Pak. j. sci. ind. res. Ser. B: biol. sci. 2024 **67B**(1) 30-41

Studies on Antioxidant and Hepatoprotective Activities of Extracts and Active Fraction of *Pachypodanthium staudtii* (Annonaceae) Stem Bark

Ezinne Chinecherem Okorafor[a], Michel Tchimene Kenne[b], Bonaventure Chinonso Obi[a]*, Wilfred Ofem Obonga[c], Theophine Chinwuba Akunne[a] and Adaobi Chioma Ezike[a]

[a]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria

[b]Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria

[c]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Enugu State, Nigeria

(received December 14, 2020; revised June 19, 2022; accepted July 18, 2022)

Abstract. This study evaluates the antioxidant and hepatoprotective activities of the stem bark extracts (methanol, PSM and dichloromethane, PSD) and active fraction of *Pachypodanthium staudtii. In-vitro* antioxidant activity was determined by DPPH, total phenolic content (TPC) and reducing power assay (RPA), while the hepatoprotective activity was verified against CCl_4-induced acute hepatotoxicity in rats. Serum biomarkers (ALT, AST, ALP), *in-vivo* antioxidant (superoxide dismutase, SOD and catalase, CAT) and malondialdehyde (MDA) levels were estimated using standard models. Histopathological studies were carried out on the liver isolates. The result showed that PSM significantly inhibited DPPH (IC_{50}=63.70 µg/mL) compared to PSD (IC_{50}=1591.58 µg/mL). PSM showed a higher phenolic content of 2536.26 mgGAE/g compared to 1916.78 mgGAE/g for PSD. Fraction A showed a phenolic content of 553.01 mgGAE/g. Significant ($P<0.05$) decrease in hepatic biomarkers (ALT, AST, ALP) was observed at varying degrees in PSM and PSD treated groups compared to CCl_4-control group. Fraction A showed significant ($P<0.05$) effect on hepatic biomarkers (ALT, AST, ALP), *in vivo* antioxidant (SOD, CAT) and MDA levels compared to the CCl_4-control group. Histopathology studies revealed restoration of liver architecture and healing from CCl_4 intoxication in the liver.

Keywords: *P. staudtii*, methanol extract, dichloromethane, antioxidant, hepatoprotective

Introduction

The liver is the largest internal organ in the body that plays a pivotal role in metabolizing most chemicals and xenobiotics. Hepatotoxicity is the liver dysfunction or damage accrued from overload of drugs or xenobiotics (Navarro and Senior, 2006). Certain medications, however, might induce liver damage even when introduced within the therapeutic limits. Hepatotoxicity may result from direct toxicity of the primary compound and a reactive metabolite or an immunologically mediated response affecting hepatocytes, biliary epithelial cells and liver vasculature (Deng *et al*., 2009; Saukkonen *et al*., 2006). It occurs through different mechanisms that may directly affect cell organelles such as mitochondria, endoplasmic reticulum, cytoskeleton, microtubules and nucleus or indirect effects through the activation and inhibition of signaling kinases, transcription factors and gene-expression profiles. The resultant intracellular stress may lead to cell death caused by either cell shrinkage (apoptosis) or swelling and lysis (necrosis) (Singh *et al*., 2011). Higher levels of some biochemical parameters such as amino-transferases (Nathwani *et al*., 2005) alkaline phosphatase (Ramaiah, 2007), total bilirubin (Dufour *et al*., 2001) and albumin (Thapa and Waila, 2007) are specifically associated with liver injury.

Oxidative stress is a redox dis-equilibrium in which the pro-oxidant/antioxidant balance shifts in favour of the pro-oxidants (Sies, 1986). A phenomenon linked to the aerobic nature of cellular metabolism in which oxygen reduction is a major event (Videla, 2009). As this process occurs, energy in the form of adenosine triphosphate (ATP) is generated in the mitochondria and by products of the cellular redox process are also generated in the

*Author for correspondence;
E-mail: bonaventure.obi@unn.edu.ng; mtchimene@yahoo.fr

form of free radicals called reactive oxygen species (ROS) and reactive nitrogen species (RNS). These can potentially damage cell function and structures (Sen *et al*., 2010; Pham-Huy *et al*., 2008). Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Antioxidant defense against oxidative stress promotes the direct extraction of pro-oxidants from the body (Panda, 2012).

Pachypodanthium staudtii is widely found in west and central Africa. In folk medicine, it is commonly used to treat gout, dropsy, oedema, worm infestations, pulmonary ailments and swellings (Burkill, 1985). The bark is used as a chest medicine for tumours (Irvine, 1961), toothache (Kerharo and Bouquet, 1950), bronchitis (Bouquet and Debray, 1974), edemas and cancer (Ngadjui *et al*., 1989). To reduce pain and inflammation, the bark is made into a paste with water and clay, mixed with leaves of *Ficus exasperata* (Moraceae) and applied topically (Sarpong *et al*., 2016). Several secondary metabolites such as flavonoids (Cavé *et al*., 1973), 2,4,5-trimethoxystyrene (Bévalot *et al*., 1976a), alkaloids (Bévalot *et al*., 1977b), lignans and bisnorlignans (Mathouet *et al*., 2007) have been reported in literature. Koona and Bouda (2006) reported the effect of 2,4,5-trimethoxystyrene on bean preservation against the bean beetle *Acanthoscelides obtectus* Say. The current study assessed the effects of methanol and dichloromethane extracts and the active fraction of *P. staudtii* for free radical scavenging and liver protective properties.

Materials and Methods

Chemicals and solvents. Methanol, ethyl acetate, n-hexane were sourced commercially and of analytical grade from Sigma-Aldrich (St. Louis, Missouri); CCl_4 (Merck Specialities Pvt. Ltd., Mumbai, India); Randox and TECO diagnostics assay kits for alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP) (Randox Laboratories Ltd., County Antrim, United Kingdom). Silymarin was sourced from Sigma-Aldrich (St. Louis, Missouri). All other routine chemicals and reagents were purchased commercially and of analytical grade.

Preparation of extract. *P. staudtii* stem barks were obtained from a forest in Uyo, Akwa Ibom State, Nigeria. The plant was authenticated at International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Enugu State and a voucher specimen (InterCEDD/1584) was deposited at the herbarium. The stem bark (2 Kg) was pulverized and soaked in methanol and dichloromethane for 48 h. The resulting filtrates were concentrated using a rotavapour to obtain a final yield of 200 g of methanol extract of *P. staudtii* (PSM) and 165.72 g of dichloromethane extract of *P. staudtii* (PSD). A quantity (100 g) of the methanol extract (PSM) was fixed on silica gel (60-200 mesh) and subjected to column chromatography using a mixture of ethyl acetate and n-hexane in different ratios. Fifty-eight (58) subfractions of 100 mL each were collected. These subfractions were pooled together based on the TLC analysis to afford fraction A (F1-9), fraction B (F10-18), fraction C (F19-27), fraction D (F28-37), fraction E (F38-47) and fraction F (F48-58).

