 500 µl of 0.8% TBA. This mixture was incubated at 100 °C for 30 minutes. TBARS produced was measured at 532 nm in UV-visible spectrophotometer.

#### **2.2.2 Determination of Protein and Non-protein Thiols**

The level of non-protein thiols in the brain homogenates were estimated using Ellman's reagent after deproteinization with TCA (5 % in 1 mmol/EDTA) following the method of Ellman [14]. For the estimation of total thiols, the tissue homogenates were not subjected to a prior deproteinization. The level of protein thiols is taken as the positive difference between total thiols and non-protein thiols.

#### **2.2.3 Sodium-Potassium Pump Assay**

The reaction system contained 3 mM MgCl, 125 mM NaCl, 20 mM KCl, 50mM Tris-HCl, pH 7.4, pro-oxidants [1mMFeSO<sub>4</sub> (10 µM) and 1mMH<sub>2</sub>O<sub>2</sub> (1µM)] and 200mM sodium azide and extract (final concentration range of 0-200mg/ml) in a final volume of 500 µl. The reaction was

initiated by addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 0.1 mM DMSO. The reaction mixture was incubated at 37 °C for 30 minutes. However, at the end of the incubation time period (30 min), the reaction was stopped by addition of 5% trichloroacetic acid. Released inorganic phosphate was measured by the method of Fiske and Subbarow [15]. Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was calculated. All the experiments were conducted at least three times and similar results were obtained. Enzyme activity was expressed as number of moles of phosphate released min/mg/protein.

#### **2.2.4 Gas Chromatography Mass Spectroscopy (GC-MS) Analysis**

Each extract was subjected to GC-MS analysis using the model instrument, GC-MS QP2010 ultra (Shimadzu co., Japan) attached with a capillary column DB-1 (0.25 µm film x 0.25mm i. d. x 30m length). Analysis was performed by injecting 1 µL of the sample with a split ratio of 20:1. Helium gas (99.9%) was used as the carrier gas at a flow rate of 1ml/min. The analysis was performed in EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 250°C (constant). The column oven temperature was set at 50°C (held for 3min), raised at 10°C per minute to 280°C (held for 3min), and finally held at 300°C for 10min. The compounds were identified after comparing the spectra configurations obtained with that of available mass spectral database (NIST and WILEY libraries) [16, 17].

#### **2.2.5 Statistical Analysis**

All values obtained were expressed as mean ± SEM. The data were analyzed by appropriate ANOVA followed by Duncan's multiple range tests where appropriate. \* and # Represents significant difference from control at p < 0.05.

### **3.0 RESULT**

#### **3.1 Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on H<sub>2</sub>O<sub>2</sub> induced lipid peroxidation in rats brain and liver homogenates**

The results in Figure 1 reveals that H<sub>2</sub>O<sub>2</sub> induced marked increase in lipid peroxidation in rat brain (panel A) and liver (panel B) homogenates. However, the ethanolic extract of both the ethanolic extract of both stem-bark and leaves of *Danielliaoliveri* exerted marked (p <0.05)

inhibitory effect on lipid peroxidation in a concentration dependent manner compared to the control with the stem-bark showing more efficacy than the leaves.

### **3.2 Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on Fe<sup>2+</sup> induced lipid peroxidation in rats liver and brain homogenates**

Similarly Figure 2 reveals that treatment with Fe<sup>2+</sup> caused an elevated level of lipid peroxidation in rat cerebral (panel A) and hepatic (panel B) tissue homogenates, but the ethanolic extract of both leaves and stem-bark of *Danielliaoliveri* exerted marked inhibitory effect on lipid peroxidation in a concentration dependent manner. However, the stem-bark exhibited more inhibitory action on the production of thiobarbituric acid reactive substances.

### **3.3 Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on lipid peroxidation in rats liver and brain homogenates induced by Fenton reaction (Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>)**

The results in Figure 3 shows that Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> provoked an increased lipid peroxidation in rat brain (panel A) and liver (panel B) homogenates, but the ethanolic extract of both leaves and stem-bark of *Danielliaoliveri* exerted profound (p<0.05) inhibitory effect on lipid peroxidation in a concentration dependent manner compared to the control. Meanwhile, the stem-bark showed more inhibitory effect on the production of thiobarbituric acid reactive substances.

