



Full Length Article

Antioxidant and Hepatoprotective Effects of *Parinari curatellifolia* Root

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Abstract

The antioxidant and hepatoprotective effects of *Parinari curatellifolia* Planch. ex Benth root methanolic extract on rats administered single (0.6 mL/kg) and repeated doses of carbon tetrachloride (0.3 mL/kg) were investigated in male albino rats allotted into six groups of five animals each. Three days pre-treatment with extract (5 mg/kg), resulted in significant decrease in malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin as well as increase in superoxide dismutase (SOD) and catalase in CCl₄-treated rats. Similarly, long term treatment with the extract at 2.5 mg/kg daily doses following 72-h administration of CCl₄ resulted in significant decrease in levels of MDA, AST, ALT, bilirubin, as well as increase in SOD and catalase. These results show that methanolic extract of the roots of *P. curatellifolia* possess significant antioxidant and hepatoprotective effects on acute and chronic liver injuries. © 2013 Friends Science Publishers

Keywords: Antioxidant; Hepatoprotective; *Parinari curatellifolia*; Oxidative stress

Introduction

Many plant products have been evaluated as alternative remedies for treatment of many infections and in the preservation of foods from toxic effects of oxidants. In addition to vitamin C and carotenoids (Miller, 1996), polyphenols, especially flavonoids and phenolic acids found in plants and certain food have been credited with significant antioxidant activity.

Many medicinal plants have been investigated recently due to their potent antioxidant activities, economic viability and relative safety (Gilani et al., 2005; Atawodi et al., 2009a, b, Atawodi, 2010; Atawodi et al., 2010; Atawodi 2011a, b; Asuku et al., 2012; Nisar et al., 2012; Rizwan et al., 2012) in reducing the incidence of free radical associated diseases like liver disorder, disturbances in lipid metabolism. However, many other plants, including *Parinari curatellifolia* have not been studied for such effects.

P. curatellifolia also called “ganza kuisa” in Hausa language of West Africa, is widely used in some parts of Northern Nigeria as a recipe in traditional management of diseases, including liver-related illnesses. It is an evergreen tropical tree of Africa origin which grows to between 10 and 13 m, and occasionally up to 26 m high. It is mostly found in various kinds of deciduous woodland, especially poorly drained areas and inland at moderate altitudes (National Research Council, 2008). It is a spreading tree with semi-circular, almost mushroom-shaped canopy depicting hues of blue-green and grey colors. The plant has a rough and corky bark, oblong-shaped alternate leaves, which are inwardly folded, very tasty and nutritious fruits that are also suitable for brewing of alcoholic beverages, and seeds that may be

eaten raw (National Research Council, 2008).

In traditional medicine, the seed is used in healing of wound and skin problems, treatment of malaria, typhoid fever, fracture and gastrointestinal disorders, while the leaf and bark extracts are deployed in the treatment of pneumonia, eye and ear diseases, and the roots for treatment of cataracts and ear pain (Palgrave, 1985) and liver disorders. Because, accumulated research findings support the importance of antioxidants in the prevention and amelioration of different diseases, including healing of wound and hepatic disorders, *Parinari curatellifolia* was evaluated for of its antioxidant and hepatoprotective properties in order to establish the relationship between these properties and its acclaimed therapeutic uses in traditional medicine.

Materials and Methods

Sample Collection and Identification

The roots of the plant were collected from Katsina State, Nigeria in June 2009. It was identified by Mallam Musa Muhammed at Ahmadu Bello University’s Department of Biological Science, Zaria, Nigeria with a voucher number, 109.

Preparation of Plant Extract

The root material of *P. curatellifolia* material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle. Pulverized material (35 g) was placed in the thimble of soxhlex extractor and extracted

first, using petroleum ether (300 mL) for 8 h each and then methanol (300 mL), three times for 5 h each. The methanol extracts were combined and dried *in vacuo* at 45°C using a rotary evaporator (Büchi Labortechnik AG, Switzerland). The methanol extracts were combined and dried *in vacuo* at 45°C using a rotary evaporator.

Animal Management

Male albino rats (7-8 weeks old) were purchased from the animal house of National Research Institute for Chemical Technology, Zaria, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and were maintained *ad libitum* on tap water and growers mash (Vitafeeds, Jos, Plateau State Nigeria) except in the last 15 h before termination of the experiment. They were weighed prior to commencement and termination of the experiment.