Test animals. Swiss albino rats (120-150 g) used for the study were procured from the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Nigeria. The animals were maintained in standard laboratory conditions and were allowed free access to food and water *ad libitum*. All animal experiments were conducted in compliance with NIH guide for the care and use of laboratory animals (Pub No: 85-23, 1985) and approved by the Institutional Ethical Committee on the use of laboratory animals.

Acute toxicity study. The study was determined using Lorke's method (1983), with slight modifications. A total of 26 mice were used for the LD_{50} study of the stem bark extracts (PSM and PSD; n=13). The study was carried out in two phases. In the first phase, nine animals for each extract were used. They were grouped into three groups of three animals each and were given oral administrations of 10, 100 and 1000 mg/Kg of the extract. Thereafter, the animals were observed for signs and symptoms of toxicity over 24 h. In the second phase, three animals respectively for each extract were given oral administration of the extract at doses of 1600, 2900 and 5000 mg/Kg. The animals were also observed over 24 h for toxicity and mortality. The remaining animals were used as control.

Phytochemical tests. The phytochemical analysis of the stem bark extracts (PSM and PSD) and fraction A were carried out using standard methods (Trease and Evans, 1986).

In-vitro* antioxidant study.** ***Total phenolic content. Total phenolic content of the stem bark extracts (PSM and PSD) and fractions (A-F) was determined employing Kim *et al.* (2003) method, with slight modifications.

About 1 mL of a 0.5 mg/mL solution of the extracts (PSM and PSD), 1 mL of a 100 µg/mL solution of each fraction (A-F) of PSM and 1 mL of blank were mixed with 0.2 mL of Folin-Ciocalteu phenol reagent, respectively. After 5 min, 1 mL of 7.6% Na_2CO_3 solution was added to the mixture, followed by 2 mL of deionized distilled water. The mixtures (in duplicate) were incubated at 40 °C for 30 min. After which, the absorbance was read at 710 nm and the average of the readings taken. The total phenolic content was determined from extrapolation of the gallic acid calibration curve made by preparing gallic acid solution and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of the extracts and fractions.

DPPH radical scavenging assay. DPPH radical scavenging assay was determined employing Patel *et al.* (2010) method, with slight modifications. A volume of 0.5 mL freshly prepared methanol solution of DPPH (0.6 mmol) was mixed with 0.5 mL of different concentrations of PSM and PSD (1000-100 µg/mL), and 0.5 mL of different concentrations of fractions A-F (100-6.125 µg/mL) (in duplicates) in test tubes and the final volume adjusted to 5 mL with methanol. The absorbance of the mixture was determined at 520 nm by using UV-visible spectrophotometer after incubating in the dark at room temperature for 30 min. This procedure was also repeated for ascorbic acid used as standard. The antioxidant activity was evaluated by comparing the absorbance at the different concentrations with that of the control (0.5 mL of DPPH and 5.5 mL of methanol). Then the free radical scavenging activities of the extracts and fractions were obtained using the equation below:

DPPH scavenging activity=100 {(Ac-As)/Ac}; where Ac=absorbance of control and As=absorbance of sample.

The concentration of each extract that produced 50% inhibition (IC_{50}) of free radical was also deduced from the log concentration-response curve.

Reducing power assay. Reducing power was determined using slight modifications by Athukorala *et al.* (2006). A volume of 0.5 mL of different dilutions (500-15.625 µg/mL) of the extracts (PSM and PSM) and fractions (A-F) and 0.5 mL of distilled water (control) in duplicates were mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Thereafter, 1.25 mL of trichloroacetic acid (TCA) was added to the reaction mixture and centrifuged for 10 min at 5000 rpm and 25 °C. The upper layer of the solution (1.25 mL) was then mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride ($FeCl_3$) and absorbance was measured at 710 nm with the UV spectrophotometer. Ascorbic acid was used as the positive control. The reducing power activity is dependent on the extent of absorbance (higher absorbance indicates higher reducing power).

Hepatoprotective study. The hepatoprotective study was achieved in two stages.

Stage 1. Animals were randomized into eight groups (n=6 each). Group I (naïve control) received the vehicle (distilled water); group II was CCl_4 control group (untreated); groups III-V received graded doses of PSM (100, 200, 400 mg/Kg BW) + CCl_4, respectively; group VI-VIII received graded doses of PSD (100, 200, 400 mg/Kg BW) + CCl_4, respectively. Groups I and II were given food and water *ad libitum* for ten days, whereas groups III –VIII were administered *P. staudtii* extracts (PSM and PSD) once daily (intragastrically) for 10 days. Eight hours after the administration of the last doses, 2 mL/Kg of CCl_4 (1:1 dilution in olive oil) was administered intraperitoneally to the animals in groups II, III-V and VI-VIII, respectively. The same process was repeated with the most active fraction (A) of PSM. Blood samples were collected through ocular puncture with the aid of a non-heparinized capillary tube and centrifuged at 3000 rpm for 10 min to obtain the serum component for hepatic biomarker analysis. Alanine amino-transferase (ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) levels were assayed using kit product of Randox and Teco diagnostics according to standard procedure (Rec, 1972; Reitman and Frankel, 1957).

Stage 2. Fraction A of PSM was subjected to further testing in stage two. Animals were randomized into six groups (n=7 each). Group I (naïve control) received the vehicle (distilled water); group II was CCl_4 control group (untreated); groups III received silymarin (100 mg/Kg, intra-gastric); groups IV-VI received graded doses of Fraction A of PSM (100, 200, 400 mg/Kg, p.o) + CCl_4, respectively, once daily (intragastrically) for ten days. Eight hours after administration of the last doses, hepatotoxicity was induced in animals (groups II-VII) as described above. After 24 h from the CCl_4 intoxication, all animals were anaesthetised with light diethyl ether and blood samples were obtained for serum biomarker analysis. Subsequently, the livers of the

animals were separately weighed and homogenised in Tris HCL buffer (pH 7.4) per gram tissue. Then, the homogenates were centrifuged for 15 min at 4000 rpm and 4 °C and the obtained supernatants were used to estimate antioxidant parameters (CAT, SOD) and malondialdehyde (MDA) levels. Serum and liver superoxide dismutase (SOD) and catalase (CAT) activities were assayed using standard methods (Akinduko *et al*., 2014; Sinha, 1972). Lipid peroxidation was assessed by measuring malondialdehyde (MDA) formation, using the method of Gutteridge and Wilkins (1982), with slight modifications.

Histopathological examination. Histopathology studies were carried out on the liver isolates (sections) from each group of the animals in the study. Serial sections of the tissue stained with haematoxylin and eosin (H&E) were examined under a light microscope (x 400) (Li *et al*., 2018).