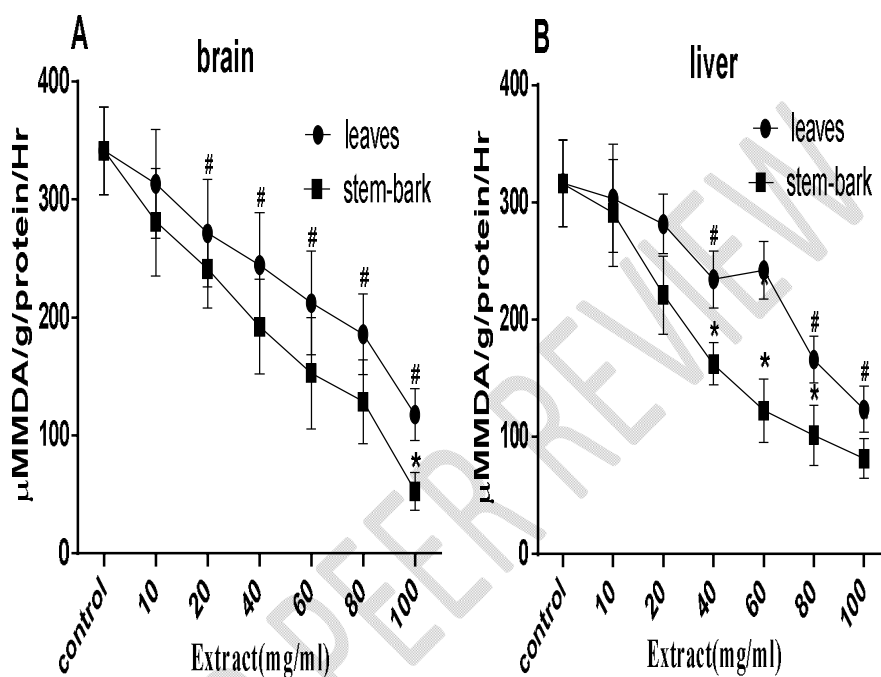


Figure 1: Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on Lipid peroxidation induced by  $\text{H}_2\text{O}_2$  in rat brain (panel A) and liver (panel B). Data are presented as mean  $\pm$  SEM of three independent experiments carried. \* and # represent significant difference from control at  $p < 0.05$ .

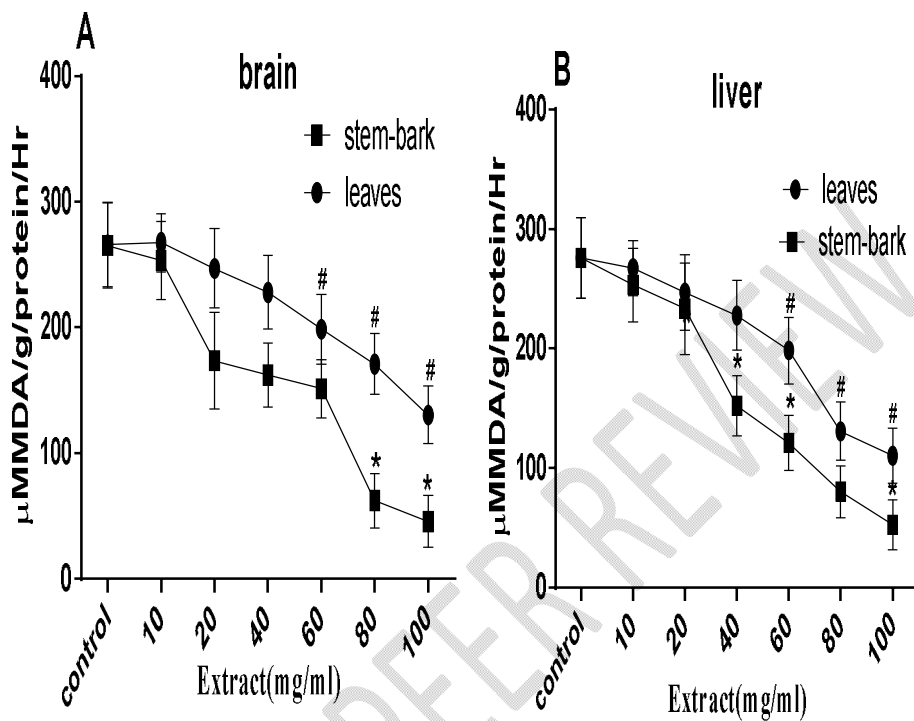


Figure 2: Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on Lipid peroxidation induced by  $\text{Fe}^{2+}$  in rat brain (panel A) and liver (panel B). Data are presented as mean  $\pm$  SEM of three independent experiments carried. \* and # Represent significant difference from control at  $p < 0.05$ .