Experimental Grouping and Treatment

The capacity of the extract to protect against oxidative stress was investigated by randomly dividing the animals into the following groups with six rats each: solvent only (corn oil); vitamin E only (50 mg/kg); Vitamin E pre-treatment + CCl₄; *P. curatellifolia* only (5 mg/kg) and *P. curatellifolia* pre-treatment + CCl₄ and CCl₄ only. However, to establish the ameliorative effect of *P. curatellifolia* on pre-existing oxidative stress condition, the following groupings were used: solvent only (corn oil); *P. curatellifolia* only; and CCl₄ pre-treatment + *P. curatellifolia*; vitamin E only and CCl₄ only. All carbon tetrachloride treatments were performed at a dose of 0.6 mL/kg from 33.3% solution in corn oil. In the experiment designed to study the protective effects of the methanol extract of the root of *P. curatellifolia* against oxidative stress, the animals were pre-treated with the extract for three days before intoxication with carbon tetrachloride, which was administered one hour after the extract treatment on the third day, while for the ameliorative effect of *P. curatellifolia* methanolic extract on pre-existing and chronic oxidative stress and liver damage conditions, carbon tetrachloride (0.6 mL/kg) was administered 1 h before extract (2.5 mg/kg) or vitamin E (50 mg/kg) on the first day, and the extract or vitamin E administration was continued for another 10 days with 72 h administration of CCl₄ (0.3 mL/kg). After animals sacrifice, blood was collected and serum separated to assay for biochemical parameters. The organs were immediately harvested, rapidly rinsed in ice-cold normal saline and stored at -20°C for analysis of malondialdehyde (MDA) as indicator of lipid peroxidation.

Animal Sacrifice

All animals were sacrificed 24 h following last administration of drug or *P. curatellifolia* extract. Animals were sacrificed under chloroform anesthesia and whole

blood was collected and allowed to stand for two hours for collection of serum. All sera samples were kept in Eppendorf tubes and stored at -20°C. The organs were immediately harvested, rapidly rinsed in ice-cold normal saline and stored at -20°C for analysis of malondialdehyde as indicator of lipid peroxidation.

Assay for Lipid Peroxidation

Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by Torres *et al.* (2004) based on the principle that peroxide intermediates generated release malondialdehyde upon cleavage. This compound reacts with thiobarbituric acid to form a coloured complex that is measured at 535 nm. In summary, the method is as follows; one milliliter of 14% trichloroacetic acid was measured into a test tube, 1 mL thiobarbituric acid (0.67%) added and 50 µL of the tissue homogenate was added. The mixture was incubated at 80°C for 30 min in a water bath, and then allowed to cool rapidly in ice for 5 min followed by centrifugation at 3000 x g for 10 min. Malondialdehyde was measured colorimetrically at 535 nm and the level of lipid peroxidation was calculated using the molar extinction coefficient of malondialdehyde.

Determination of the Activity of Endogenous Antioxidant Enzymes

The ability of the extracts to boost the capacity of antioxidant enzymes was evaluated by determining the activity of two endogenous antioxidant enzymes, namely catalase (CAT) and superoxide dismutase (SOD) as follow:

Catalases (CAT): Catalase (CAT) activity was measured using the procedure reported by Abei (1974). Briefly, the method is as follows: 10 µL of serum was added to test tube containing 2.80 mL of 50 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM H₂O₂ and the decomposition rate of H₂O₂ was measured at 240 nm for 5 min. on a spectrophotometer (Jenway 640 UV/Vis). A molar extinction coefficient of 0.0411 mM⁻¹cm⁻¹ was used to calculate catalase activity.

Superoxide dismutase (SOD): Superoxide dismutase activity was evaluated according to the method described by Martin *et al.* (1987). The method can be summarized thus; exactly 920 µL of assay buffer was added into clean test tube containing 40 µL of sample, mixed and incubated for 2 min at 25°C, following which 40 µL of hematoxylin solution was added, mixed quickly and the absorbance was measured immediately at 560 nm.

Determination of Liver Function Parameters

Aspartate aminotransferase and alanine aminotransferase were determined colorimetrically at 546 nm using Randox assay kits based on the principle described by Reitman and Frankel (1957). Also, using the Randox kit, the colorimetric assay method for conjugated bilirubin was employed

(Jendrassik and Grof, 1938).

Determination of Packed Cell Volume (PCV)

Whole blood samples were collected into heparinized capillary tubes, filled up to about 2/3 the length, sealed with plasticine and centrifuged at 3,000 rpm for 10 min. Packed cell volume was determined using hematocrit reader, and the hemoglobin concentration was calculated from the PCV values.