Statistical analysis. Data were presented as means ± S.E.M using SPSS (version 23). Statistical significance among groups was determined by ANOVA, followed by the Post Hoc Dunnet's test. P-values=0.05 were considered statistically significant in all cases.

Results and Discussion

The result of the phytochemical analysis of PSM, PSD and the most active fraction (fraction A) are presented in Table 1. The presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, tannins and proteins was observed in the PSM, while only alkaloids, cardiac glycosides and proteins were noted in the PSD. Alkaloids, terpenoids, saponins and proteins were markedly present in fraction A of the PSM. The total phenolic content (TPC) assay revealed that PSM had a higher phenolic content of 2536.26 mgGAE/g compared to 1916.78 mgGAE/g for PSD. The total phenolics varied among the various fractions (A-F), with the highest amounts observed in fraction A (553.01 mgGAE/g) at 100 µg/mL (Fig. 1).

PSM exhibited significant DPPH scavenging activity (IC_{50} of 63.70 µg/mL), suggesting good and better antioxidant activity (represented as percentage inhibition in Fig. 2), unlike PSD, which showed minimal antioxidant activity with an IC_{50} value of 1591.58 µg/mL. Among the PSM fractions, only fraction A, with IC_{50}=96.17 µg/mL (like ascorbic acid, IC_{50}=13.68 µg/mL), demonstrated significant concentration-dependent DPPH radical scavenging ability (Fig. 3).

The reducing power assay result showed a concentration dependent increase in absorbance value with the highest observed in PSM (1.28) and ascorbic acid (1.30) at 500 µg/mL, respectively (Fig. 2). The reducing power was also most significant for fraction A, with a maximal absorbance of 1.12 at 500 µg/mL (Fig. 3).

The results of hepatoprotective activities on CCl_4-induced acute hepatotoxicity are presented in Table 2. The acute toxicity effects on administration of CCl_4 were indicated by the significant rise ($P<0.05$) in ALT, AST and ALP levels compared to pre-treatment levels.

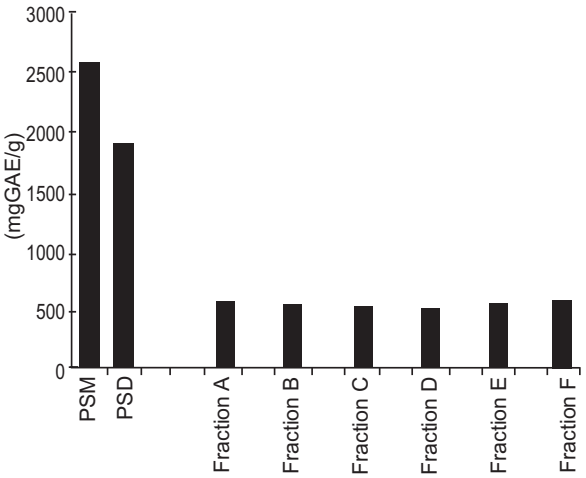

Fig. 1. Total phenolic contents in *Pachypodanthium staudtii* extracts and various fractions.

Table 1. Phytochemical constituents of PSM, PSD and fraction A

Phyto-constituents	PSM	PSD	Fraction A (PSM)
Alkaloids	++	++	++
Saponins	-	-	++
Tannins	+	-	-
Flavonoids	+	-	-
Steroids	-	-	-
Terpenoids	+	-	+
Cardiac glycosides	+	++	-
Protein	-	+	+
Carbohydrate	-	-	-

Key: - = absent; + = present in moderate concentration; ++ =present in high concentration; PSM=methanol extract of *Pachypodanthium staudtii*; PSD=dichloromethane extract of *Pachypodanthium staudtii*.

Table 2. Effect of PSM and PSD on serum biomarkers (ALT, AST, ALP) in CCl_4-induced hepatotoxic rats (means ± SEM)

Treatment	Dose (mg/Kg)	ALT (U/L)		ALT (U/L)		ALT (U/L)	
		pre-treatment	post-treatment	pre-treatment	post-treatment	pre-treatment	post-treatment
Naïve	-	37.50±3.48	22.50±6.25	72.17±1.07	71.30±10.47	96.18±6.79	47.42±9.34
CCl_4-control	-	38.57±2.15	81.40±2.56*	68.70±3.19	156.52±4.12*	88.70±5.26	138.82±3.63*
PSM	100	30.36±4.07	80.78±1.04* (0.76)	93.91±6.24	152.17±3.07* (2.77)	39.86±8.13	107.94±12.10*,# (22.24)
	200	39.64±6.27	79.64±0.91* (2.16)	89.57±10.16	148.70±6.36* (4.99)	55.36±17.62	98.18±10.21*,# (29.27)
	400	48.92±4.54	64.64±1.10*,# (32.87)	71.30±4.26	121.74±9.33*,# (22.22)	44.04±9.75	92.38±3.71*,# (33.45)
PSD	100	40.00±4.54	77.50 ±1.07* (4.79)	79.13±4.22	149.57±2.22* (4.44)	36.92±8.35	120.90±12.74* (12.90)
	200	34.28±5.18	78.57±1.38* (3.47)	60.00±5.22	153.91±4.48* (1.66)	42.84±11.66	108.00±4.80*,# (22.20)
	400	38.21±2.74	73.93±1.84* (9.17)	59.13±4.88	146.09±8.76* (6.66)	52.38±.55	92.09±1.99*,# (33.66)

Values are the means of 6 rats. *, # P<0.05 compared with pre-treatment and CCl_4-control group values, respectively (One-way ANOVA; Dunnett's post hoc). PSM=methanol extract of *P. staudtii*; PSD=dichloromethane extract of *P. staudtii*. Values in parenthesis represent percentage reduction relative to CCl_4-control group.

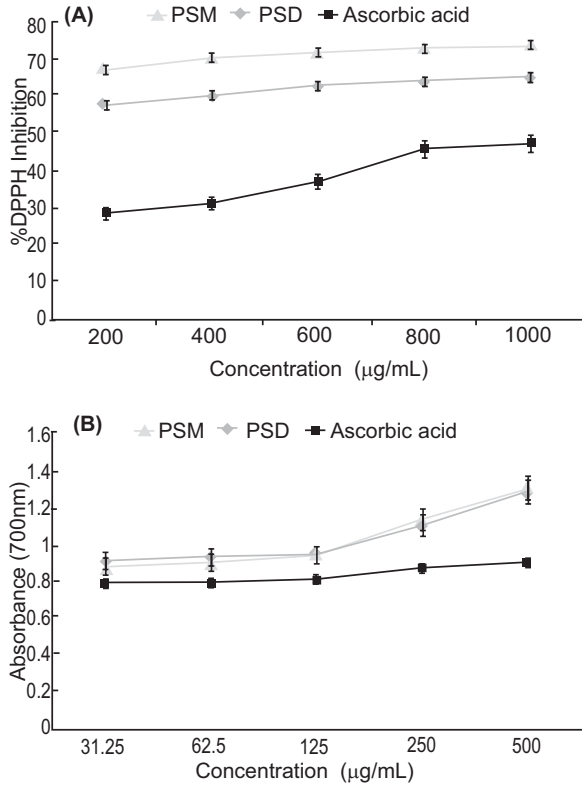

Fig. 2. **(A)** % inhibition of DPPH radical for *Pachypodanthium staudtii* extracts. **(B)** Reducing power capacity for *Pachypodanthium staudtii* extracts.