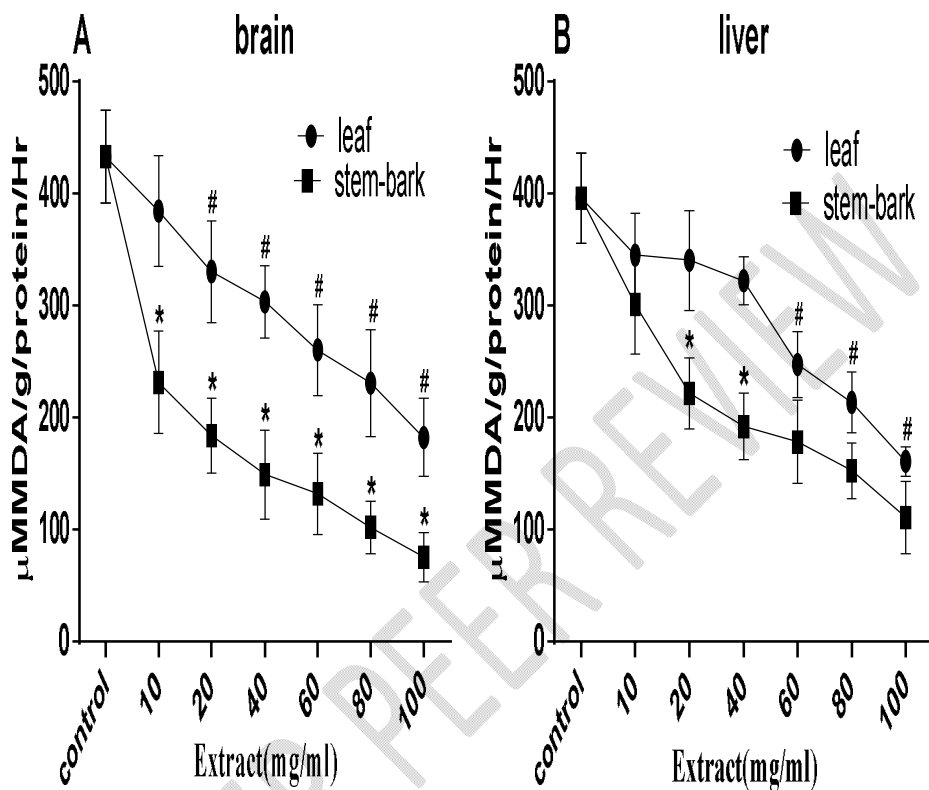


Figure 3: Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on Lipid peroxidation induced by  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  (Fenton reaction) in rat brain (panel A) and liver (panel B). Data are presented as mean  $\pm$  SEM of three independent experiments carried. \* and # represent significant difference from control at  $p < 0.05$ .

### 3.4 Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on thiol level in Fenton reaction treated rat brain and liver homogenate

As displayed in Figure 4 (panel A),  $\text{H}_2\text{O}_2$  inhibited the activity of cerebral  $\text{Na}^+/\text{K}^+$ -ATPase in the rat cerebral tissue homogenate, but treatment with ethanolic extract of both the leaves and stem-bark of *Danielliaoliveri* restored  $\text{Na}^+/\text{K}^+$ -ATPase activity. Similar result was observed when  $\text{Fe}^{2+}$

was to assault the pump (panel B), while the stem-bark displayed more efficacy than the leaves, with increasing extract concentration.

### **3.5 Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliverion* thiol level in Fenton reaction treated rat brain and liver homogenate**

The results presented in figure 5 reveal that  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  treatment caused a depletion in the level of both protein (A and B) and non-protein (C and D) thiols. However, treatment with the ethanolic extract of both the leaves and stem-bark extracts of *Danielliaoliveri* significantly ( $p < 0.05$ ) raised the levels of both protein and non-protein thiols in concentration dependent manner with the stem-bark exhibiting more increasing potential.

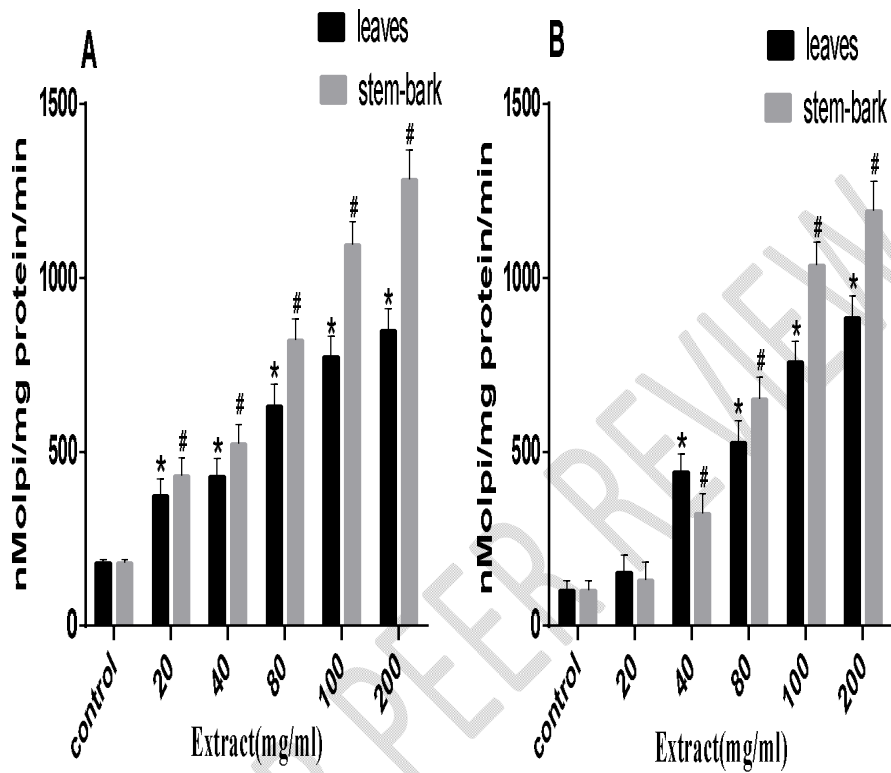


Figure 4: Effect of ethanolic extract of leaves and stem-bark of *Danielliaoliveri* on cerebral  $\text{Na}^+/\text{K}^+$ -ATPase activity in  $\text{H}_2\text{O}_2$  (panel A) and  $\text{Fe}^{2+}$  (panel B) treated rat brain homogenates. Data are presented as mean  $\pm$  SEM of three independent experiments carried. \* and # represent significant difference from control at ( $p < 0.05$ ).