Statistical Analysis

The results obtained were statistically Analyzed using Analysis of Variance (ANOVA) and students t-test for significant difference between the grouped means at 95% confidence level ($p < 0.05$).

Results

Malondialdehyde (MDA) Levels in the Liver and Kidney

Carbon tetrachloride caused significant increase ($p < 0.05$) in the level of malondialdehyde in the liver compared to the untreated control group. However, treatment with *P. curatellifolia* root extract significantly reduced the MDA level in CCl₄-treated group ($p < 0.05$; Table 1), while the group on vitamin E and extract alone experienced the lowest level of lipid peroxidation. Similar effect was observed for lipid peroxidation in the kidney, although the effect was less pronounced than in the liver (Table 1).

After initial pre-treatment and 72 h dosing with CCl₄ with concomitant daily treatment with methanolic extract of the root of *P. curatellifolia* (2.5 mg/kg; Table 2), the MDA levels in the liver of the CCl₄ intoxicated group also showed a significant increase ($p < 0.05$) over that of untreated control, but treatment with either *P. curatellifolia* (2.5 mg/kg) or vitamin E significantly reduced the liver MDA level (Table 2), and to a lesser extent, the levels of MDA in the kidney. However, MDA levels in the groups chronically intoxicated with CCl₄ (Table 2) was generally higher than that acutely intoxicated with CCl₄ (Table 1).

Endogenous Antioxidant Enzymes

The activity of liver superoxide dismutase which was depleted in CCl₄-intoxicated group was found to be nominally improved to the same extent by either vitamin E or *P. curatellifolia* pre-treatment, while the improvement in liver catalase activity was statistically significant in both cases (Table 1). Similar trend was observed in the chronic carbon tetrachloride intoxication model, but here the capacity of the either the extract or the vitamin E to boost the level of the endogenous antioxidant enzymes was greatly diminished (Table 2).

Liver Function Parameters in CCl₄-Treated Rats

Pre-treatment with methanolic extract of the root of *P. curatellifolia* significantly ($P < 0.05$) decreased the CCl₄-induced elevation in the levels of serum alanine transaminases (ALT) activity, aspartate transaminases (AST), total bilirubin and conjugated bilirubin upon subsequent treatment with carbon tetrachloride (Table 3), but there was no statistical difference ($p > 0.05$) between the effects exhibited by the extract pre-treatment and vitamin E pre-treatment. Under repeated CCl₄ intoxication, similar trend was observed for most of these liver function parameters (Table 4).

PCV and Hemoglobin Concentration

P. curatellifolia extract or vitamin E pre-treatment significantly prevented the reduction in the PCV induced by the subsequent administration of CCl₄ (Fig. 1 and 2), but the effect on hemoglobin concentration was minimal. The effect of the extract on chronically intoxicated animals followed the same trend (Fig. 3 and 4).

Discussion

In current investigation, antioxidant and hepatoprotective effects of methanolic extract of *P. curatellifolia* root was evaluated in rats on acute CCl₄ intoxication following three days pre-treatment and daily treatment following chronic CCl₄ intoxication.

Carbon tetrachloride (CCl₄) toxicity is dependent on the activity of mixed-Function Oxidase (MFO), abundant in liver tissues to form free radical intermediates (Recknagel *et. al.*, 1977; Stageman, 1981). Due to these reasons, higher level of MDA was observed in the liver compared to the kidneys subjected to CCl₄ intoxication. However, levels of MDA in the kidneys of CCl₄ -treated rats was significantly higher than in untreated groups suggesting that the oxidative stress in the kidneys and the mitigating effects of natural substances may also be investigated in animals on CCl₄ intoxication model.

The results for the evaluation of MDA levels (Tables 1 and 2) showed significant decrease ($p < 0.05$) in the extract-treated group compared to the control, confirming the potency of the extract in preventing toxicity and consequent lipid peroxidation caused by CCl₄ on the albino rats. There was no significant difference between the effects exhibited by the extract and that of the known antioxidant, vitamin E, suggesting that like tocopherol (Packer *et. al.*, 2001), methanol extract of *P. curatellifolia* is capable of protecting the cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction, and thus prevent the oxidation reaction from proceeding to completion by removing free radical intermediates.