The hepatoprotective effects were represented as percentage reduction in elevated serum ALT, AST and ALP levels compared to CCl_4-control values as shown in Table 2. PSM administration produced better dose-dependent hepatoprotective effects than PSD. Pre-treatment with a high dose (400 mg/Kg) of PSM significantly (P<0.05) decreased the level of elevated ALT and AST compared to the CCl_4-control group. No significant difference (P>0.05) in ALT and AST levels was observed in the PSD-treated group. However, a significant decrease in ALP was observed in PSM (at all doses) and PSD (200 and 400 mg/Kg) pretreated animals compared to the CCl_4- control group. Conversely, fraction A and silymarin (100 mg/Kg) expressed an ability to counteract hepatotoxicity by decreasing elevated levels of the marker enzymes. Pre-treatment with fraction A caused significant (P<0.05) and dose dependent decrease in ALT and ALP (at all doses) and AST (200 and 400 mg/Kg) compared to CCl_4-control group (Table 3).

Relative to the CCl_4- control group, SOD and CAT levels in the liver and serum decreased significantly compared with the naïve control group. Pre-treatment with fraction A significantly (P<0.05) increased hepatic and serum SOD levels, with the 400 mg/Kg dose exhibiting the highest percentage change in SOD levels. The CAT levels in the liver and serum were significantly

(P<0.05) increased on pre-treatment with fraction A except at 100 mg/Kg dose with no significant difference (P>0.05) in serum CAT level compared to the CCl_4-control group. Pre-treatment with fraction A (100, 200 and 400 mg/Kg) significantly (P<0.05) and in a dose dependent manner lowered the elevated MDA content level when compared to the CCl_4- control group. The standard drug (silymarin, 100 mg/Kg) also significantly (P<0.05) restored hepatic and serum SOD and CAT levels and decreased elevated MDA content compared to the CCl_4- control group (Table 4,5).

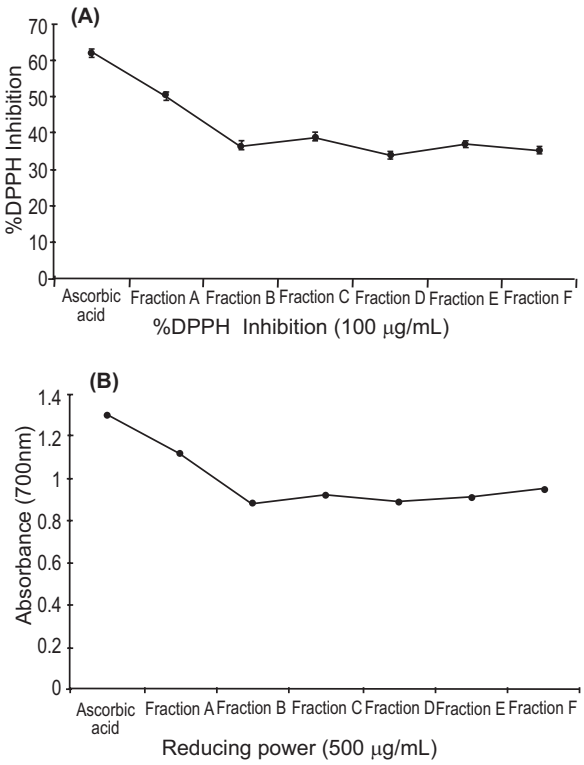

Fig. 3. **(A)** % inhibition of DPPH radical for methanol extract of *Pachypodanthium staudtii* (PSM) fractions. **(B)** Reducing power capacity for PSM fractions.

The liver sections of the CCl_4- control group showed hepatic cells with severe toxicity characterized by congestion, marked necrosis, inflammatory infiltration, fatty changes and vacuole degeneration. Pre-treatment with fraction A (400 mg/Kg) and silymarin (100 mg/Kg) showed significant liver protection against CCl_4-induced hepatic cell damage evident as near restoration to normal liver structure, characterized by mild necrosis and degeneration and reduction of inflammatory infiltration. This corresponded with the levels of the enzyme markers and antioxidants (Fig 4. 1a-f).

Potential antioxidant plants express their activity through various mechanisms such as free radical scavenging, prevention of continued hydrogen abstraction, termination of radical chain initiation (and propagation), binding of transition metal ion catalysts, peroxide decomposition and reduction process (Gulein *et al*., 2002). Another possible mechanism of antioxidant activity of plant materials could be their ability to donate hydrogen and scavenge free radicals, which may be related to the presence of phyto-constituents (Anirban *et al*., 2012). In the present study, chromatographic separation of the methanol extract of *P. staudtii* yielded five solvent fractions that were subjected to pharmacological screening.

The antioxidant capacity of plant extracts is directly related to the total phenolic content (TPC) of plant material (Liu *et al*., 2008). Generally, phenolics are powerful antioxidants. The antioxidative properties of polyphenols stem from their ability to donate hydrogen or oxygen atom, free radical chain-breaking function and their capacity to chelate metal ions (Kandhasamy

Table 3. Effect of fraction A on serum biomarkers (ALT, AST, ALP) in CCl_4-induced hepatotoxic rats (means±SEM)

Treatment	Dose (mg/Kg)	ALT (U/L)	AST (U/L)	ALP (U/L)
Naïve	-	24.76±1.63	25.70±1.28	49.08±12.16
CCl_4-control	-	150.52±10.59*	128.70±11.71*	116.85±10.58*
Silymarin	100	86.73±14.45$^{*,\#}$(42.37)	95.22±6.77$^{*,\#}$(26.01)	30.74±4.06$^{\#}$ (73.69)
Fraction A	100	93.00±8.10$^{*,\#}$(38.21)	122.10±17.47*(5.12)	63.45±7.82$^{*,\#}$ (45.69)
Fraction A	200	86.78±9.81$^{*,\#}$ (42.34)	94.80±17.00$^{*,\#}$(26.34)	55.92±4.15$^{\#}$ (52.14)
Fraction A	400	78.66±5.15$^{*,\#}$ (47.74)	82.00±7.59$^{*,\#}$(36.28)	44.39±7.36$^{\#}$ (62.01)

Values are the means of 7 mice= $^{*,\#}$ P<0.05 compared with naïve group and CCl_4-control group values (One-way ANOVA; Dunnett's post hoc). PSM=methanol extract of *P. staudtii*; PSD=dichloromethane extract of *P. staudtii*. Values in parenthesis represent percentage reduction relative to CCl_4-control group.