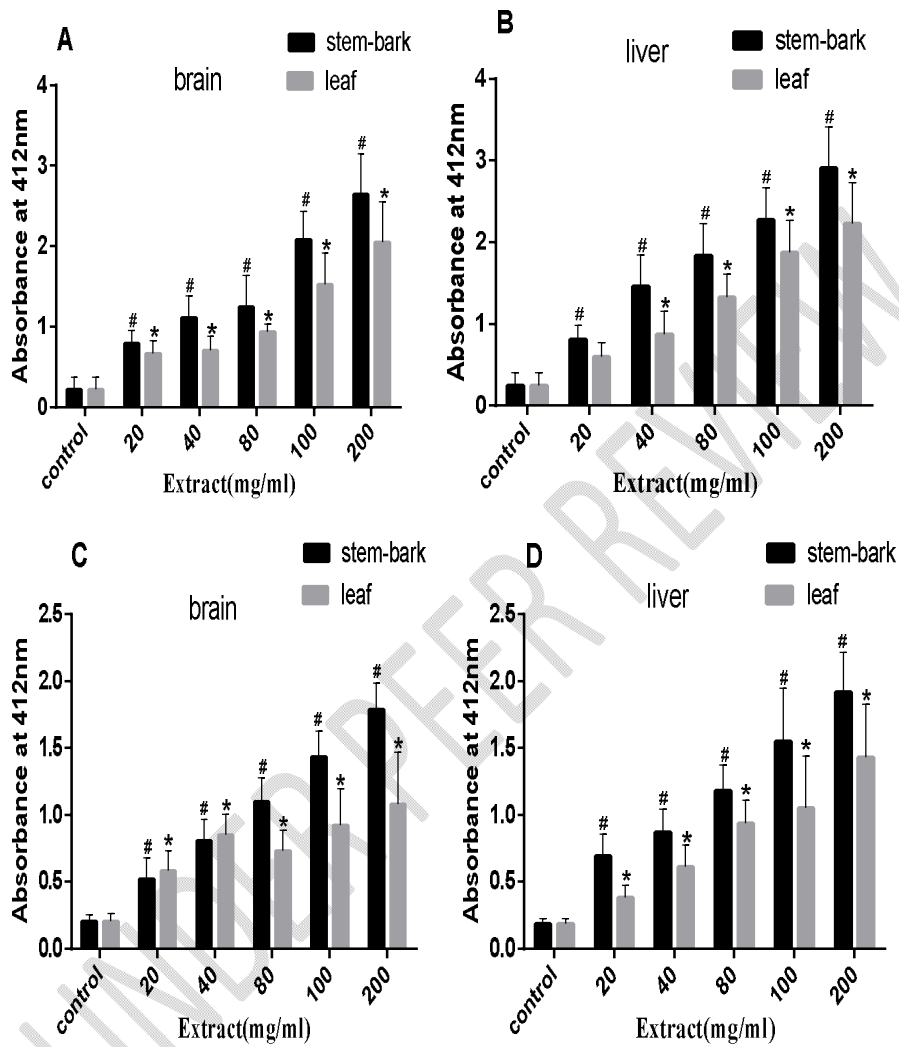


Figure 5: Effect of ethanolic extract of stem-bark and leaf of *Danielliaoliveri* on protein thiols (panel A and B) and non-protein thiols (panel C and D) level in Fenton reaction treated rat brain and liver homogenate respectively, Data are presented as mean  $\pm$  SEM of three independent experiments carried. \* and # represent significant difference from control at ( $p < 0.05$ ).

### 3.6 GC-MS Result

The compounds in the leaves and stem-bark of *Danielliaoliveri* with their retention time (RT), quantity and peak area in percentage are presented in table 1 and table 2 respectively. GC-MS analysis of ethanolic extract of *Danielliaoliveri* revealed the presence of 10 compounds in the stem bark including 2-Methyl-Z, Z-3,13-octadecadienal, Desulphosinigrin, Dimethyl-cyanophosphine, (Methylthio)-acetonitrile, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester and 4H-1,2,4-triazole-3,5-diamine (table 1) and 21 compounds in stem-bark including methyl ester, 9,17-Octadecadienal, trans-13-Octadecenoic acid, 9,12-Octadecadienoic acid (Z,Z), trans-1-Butyl-2-methylcyclopropane, 4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, cis-9-Tetradecenoic acid, heptyl ester, Benzo[h]quinolone, 2-Ethylacridine and 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7- tetrahydro-, isopropyl ester (table 2).