Table 1: MDA and antioxidant enzymes level in rats administered single dose of CCl₄ following three days pre-treatment with *Parinari curatellifolia* root methanolic extract (5 mg/kg)

Group	Treatment	Liver MDA (nmol/mg protein)	Kidney MDA (nmol/mg protein)	Liver Catalase (U/mL)	Liver SOD (U/mL)
I	Solvent only	0.162±0.04 ^a	0.103±0.03 ^a	2.61±0.64 ^a	52.6±3.65 ^a
II	Solvent + CCl ₄	0.213±0.05 ^b	0.134±0.02 ^b	1.39±0.19 ^b	43.8±2.59 ^b
III	Vitamin E only	0.142±0.02 ^c	0.056±0.02 ^c	4.11±0.84 ^c	55.20±4.09 ^b
IV	Vitamin E + CCl ₄	0.181±0.04 ^{ac}	0.115±0.04 ^a	2.56±0.51 ^a	53.60±3.05 ^a
V	Extract only	0.150±0.04 ^c	0.075±0.02 ^c	4.01±0.26 ^c	55.20±4.21 ^b
VI	Extract + CCl ₄	0.186±0.05 ^{ac}	0.116±0.01 ^a	2.49±0.43 ^a	53.20±2.17 ^a

Table 2: MDA and antioxidant enzymes Levels in rats administered repeated doses of CCl₄ with concomitant *Parinari curatellifolia* root methanolic extract (2.5 mg/kg) for twelve days

Group	Treatment	Liver MDA (nmol/mg protein)	Kidney MDA (nmol/mg protein)	Liver Catalase (U/ml)	Liver SOD (U/ml)
i	Solvent only	0.197±0.06 ^a	0.104±0.03 ^a	2.11±0.65 ^a	53.8±4.76 ^a
ii	Solvent + CCl ₄	0.278±0.05 ^b	0.134±0.02 ^b	1.31±0.23 ^b	32.6±5.32 ^b
iii	Vitamin E + CCl ₄	0.212±0.05 ^a	0.115±0.03 ^a	1.94±0.45 ^{ab}	38.2±4.02 ^b
iv	Extract + CCl ₄	0.217±0.06 ^a	0.116±0.04 ^a	2.03±0.53 ^{ab}	38.0±6.08 ^b
v	Extract only	0.165±0.05 ^c	0.075±0.02 ^c	3.22±0.75 ^c	58.0±3.74 ^a
vi	Vitamin E only	0.156±0.05 ^c	0.056±0.01 ^c	3.36±0.64 ^c	57.8±2.59 ^a

Table 3: Total and direct bilirubin, ALT and AST level in rats administered single dose of CCl₄ following three days pre-treatment with *Parinari curatellifolia* root methanolic extract (5 mg/kg)

Group	Treatment	Total bilirubin (μmol/L)	Direct bilirubin (μmol/L)	ALT (U/L)	AST (U/L)
i	Solvent only	16.31±3.2 ^a	3.640±1.42 ^a	6.55±1.23 ^c	13.18±2.5 ^a
ii	Solvent + CCl ₄	34.95±10.4 ^b	6.740±1.02 ^b	10.73±3.18 ^b	17.27±4.5 ^b
iii	Vitamin E only	15.24±2.6 ^a	2.952±1.14 ^a	7.18±1.74 ^c	10.73±2.1 ^c
iv	Vitamin E + CCl ₄	23.59±4.1 ^c	5.510±1.50 ^b	8.73±2.28 ^a	13.73±4.6 ^a
v	Extract only	15.72±1.1 ^a	3.001±1.35 ^a	7.36±3.35 ^c	11.55±1.2 ^c
vi	Extract + CCl ₄	25.33±4.9 ^{bc}	5.703±0.71 ^b	9.00±2.47 ^a	14.55±2.7 ^a

Table 4: Total and direct bilirubin, ALT and AST Levels in rats administered repeated doses of CCl₄ with concomitant *Parinari curatellifolia* root methanolic extract (2.5 mg/kg) for twelve days

Group	Treatment	Total bilirubin (μmol/L)	Direct bilirubin (μmol/L)	ALT Activity (U/L)	AST Activity (U/L)
i	Solvent only	25.71±0.22 ^a	6.55±3.63 ^a	12.73±4.7 ^a	11.20±2.5 ^a
ii	Solvent + CCl ₄	49.95±0.14 ^b	12.25±5.25 ^b	20.93±70.9 ^b	21.13±6.5 ^b
iii	Vitamin E + CCl ₄	45.69±0.68 ^c	9.74±3.39 ^c	12.40±3.4 ^a	18.40±3.2 ^c
iv	Extract + CCl ₄	45.88±0.02 ^c	10.23±2.81 ^c	13.07±4.2 ^a	18.87±3.6 ^c
v	Extract only	21.46±1.23 ^a	5.41±3.0 ^a	09.47±1.9 ^c	11.20±1.2 ^a
vi	Vitamin E only	19.98±1.21 ^a	5.11±2.8 ^a	08.00±2.3 ^c	10.60±2.7 ^a