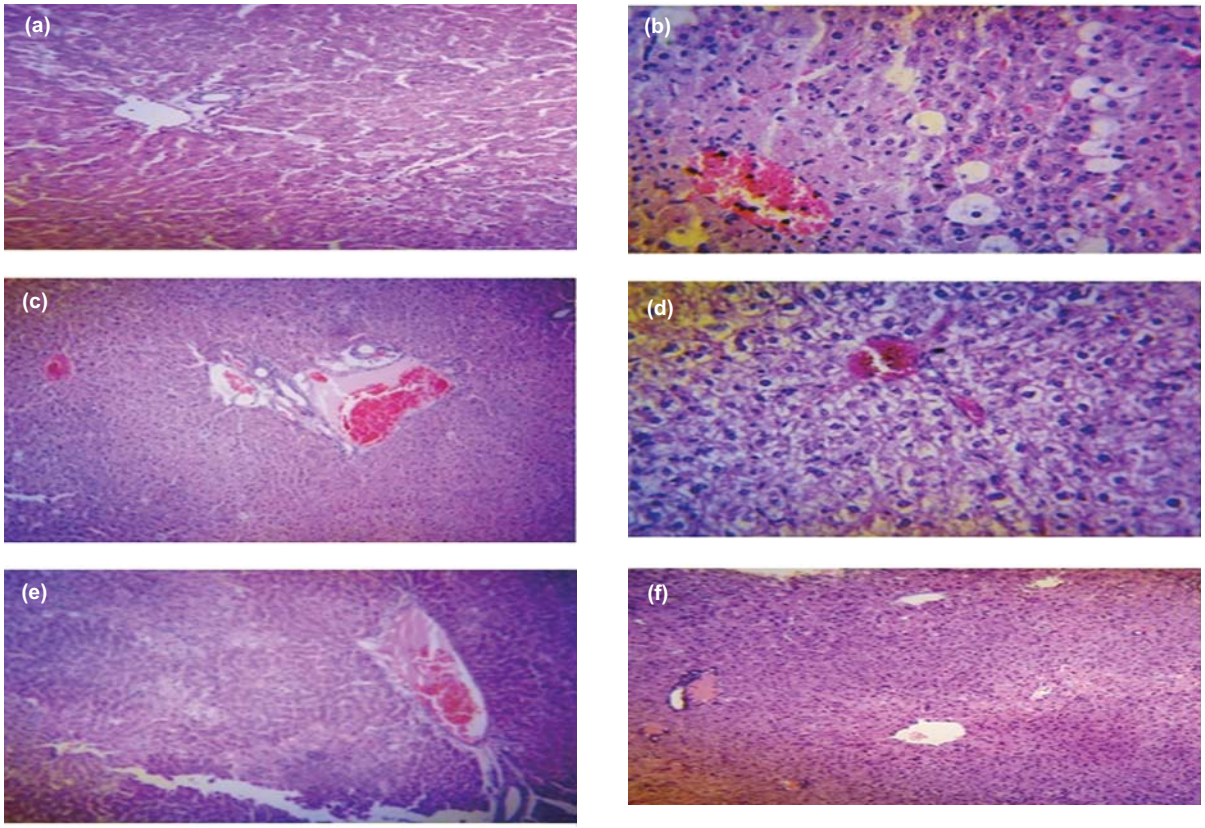

Fig. 4. Photomicrographs (H & E stained) of liver isolates (sections) **a**=naïve group showing normal liver cells and central vein architecture; **b**=CCl_4-control group (untreated) showing marked necrosis, fatty changes, inflammatory infiltrations, vacuolar degenerations and congestion; **c**=Silymarin (100 mg/Kg) pre-treatment showing preserved liver architecture with less fatty changes and mild/patchy necrosis and congestion; **d**=fraction A (100 mg/Kg) pre-treatment shows mild necrosis, inflammatory infiltration, vacuolar degeneration and congestion; **e**=fraction A (200 mg/Kg) pre-treatment showing few inflammatory cells and necrosis; **f**=fraction A (400 mg/Kg) pre-treatment showing preservation of liver architecture and a pattern of less necrosis.

Table 4. Effect of fraction A on *in vivo* antioxidant status in CCl_4-induced hepatotoxic rats (means±SEM)

Treatment	Dose (mg/Kg)	CAT		SOD	
		Liver (µmol)	Serum (U/mL)	Liver (µmol)	Serum (U/mL)
Naïve	-	23.31±4.24	2.60±0.44	14.75±0.92	14.40±1.93
CCl_4-control	-	2.18±1.06*	0.36±0.16*	3.66±0.97*	9.17±1.46*
Silymarin	100	18.19±2.89$^{\#}$ (-73.44)	2.79±0.77$^{\#}$ (-≥200)	10.88±0.68$^{\#}$ (-197.26)	12.94±0.74$^{\#}$ (-41.11)
Fraction A	100	10.75±3.91$^{*,\#}$ (-39.31)	0.98±0.17$^{*,\#}$ (-172.22)	7.35±0.94$^{*,\#}$ (-100.81)	10.59±0.74 (-15.48)
Fraction A	200	17.48±7.71$^{\#}$ (-70.18)	1.05±0.43$^{*,\#}$ (-191.66)	9.17±1.20$^{*,\#}$ (-150.54)	11.29±1.32$^{\#}$ (-23.11)
Fraction A	400	21.77±2.70$^{\#}$ (-89.86)	2.55±0.67$^{\#}$ (-=200)	12.19±0.22$^{\#}$ (-=200)	13.23±0.23$^{\#}$ (-44.27)

Values are the means of 7 mice=$^{*,\#}$ P<0.05 compared with naïve group and CCl_4-control group values (one-way ANOVA; Dunnett's post hoc). PSM=methanol extract of *P. staudtii*; PSD=dichloromethane extract of *P. staudtii*. Values in parenthesis represent percentage increase (-) relative to CCl_4-control group.

Table 5. Effect of fraction A on MDA levels in CCl_4-induced hepatotoxic rats (means±SEM)

Treatment	Dose (mg/Kg)	MDA
Naïve	-	1.48±0.04
CCl_4-control	-	3.59±0.13*
Silymarin	100	1.79±0.01$^{\#}$ (50.13)
Fraction A	100	2.11±0.03$^{*,\#}$ (41.22)
Fraction A	200	1.94±0.11$^{\#}$ (45.96)
Fraction A	400	1.68±0.00$^{\#}$ (53.20)

Values are the means of 7 mice=$^{*,\#}$ P<0.05 compared with naïve group and CCl_4-control group values (One-way ANOVA; Dunnett's post hoc). PSM=methanol extract of *P. staudtii*; PSD=dichloromethane extract of *P. staudtii*. Values in parenthesis represent percentage reduction relative to CCl_4-control group.

and Sun, 2013). Matthew and Abraham (2006) attributed the inhibition of lipid peroxidation by several chemical mechanisms (such as free radical quenching, electron transfer, radical addition and radical recombination) to high amounts of phenolic compounds and other chemical components in an extract. Thus, a TPC-rich fraction could exhibit both antioxidant and hepatoprotective activities.