Table 1: The GC-MS composition for ethanolic extract of *Danielliaoliveri* leaf.

S/N	Retention Time	Name of Compound	Area %	Quantity
1	5.1484	2-Methyl-Z, Z-3,13-octadecadienol	24.6896	90
2	10.1027	Desulphosinigrin	-1.2528	64
3	10.8212	Dimethyl-cyano-phosphine	21.7141	43
4	10.888	(Methylthio)-acetonitrile	16.0457	38
5	13.9237	Hexadecanoic acid, methyl ester	9.997	55
6	16.232	13-Octadecenal, (Z)	10.4507	46
7	16.3562	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	11.0187	76
8	26.9988	Carvacrol, TBDMS derivative	2.4666	38
9	27.1642	1,2-Benzisothiazol-3-amine, TBDMS Derivative	3.7845	46
10	27.2324	4H-1,2,4-triazole-3,5-diamine	1.0858	55

Comment [U1]: Indicate, time unit (Min)?

Table 2: The GC-MS composition for ethanolic extract of *Danielliaoliveri* bark.

Comment [U2]: Indicate if it is bark-stem?

Comment [U3]: Indicate, time unit (Min)?

S/N	Retention Time	Name of Compound	Area %	Quantity
	12.9315	Hexadecanoic acid, methyl ester	4.4452	93
2	12.9622	Hexadecanoic acid, methyl ester	4.116	90
3	13.004	Hexadecanoic acid, methyl ester	8.6864	94
4	13.9321	Hexadecanoic acid, methyl ester	3.8867	93
5	15.9503	9,17-Octadecadienal, (Z)	7.2818	86
6	16.1181	trans-13-Octadecenoic acid, methyl ester	7.7952	64
7	16.1374	9,12-Octadecadienoic acid (Z, Z)	4.1932	80
8	16.2398	9,12-Octadecadienoic acid (Z, Z), methylester	8.5446	99
9	16.3682	trans-13-Octadecenoic acid, methyl ester	7.3961	76
10	16.7784	trans-1-Butyl-2-methylcyclopropane	14.7079	38

11	22.6438	6-Octadecenoic acid, (Z)	1.8534	53
12	26.8291	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	1.2766	47
13	27.0537	cis-9-Tetradecenoic acid, heptyl ester	0.9637	44
14	27.1585	Cholesta-22,24-dien-5-ol	13.0696	47
15	27.2389	1,2-Benzisothiazol-3-amine, TBDMS derivative	1.2938	38
16	27.7114	Benzo[h]quinolone	4.2354	41
17	29.0025	2-Ethylacridine	4.5595	60
18	29.078	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	0.319	64
19	31.1741	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	1.1385	46
20	33.0877	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(3-fluoroanilino) propionate	0.7294	17
21	33.1237	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-fluoroanilino) propionate	0.2125	25

#### 4.0 DISCUSSION

About 80% of the population uses medicinal plants for therapy [3,4] especially in developing countries, notably in West Africa where synthetic drugs are not often affordable. Traditionally, the leaves, fruits, seeds, bark and root of *Danielliaoliveri* have been used in the treatment of various ailments in Nigeria and some West African countries. In Nigeria it has been used in combination with other plants in management of HIV/AIDs patients [18]. The leaves are used traditionally to treat diabetes, yellow fever and as aphrodisiac [18].

Lipid peroxidation has become a great challenge in many organisms especially humans, causing a great challenge due to generation of reactive oxygen species and nitrogen species. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers [19]. This causes oxidative damage of cells leading to several diseases, which may eventually result to cell death [9]. Oxidative stress by ROS usually involves a chain of reactions which leads to damage of cell constituents such as proteins, lipid and nucleic acids. In aerobic organisms, membrane phospholipids are continually subjected to oxidation by both endogenous and exogenous sources. Lipid peroxidation has been implicated as a major mechanism in the generation of diseases and disorders such as cancer, cardiovascular and neurodegenerative diseases that function through alteration of the integrity, fluidity, and permeability of the membranes [20]. In the present study, two pro-oxidants,  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  were employed in inducing lipid peroxidation in the liver and brain homogenates and the possible antioxidant effects of the extract were evaluated.

$\text{H}_2\text{O}_2$  can be regarded as an effective oxidant of a large variety of biomolecules including lipids as research demonstrated that  $\text{H}_2\text{O}_2$  constituted a marked increase in lipid peroxidation in cerebral and hepatic tissues of rat [8, 21, 22]. As shown in Figure 1,  $\text{H}_2\text{O}_2$  induced marked increase in lipid peroxidation in both rat brain (panel A) and liver (panel B) homogenates. However, treatment with ethanolic extract of both the leaves and stem-bark inhibited the formation of thiobarbituricacid reactive substances in concentration dependent manner. This effect was

significant ( $p < 0.05$ ) when compared with the control with the stem-bark having more potency than the leaves.

In like manner,  $Fe^{2+}$ , a strong prooxidant induced marked increase in the formation of thiobarbituric acid reactive substances in rat brain (Figure 2, panel A) and liver homogenates (Figure 2, panel B), but upon treatment with the ethanolic extract of leaves and stem-bark of *Danielliaoliveri*, lipid peroxidation was profoundly inhibited when compared with the control with the stem-bark exhibiting more efficacy.

Furthermore, the effect of the extract was further tested on the oxidative assault induced by the combination of  $Fe^{2+}$  and  $H_2O_2$  (fenton reaction). The results displayed in figure 3 reveal that  $Fe^{2+}$  and  $H_2O_2$  (fenton) induced increased production of lipid peroxidation adducts in both the hepatic (panel A) and cerebral (panel B) tissue homogenates. However, treatment with ethanolic extract of both stem-bark and leaves of *Danielliaoliveri* exerted marked inhibitory effects, with the stem-bark possessing more antioxidant potential than the leaves. The potency of the extracts might be due to the presence of active antioxidant compounds such as flavonoids, some lignans, flavones, anthocyanins and phenols. According to many researchers, flavonoids and flavones are the main constituents responsible for antioxidant and anti-radical properties [23]. Therefore, *Danielliaoliveri* may be effective in the management of chronic diseases with etiology in oxidative stress. The results herein are in line with the findings of Rossato and Bioportfolio, [24,25] on antioxidant properties of stem-bark of *Danielliaoliveri* plant. This is also similar to the findings of Onoja et al. [26] in which *Danielliaoliveri* leaves were found to possess strong antilipid peroxidation and antioxidant activities. The phytochemical screening of *Danielliaoliveri* leaves revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins and terpenes/sterols. *Danielliaoliveri* extract was found to possess DPPH radical scavenging ability in concentration-dependent manner and this activity has been shown to be related to its lipid peroxidation inhibitory property [27]. The result herein is also in correlation with the findings of Kning, et al. [28], on the effect of ethanolic extract of roots and seeds of *Danielliaoliveri* on thiol and lipid peroxidation in rats treated with cadmium. [29] reported the DPPH radical scavenging activity as well as the ability of stem bark ethanolic extract of *Danielliaoliveri* to increase the level of antioxidant enzymes in the body. Noteworthy is the fact that the antioxidant activity of flavonoids and phenolic compounds depends largely on the presence of free OH groups [30].

Several works have documented that flavonoids exhibit antioxidant, free radical scavenging and anti-lipoperoxidation activities. Moreso, some essential flavonoids such as rutin, narcissin and quercetin have been successfully isolated from *Danielliaoliveri*[31] which could be instrumental to the reported biological activities of the plant. It has also been suggested that the stem bark ethanolic extract of *D. oliveri* possess hepatoprotective effects and this has been linked to the residing phytochemicals such as tannins, flavonoids, terpenes, saponins, cardiac glycosides, steroids, and phenolic compounds [32]. Interestingly, numerous findings suggest that compounds with antioxidant activity are able to impede the action of free radicals that play a role in the pathogenesis of diseases such as cancer, atherosclerosis, liver disorders and Alzheimer disease [33].

Thiols (free thiol groups) are thought to play a protective role against oxidative stress through ROS scavenging and are an important component of the *in vivo* antioxidant buffer capacity [28]. Therefore, serum-free thiol significantly predicted the risk of oxidative stress. The results of thiols analysis revealed that fenton reaction ( $H_2O_2$  and  $Fe^{2+}$ ) led to a marked ( $p < 0.05$ ) reduction in both protein thiols and non-protein thiols (figure 4) levels in rat brain and liver homogenates. Contrarily, treatment with ethanolic extract of both the leaves and stem-bark significantly ( $p < 0.05$ ) elevated the levels of both protein thiols (panel A and B) and non-protein thiols (panel C and D) when compared with compared to the control with the stem-bark having more potency.

Evidences suggest that thiol compounds react with oxygen radicals by donating hydrogen to the radical yielding sulfur-centered thiyl radical. The thiyl radicals formed in the reaction of thiol compounds with free radicals undergo several secondary reactions and the effect or efficacy of thiol compounds as antioxidant depends on the balance and fates of thiyl radicals. One of such reactions is the reversible addition of thiyl radical to unsaturated fatty acids, followed by elimination with concomitant isomerization of fatty acids from cis form to trans form thereby impairing the antioxidant efficacy of thiols [34,35]. With increasing evidence of the deleterious effects of trans fatty acids on human health [36,37], the thiyl radicals are receiving renewed attention [38,39].

Under conditions of moderate oxidative stress, oxidation of Cys residues can lead to the reversible formation of mixed disulfides between protein thiol groups and low-molecular-mass

thiols (S-thiolation), particularly with GSH (S-glutathionylation) [40]. A characteristic hallmark of many pathophysiological conditions is a decrease in the GSH: GSSG ratio. S-Glutathionylated proteins accumulate under oxidative/nitrosative stress conditions when GSSG accumulates in cells and undergo disulfide exchange reactions with protein thiols. S-Glutathionylated proteins have been investigated as possible biomarkers of oxidative/nitrosative stress in some human diseases, such as renal cell carcinoma and diabetes. Glutathionylated hemoglobin is increased in patients with type 1 and type-2 diabetes, hyperlipidemia and uraemia associated with haemodialysis or peritoneal dialysis [41]. It could therefore be suggested from our result that the extracts probably counteracted the harmful reaction of thiyl radicals and as well increased the GSH: GSSG ratio in the rat tissues.