The DPPH assay is a widely accepted model for assessing the radical scavenging activity of antioxidant compounds. The antioxidant's ability to donate hydrogen to the DPPH radical may account for its radical scavenging property (Moulisha *et al*., 2010). In this study, the methanol and dichloromethane extracts of *P. staudtii* (PSM and PSD) exhibited a concentration dependent DPPH radical scavenging activity, suggesting an excellent antioxidant activity comparable to ascorbic acid. However, PSM was the most efficient, with an IC_{50} of 63.70 µg/mL, compared to PSD, which had limited antioxidant activity with an IC_{50} of 1591.58 µg/mL. This implies that PSM is an effective free radical scavenger and a primary antioxidant that may alleviate the deleterious actions of free radicals in the body. This could be attributed to its high flavonoid and terpenoid content. The same cannot be surmised for PSD as it had very little free radical scavenging ability. The various fractions (A-F) of PSM exhibited scavenging capacity of DPPH above 30%, with the highest percentage inhibition (IC_{50}=96.17 µg/mL) observed in fraction A at 100 µg/mL. This may be due to the ability to donate hydrogen in the system, making a non-radical DPPH-H, which inhibits lipid peroxidation (Rekka and Kourounakis, 1991; Blois, 1958).

The reducing power assay X-ray the ability of *P. staudtii* extracts and fractions to convert Fe^{3+} to Fe^{2+}, which indicates its antioxidant capacity (Diplock, 1997). The high absorbance value exhibited by PSM, indicated higher reducing capability (Padmanabban and Jangle, 2012). Similarly, fraction A had a higher absorbance value than the crude extracts, indicating a substantial reductive activity. The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown to exert antioxidant activity by chain-breaking function and high reactivity as hydrogen atoms (Mbaoji *et al*., 2020).

Based on the *in vitro* findings, the extracts and fraction A were investigated further *in vivo* to establish the protective impact against reactive oxygen species (ROS) and oxidative stress. Animals subjected to carbon tetrachloride (CCl_4) developed significant hepatocellular damage as evident from the increase in serum alanine amino-transferase (ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) levels compared to pre-treatment levels. CCl_4 is a well-known hepatotoxicant employed in hepatotoxicity studies (Ebaid *et al*., 2021). CCl_4-induced toxicity may result from covalent interactions with critical target molecules such as DNA, lipids, protein or carbohydrates and changes in the endoplasmic reticulum, resulting in the loss of metabolic enzymes in the intracellular structures. The toxic metabolite, $CC1_3$ radical, is produced, which further reacts with oxygen to give trichloromethyl peroxyl radical. This radical binds covalently to the unsaturated lipid membrane causing marked elevation of lipid peroxides that gives rise to a sequel of other pathological events, including elevated serum hepatic enzymes, depletion of glutathione, decreased protein synthesis, triacylglycerol accumulation, increased lipid peroxidation, destruction of calcium homeostasis and hepatocyte damage (Weber *et al*., 2003). Extracts of *P. staudtii* and fraction A exhibited varying degrees of hepatoprotective effects in elevated serum ALT, AST and ALP levels compared to CCl_4- control group, with PSM demonstrating a better dose dependent activity than PSD. However, the percentage reduction in fraction A was more prominent, hence a better dose dependent hepatoprotective effect than PSD and comparable to PSM. The attenuation in elevated AST and ALT levels by an extract (or fraction) is associated with plasma membrane stabilization which promotes the repair of hepatic tissue damage caused by CCl_4 (Panda *et al*., 2009). This is consistent with the accepted view that

serum transaminase levels return to normal as the hepatic parenchyma heals and hepatocytes regenerate (Deshraj and Chandrashekhar, 2012).

The most sensitive antioxidant enzymatic indices in liver injury caused by reactive oxygen species (ROS) and oxidative stress are serum activities of superoxide dismutase (SOD) and catalase (CAT). The reduction observed in the activities of SOD and CAT in CCl_4-control group substantiates that ROS production is prominent. The heightened release of ROS is the first step in a series of events leading to membraned cell organelle lipid peroxidation, resulting in apoptosis and necrosis (Ebaid *et al*., 2013). Pre-treatment with fraction A led to an increase in both hepatic and serum SOD and CAT levels on induction of hepatotoxicity with CCl_4. This suggests an ability to ameliorate the deleterious oxidative effects of ROS, hence contributing to the protective effect on hepatocytes. Previous report also indicates that an increase in liver cell lipid peroxidation is the destructive process implied in liver injury in CCl_4-induced hepatotoxicity (Muriel, 1997). Thus, elevated levels of MDA content (a lipid peroxidative substance) indicate tissue damage and the production of free radicals as the balance between the radicals and antioxidants was disrupted in favour of the radicals. Fraction A significantly reduced MDA levels on pre-administration and restored this balance. Silymarin, a hepatoprotective compound isolated from *Silybum marianum* (milk thistle) plant, was reported to exhibit cell regenerating functions, antioxidant activities and a protective effect on plasma membrane of hepatocytes against different hepatotoxic agents (Surai *et al*., 2015). In this context, the hepatoprotective activity of fraction A of PSM might be similar to the protective effect on the plasma membrane of the hepatocytes or to cell regenerating function associated with the actions of silymarin.

Liver injury is associated with inflammation, which is generally believed to accelerate the progression of liver diseases (Horiguchi *et al*., 2010). The lipid peroxidation and damaged hepatocytes caused by the trichloromethyl radicals and by-products when CYP2E1 metabolizes CCl_4 generate free radicals, thereby activating Kupffer cells/macrophages to produce pro-inflammatory and anti-inflammatory cytokines that control the progression of liver inflammation and injury (Horiguchi *et al*., 2010; Basu, 2003). Since fraction A was proven to exhibit radical scavenging activities from the *in vitro* antioxidant model suggesting hepatoprotection by alleviation of oxidative stress effects and lipid peroxidation sequelae as surmised from the premise above, then anti-inflammatory activity could likewise be suggested as another possible mechanism through which fraction A expresses its hepato-protective action as it also reduced inflammatory infiltrations. This notion is further based on a previous report that the ethanol stem bark extract of *P. staudtii* demonstrated anti-inflammatory properties (Sarpong *et al*., 2016).

Furthermore, the antioxidant, free radical scavenging, and lipid peroxidation inhibition abilities of several phyto-constituents from plants have been reported in previous studies (Salehi *et al*., 2020; Soon *et al*., 2013). Flavonoids and terpenoids are well documented for their hepatoprotective effects, particularly through antioxidant and free radical scavenging pathways (Maheshwari *et al*., 2011). These were not found in the PSD. However, alkaloids in both PSM and PSD also accounted for the antioxidant activity (Wang *et al*., 2020).