Glutathione, an intracellular multifunctional non-enzymatic antioxidant known for its role as a major redox (thiol-disulphide) buffer of the cell, is abundant in cytosol (1–11 mM), nuclei (3–15 mM), and mitochondria (5–11 mM) [42]. Glutathione and other thiol compounds play an important role in the defense network against oxidative stress *in vivo* by maintaining redox balance and homeostasis [43-45]. Furthermore, thiol compounds are present in intracellular and extracellular fluids in high concentrations and react with reactive hypochlorous acid and hypothiocyanous acid produced by myeloperoxidase [46,47]. They can also act as an electron or hydrogen donor to scavenge active free radicals and repair damaged molecules [48]. Notably, pulse and ionizing radiation studies contributed extensively to the understanding of the mechanisms and kinetics of the reactions involving thiol compounds and thiyl radicals [49,50]. However, the reactivity of thiol compounds toward free radicals, especially peroxy radicals, has not been measured systematically. Peroxy radicals are important as chain carrying species in lipid peroxidation, one of the important reactions *in vivo*.

Apart from their role in defense against free radicals, thiols share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level. Decreased levels of thiols have been noted in various medical disorders including chronic renal failure and other disorders related to kidney, cardiovascular disorders, stroke and other neurological disorders, diabetes mellitus, alcoholic cirrhosis and various other disorders. Therapy using thiols has been under investigation for certain disorders [51].

The sodium-potassium pump ( $\text{Na}^+/\text{K}^+$  ATPase) is the membrane-bound enzyme that maintains the  $\text{Na}^+$  and  $\text{K}^+$  gradients across the plasma membrane of animal cells. Because of its importance in many basic and specialized cellular functions, this enzyme is necessary for adaptation to changing cellular and physiological stimuli [11]. It is known that  $\text{Na}^+/\text{K}^+$  ATPase plays a crucial role in cell signalling.  $\text{Na}^+/\text{K}^+$  ATPase regulates cell motility, cell proliferation, glycogen synthesis, intracellular calcium and sodium homeostasis, calcium signalling, apoptosis etc. [52,53,54].  $\text{Na}^+/\text{K}^+$  ATPase is sensitive to ROS insult. It is known that increases in superoxide anion/hydrogen peroxide can modify the  $\text{Na}^+/\text{K}^+$  ATPase  $\alpha$  and  $\beta$  subunit to inhibit its enzymatic (ion exchange) activity [55,56].

As shown in Figure 5,  $\text{H}_2\text{O}_2$  (panel A) and  $\text{Fe}^{2+}$  (panel B) inhibited the activity of  $\text{Na}^+/\text{K}^+$ -ATPase in the brain homogenate. Interestingly, intervention of the leaves and stem-bark of *Danielliaoliveri*, exerted an increase in activity of the enzyme. Meanwhile, the stem exhibited higher potency. This suggests the extracts might be having some modulatory/pharmacological effects on the nervous system. Sodium pump dysfunction has been linked to some neurological disorders such as Alzheimer's disease, diabetes, bipolar disorder and epilepsy during which there is often an imbalance in the amount of neurotransmitters in the cell due to persistent depolarization across the cell membrane [57-59]. Reduced activity of the electrogenic enzyme in several free-radical linked degenerative and metabolic pathologies have been an area of intense study [58,59] as it was suggested that reactive oxygen species (ROS) may indirectly inhibit the enzyme [60,61]. Though biochemical mechanisms of free radicals-induced pump dysfunction is obscure, there are speculations that ROS may attack membrane lipids anchoring the enzyme resulting in altered membrane viscosity, which eventually destroys enzyme's function [60,61]. In addition, ROS have been reported to cause loss of activity of the transmembrane enzyme by attacking and altering the amino acid residues of the enzyme [62], while some works suggested that the enzyme's loss of activity may result from the attack of the aldehydic products of lipid peroxidation [63,64].

Ideally, our model herein probably studied the possible link between oxidative stress and the transmembrane enzyme's transport function. In the mechanistic point of view, it could be that the prooxidants disorganized the arrangement of lipid bilayer of the brain cell membrane which consequently led to a concomitant alteration in the integrity of the membrane as well as its

fluidity. As one would expect, these cascade of events may lead to a drastic shut down of the membrane bound protein activity. It is therefore rational to suggest that •OH radical generated from Fenton reactions which assaulted and peroxidized membrane lipids or the aldehydic product of lipid peroxidation ultimately disrupted the transmembrane electrogenic pump structure and consequently caused loss of its activity.