The LD_{50} study revealed no obvious toxicity or mortality for extracts of *Pachypodanthium staudtii* stem bark at the highest dose (5 g/Kg BW) after 48 h of administration and hence may indicate safety on oral administration.

Conclusion

Findings from this study revealed that *Pachypodanthium staudtii* stem bark exhibited significant antioxidant activities through free radical scavenging, high amounts of phenolic compounds and high reducing power ability. Also, protection against CCl_4-induced hepatotoxicity in mice may be attributed to its antioxidant effects. This study justifies the folkloric use of *P. staudtii* (stem bark) in treating pain, inflammation, swellings and oedema. However, further studies are ongoing to identify the active principles responsible for these activities, as well as the mechanisms therein.

Conflict of Interest. The authors declare that they have no conflict of interest.

References

Akinduko, D.S., Olatosin, T.M., Uche, C.Z. 2014. Antioxidant capacity of *Moringa oleifera* seed oil against CCl_4 induced hepatocellular lipid peroxidation in Wistar albino rats. *European Journal of Experimental Biology*, **4:** 514-518.

Anirban, B., Satadru, B., Soumitra, D.M., Monojit, D.,

Moulisha, B. 2012. The antioxidant effects of *Eupatorium triplinerve, Hygrophila triflora* and *Pterocarpus marsupium*-a comparative study. *European Journal of Applied Sciences*, **4:** 136-139.

Athukorala, Y., Kim, K.N., Jeon, Y.J. 2006. Anti-proliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Eckloniacava*. *Food and Chemical Toxicology*, **44:** 1065-1074.

Basu, S. 2003. Carbon tetrachloride induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. *Toxicology*, **189:** 113-127.

Bévalot, F., Leboeuf, M., Bouquet, A., Cavé, A. 1977a. Alcaloïdes des Annonacées: Alcaloïdes des écorces de tiges et de racines de *Pachypodanthium confine* Engl. et Diels. *Annales Pharmaceutiques Françaises*, **35:** 65-72.

Bévalot, F., Leboeuf, M., Bouquet, A., Cavé, A. 1976b. Isolement du trimethoxy-2,4,5-styrène à partir du *Pachypodanthium confine* Engl. et Diels. Annonacées. *Plantes Médicinales Phytothérapie*, **10:** 179-181.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, **181:** 1199-1150.

Bouquet, A., Debray, M. 1974. *Plantesmédicinales de Côte d'Ivoire*, 232 pp., Mission O.R.S.T.O.M 32 ème Ed. Paris, France.

Burkill, H.M., 1985. The Useful Plants of West Tropical Africa, **vol. 1,** 2nd edition, Royal Botanic Gardens, Kew, UK. https://plants.jstor.org/compilation/pachypodanthium.staudtii.

Cavé, A., Bouquet, A., Paris, R.R. 1973. Sur le pachypodol (dihydroxy 5,40-trimethoxy-3,7,3 0-flavone ou quercetol-3,7,3 0-trimethyléther), nouveau flavonoide isolé du *Pachypodanthium confine* Engler et Diels (Annonacées). *Comptes Rendus de l'*Académie *des Sciences*, **276:** 1899-1901.

Deng, X., Luyendyk, J.P., Ganey, P.E., Roth, R.A. 2009. Inflammatory stress and idiosyncratic hepatotoxicity: hints from animal models. *Pharmacological Reviews*, **61:** 262-282.

Deshraj, S.C., Chandrashekhar, D.U. 2012. Hepatoprotective and antioxidant activity of *Thespesia lampas* (Cav.) Dalz and Gibs. *Phytopharmacology*, **2:** 114-122.

Diplock, A.T. 1997. Will the "good fairies" please prove to us that vitamin E lessens human degenerative disease? *Free Radical Research*, **27:** 511-532.

Dufour, D.R., Lott, J.A., Nolte, F.S., Gretch, D.R., Koff, R.S., Seeff, L.B. 2001. Diagnosis and monitoring of hepatic injury. II. recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemistry*, **47:** 1133-1135.

Ebaid, H., Al-Tamimi, J., Hassan, I., Habila, M.A., Rady, A.M., Alhazza, I.M., Ahmed, A.M. 2021. Effect of selenium nanoparticles on carbon tetrachloride- induced hepatotoxicity in the Swiss albino rats. *Applied Sciences*, **11:** 3044.

Ebaid, H., Bashandy, S.A., Alhazza, I.M., Rady, A., El-Shehry, S. 2013. Folic acid and melatonin ameliorate carbon tetrachloride- induced hepatic injury, oxidative stress and inflammation in rats. *Nutrition and Metabolism*, **10:** 20.

Gülein, I., Oktay, M., Küfrevioglu, O.I., Aslan, A. 2002. Determination of antioxidant activity of lichen *Cetraria islandica* (L). *Journal of Ethnopharmacology*, **79:** 325-329.

Gutteridge, J.M., Wilkins, S. 1982. Copper dependent hydroxyl radical damage to ascorbic acid; the formation of thiobarbituric acid reactive products. *FEBS Letters*, **137:** 327-330.

Horiguchi, N., Lafdil, F., Miller, A.M., Park, O., Wang, H., Rajesh, M., Mukhopadhyay, P., Fu, X.Y., Pacher, P., Gao, B. 2010. Dissociation between liver inflammation and hepatocellular damage induced by carbon tetrachloride in myeloid cell specific signal transducer and activator of transcription 3 gene knockout mice. *Hepatology*, **51:** 1724-1734.

Irvine, F.R. 1961. *Woody Plants of Ghana*, 6 pp., Oxford University Press, London, UK.

Kandhasamy, S., Sun, C.K. 2013. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight and Arn. *Saudi Journal of Biological Sciences*, **20:** 319-325.

Kerharo, J., Bouquet, A. 1950. *Plantes Médicinales et Toxiques de la Côte d'Ivoire Haute Volta*. Mission d'étude de la pharmacopée indigèneen A.O.F., 301 pp., Vogot Frères, Paris, France.

Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y., Lee, C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry*, **51:** 6509-6515.

Koona, P., Bouda, H. 2006. Biological activity of *Pachypodanthium staudtii* (Annonaceae) against the bean beetle *Acanthoscelides obtectus* Say (Coleptera: Bruchidae). *Journal of Applied Sciences*

Research, **2:** 1129-1131.

Li, S., Wang, X., Zhang, J., Li, J., Liu, X., Ma, Y., Zheng, L. 2018. Exenatide ameliorates hepatic steatosis and attenuates fat mass and FTO gene expression through PI3K signalling pathway in non-alcoholic fatty liver disease. *Brazilian Journal of Medical and Biological Research*, **51:** 1-10.

Liu, H., Qiu, N., Ding, H., Yao, R. 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research International*, **41:** 363-370.

Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology*, **54:** 272-89.

Maheshwari, D.T., Kumar, M.S.Y., Verma, S.K., Singh, V.K., Singh, S.N. 2011. Antioxidant and hepato-protective activities of phenolic rich fraction of seabuckthorn (*Hippophae rhamnoides* L.) leaves. *Food and Chemical Toxicology*, **49:** 2422-2428.

Mathouet, H., Elomri, A., Lameiras, P., Daïch, A., Vérité, P. 2007. An alkaloid, two conjugate sesquiterpenes and a phenylpropanoid from *Pachypodanthium confine* Engl. and Diels. *Phytochemistry*, **68:** 1813-1818.

Matthew, S., Abraham, T.E. 2006. *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology*, **44:** 198-206.

Mbaoji, F.N., Nweze, J.A. 2020. Antioxidant and hepatoprotective potentials of active fractions of *Lannea barteri* Oliv. (Anarcadiaceae) in rats. *Heliyon*, **6:** 1-10.

Moulisha, B., Pallab, K.H., Ashoke, K.G. 2010. Antioxidant and free-radical-scavenging effects of fruits of *Dregea volubilis*. *Journal of Natural Science*, *Biology and Medicine*, **1:** 29-34.

Muriel, P. 1997. Peroxidation of lipids and liver damage. In: *Antioxidants, Oxidants and Free Radicals*, Baskin, S.I., Salem, H. (Eds), pp. 237-252, Taylor and Francis, Washington, DC, USA.

Nathwani, R.A., Pais, S., Reynolds T.B., Kaplowitz, N. 2005. Serum alanine amino-transferase in skeletal muscle diseases. *Hepatology*, **41:** 380-382.

Navarro, V.J., Senior, J.R. 2006. Drug-related hepatotoxicity. *The New England Journal of Medicine*, **354:** 731-739.

Ngadjui, B.T., Lontsi, D., Ayafor, J.F., Sondengam, B.L. 1989. Pachypophyllin and pachypostaudins A and B: three bisnorlignans from *Pachypodanthium staudtii*. *Phytochemistry*, **28:** 231-234.

Padmanabhan, P., Jangle, S.P. 2012. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *International Journal of Pharmaceutical Sciences and Drug Research*, **4:** 143-146.

Panda, B.N., Gaur, K., Nema, R.K., Sharma, C.S., Abhishek, K.J., Jain, C.P. 2009. Hepatoprotective activity of *Jatropha gossypifolia* against carbon tetrachloride induced hepatic injury in rats. *Asian Journal of Pharmaceutical and Clinical Research*, **2:** 50-54.

Panda, K.S. 2012. Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. In: *Antioxidant Enzyme*, El-Missiry, M.A. (eds), pp. 381, IntechOpen, Croatia.

Patel, A., Patel, A., Patel, A., Patel, N. 2010. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). *Pharmacognosy Research*, **2:** 152-158.

Pham-Huy, L.A., He, H., Pham-Huy, C. 2008. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, **4:** 89-96.

Pin-Der-Duh, X. 1998. Antioxidant activity of burdock (*Arctiumlappa* Linne): its scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemists Society*, **75:** 455-461.

Ramaiah, S.K. 2007. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, **45:** 1551-1557.

Rec, G.S.C.C. 1972. Determination of alkaline phosphatase. *Journal of Clinical Chemistry and Clinical Biochemistry*, **10:** 281-91.

Reitman, S., Frankel, S.A. 1957. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Pathology*, **28:** 56-63.

Rekka, E., Kourounakis, P.N. 1991. Effect of hydroxyethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity-some structural aspects. *Journal of Pharmacy and Pharmacology*, **43:** 486-491.

Salehi, B., Azzini, E., Zucca, P., Varoni, E.M., Kumar, N.V.A., Dini, L.D., Panzarini, E., Rajkovic, J., Fokou, P.V.T., Peluso, I., Mishra, A.P., Nigam, M., El Rayess, Y., Beyrouthy, M.E., Setzer, W.N.,

Polito, L., Iriti, M., Sureda, A., Quetglas-Llabrés, M.M., Martorell, M., Nartins, N., Sharifi-Rad, M., Estevinho, L.M. 2020. Plant-derived bioactives and oxidative stress-related disorders: a key trend towards healthy aging and longevity promotion. *Applied Sciences*, **10:** 947.

Sarpong, F.M., Amponsah, I.K., Armah, F.A., Yakubu, J., Gyamfi, R. 2016. Anti-inflammatory and antioxidant activity of the ethanolic stem bark extract of *Pachypodanthium staudtii* Engl. and diels (family annonaceae). *World Journal of Pharmaceutical Sciences*, **4:** 73-77.

Saukkonen, J.J., Cohn, D.L., Jasmer, R.M., Schenker, S., Jereb, J.A., Nolan, C.M., Peloquin, C.A., Gordin, F.M., Nunes, D., Strader, D.B., Bernado, J., Venkataramanan, R., Sterling, T.R. 2006. An official ATS statement: hepatotoxicity of antituberculosis therapy. *American Journal of Respiratory and Critical Care Medicine*, **174:** 935-952.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y.S.R., De, B. 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research*, **3:** 91-100.

Sies, H. 1986. Biochemistry of oxidative stress. *Angewandte Chemie International Edition in English*, **25:** 1058-1071.

Singh, A., Bhat, T.K., Sharma, O.P. 2011. Clinical biochemistry of hepatotoxicity. *Journal of Clinical Toxicology*, **6:** 1-19.

Sinha, A.K. 1972. Colometric assay of catalase. *Analytical Biochemistry*, **47:** 389-394.

Soon, H.T., Chung, Y.L., Hazrina, H., Aditya, A., Javad, P.M., Won, F.W., Shiau-Chuen, C., Mohd, R.M., Khalijah, A. 2013. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. *Molecules* **18:** 9770-9784.

Surai, P.F. 2015. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. *Antioxidants*, **4:** 204-247.

Thapa, B.R., Walia, A. 2007. Liver function tests and their interpretation. *Indian Journal of Pediatrics*, **74:** 663-671.

Trease, G.E., Evans, W.C. 1983. *Textbook of Pharmacognosy*, 12th ed., pp. 343-383, Bailliere Tindal, London, UK.

Videla, L.A. 2009. Oxidative stress signaling underlying liver disease and hepatoprotective mechanisms. *World Journal of Hepatology*, **1:** 72-78.

Wang, R., Zhou, J., Shi, G., Liu, Y., Yu, D. 2020. Aporphine and phenanthrene alkaloids with antioxidant activity from the roots of *Stephania tetrandra*. *Fitoterapia*, **143:** 10455.

Weber, L.W.D., Boll, M., Stampfl, A. 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology*, **33:** 105-136.