GCMS analysis involves identification of different compounds in a test sample using a hyphenated analytical method that couples the separation properties of gas-liquid chromatography with mass spectrometry detection [65]. The results of GCMS analysis of ethanolic extract of *Danielliaoliveri* revealed the presence of 10 compounds in the leaves including 2-Methyl-Z, Z-3,13-octadecadienal, Desulphosinigrin, Dimethyl-cyano-phosphine, (Methylthio)-acetonitrile, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester and 4H-1,2,4-triazole-3,5-diamine (table 1), while 21 compounds were found in the stem bark including hexadecanoic acid, methyl ester, 9,17-Octadecadienal, trans-13-Octadecenoic acid, 9,12-Octadecadienoic acid (Z,Z), trans-1-Butyl-2-methylcyclopropane, 4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, cis-9-Tetradecenoic acid, heptyl ester, Benzo[h]quinolone, 2-Ethylacridine, 1H-Indole-2-carboxylic acid and 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester (table 2). Compound such as 1,2-Benzisothiazol-3-amine, TBDMS Derivative and Hexadecanoic acid, methyl ester were present in both extracts. Some of these chemicals especially the acids have been demonstrated to be bioactive [66,67]. There are reports that phenols [68] and carboxylic acids [69] are responsible for the antimicrobial activity in medicinal plants.

Hexadecanoic acid (palmitic acid) is a fatty acid and it has been suggested as an active antimicrobial and antidiarrhoeal agent [70]. It was also documented by Yakubu *et al.* [71] that Hexadecanoic acid is an anti-inflammatory agent and antioxidant. It has been suggested that 9,12-Octadecadienoic acid oleic acid prevent the development of adreno-leuko dystrophy (ALD), a disease that damages the brain and the adrenal glands. Additionally, oleic acid performs some regulatory functions in body in biochemical processes such as blood-pressure, immune response and blood-clotting as well as possibly increasing the level of high-density lipoprotein-cholesterol (HDL) and decreasing low-density lipoprotein-cholesterol (LDL) [72].

Alkaloids such as 2-Ethylacridine are widely used in medicine as antimicrobial, antioxidant, antiseptic and cancer-preventive [73]. Hexadecanoic acid plays an essential role in the anti-inflammation process which by helping to design a specific inhibitor of phospholipase A [74]. 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester antidiabetic through insulin sensitizing and glucose lowering effects [75]. In addition, most of the indole derivatives act as an activators of glycogen synthase enzyme, which is involved in the glycogen synthesis pathway [76].

Also, indole derivatives have been reported to possess Indole derivatives possess various biological activities, i.e., antiviral [77], anti-inflammatory and analgesic [78], anticancer [79], anti-HIV [80], antioxidant [81], antimicrobial [82], antitubercular [83], antidiabetic, antimalarial [84], anticholinesterase activities [85], etc. which created interest among researchers to synthesize a variety of indole derivatives.

Benzisothiazoles are antimicrobial, anticancer, anthelmintic and antidiabetic [86]. trans-13-Octadecenoic acid, methyl ester has been reported to be antimicrobial, hepatoprotective, antihistaminic, hypocholesterolemic and antieczemic [86]. 9,12-Octadecadienoic acid (Z, Z) is anti-inflammatory, hypocholesterolemic, anticancer, hepatoprotective, nematocide, insectifuge, antihistaminic, anemia-genic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary and insectifuge [86]. 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene is an antibacterial agent [87], 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester is said to be antidiabetic [75,76] and benzo[h]quinolones possesses antibacterial activity [88] and induces anti-cancer activity by oxidative stress-mediated DNA damage [89].

Considering the numerous biological properties attributed to these compounds, it is worth mentioning that the observed pharmacological activities of this plant are linked to the revealed bioactive compounds present and the higher potency of the stem bark is justified by the presence of more bioactive constituents in found in it.

## 5.0 CONCLUSION

Herein, *Danielliaoliveri* leaves and stem-bark ethanolic extract exhibited a strong antioxidant capacity in rat brain and liver homogenates treated with  $\text{H}_2\text{O}_2$  or  $\text{Fe}^{2+}$  or  $\text{H}_2\text{O}_2/\text{Fe}^{2+}$  (Fenton reaction). The extracts also resuscitated the activity of the prooxidants' assaulted  $\text{Na}^+/\text{K}^+$  ATPase as well as thiol level in the tissues. However, the stem bark elicited higher potency. This might be due to the presence of the bioactive compounds revealed by GC-MS analysis which also buttress the report that the plant can be a renewable source of anti-oxidants for commercial and medicinal uses as well as effective drug in the management of chronic diseases with etiology in oxidative stress.

## 6.0 RECOMENDATION

Considering the fact that compounds with antioxidant activity are able to impede the action of free radicals that play a role in the pathogenesis of diseases such as cancer, atherosclerosis, liver disorders and Alzheimer disease, *Danielliaoliveri* leaves and stem-bark can be used for treatment and management of diseases associated to oxidative stress, neurodegeneration consequent to the detected bioactive compounds. More research need to be carried out on leaves and stem-bark of *Danielliaoliveri* towards the development of novel drugs with little or no side effect.

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