Values denote mean± SD (n=5)

Values with different superscript along a row are statistically significant (P<0.05)

Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase are the body's main defence against oxidative stress. Pre-treatment of experimental animals with methanolic extract of the root of *Parinari curatellifolia* appeared to have improved free radical scavenging capacity through boosted levels of superoxide dismutase and catalase when compared to CCl₄ control group, where the level was significantly depleted (Tables 1 and 2). Similarly, in groups chronically dosed with CCl₄, but treated daily with extract of *P. curatellifolia* experienced significant reduction (P < 0.05) in MDA levels suggesting that the extract can be used to prevent as well as ameliorate pre-existing oxidative stress conditions.

When certain types of cells are damaged, they may

leak enzymes into the blood, where they can be measured as indicators of cell's damage. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are some of such enzymes in the liver. Their levels are markedly elevated in hepatitis and from other acute liver injuries (Stageman, 1981), and have thus been found diagnostic in patients with hepatic disorders (Packer *et al.*, 2001), as values between 20 and 100 times the upper limit of the reference range are not unusual in hepatocellular injury (Stageman, 1981).

In line with this, significant elevation (p<0.05) in the level of alanine aminotransferase (ALT) and aspartate aminotransferase AST (Tables 3 and 4) was observed in the control group compared to the normal group. However, like

the vitamin E group, pre-treatment with the methanol extract of *P. curatellifolia* root significantly ($p < 0.05$) depressed levels of markers of hepatocellular damage upon subsequent intoxication with CCl_4 (Tables 3 and 4). This is a strong indication of the protective effect of the methanol extract of

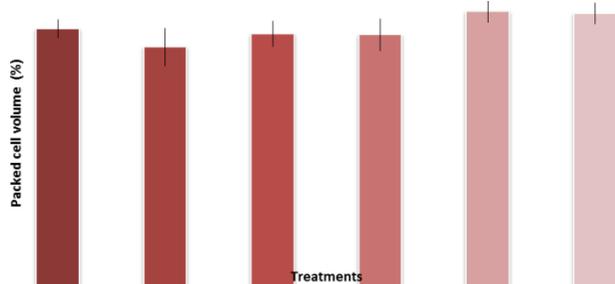


Fig. 1: Packed cell volume levels of rats administered single dose of CCl_4 (0.6 mL/kg) following three days pre-treatment with *Parinari curatellifolia* root methanolic extract (5 mg/kg)

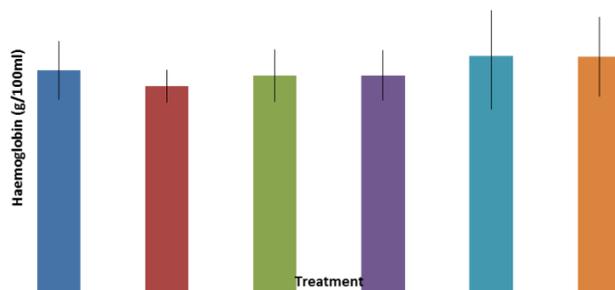


Fig. 2: Hemoglobin levels of rats administered single dose of CCl_4 (0.6 mL/kg) following three days pre-treatment with *Parinari curatellifolia* root methanolic extract (5 mg/kg)

P. curatellifolia root against hepatocellular injury.

Consistent with the results of AST, ALT, the level of bilirubin (total and conjugated) and packed cell volume (PCV) strongly support the hepatoprotective effect of *P. curatellifolia* methanolic root extract, as significant decrease was demonstrated in the extract-treated group compared to the CCl_4 control (group 2). This strongly suggests the potency of the extract in combating liver toxicity and the subsequent peroxidation caused by CCl_4 on the albino rats, as there was no significant difference between the effects exhibited by the extract and vitamin E (Tables 3 and 4).

Similarly, in chronically intoxicated animals, the packed cell volume (PCV) level in CCl_4 control group decreased significantly (Figs. 1-4), the decreased levels of PCV in the control group agrees with the report of Xie *et al.* (2005), in the same way as the increased levels of bilirubin (total and conjugated). The decreased level of PCV and increased level of total bilirubin are respectively consistent with increased level of red blood cell

destruction associated with CCl_4 intoxication and haem degradation that accompanies red blood cell destruction (Cooper *et al.*, 1977). However, the significantly lower levels of these parameters in extract-treated animals

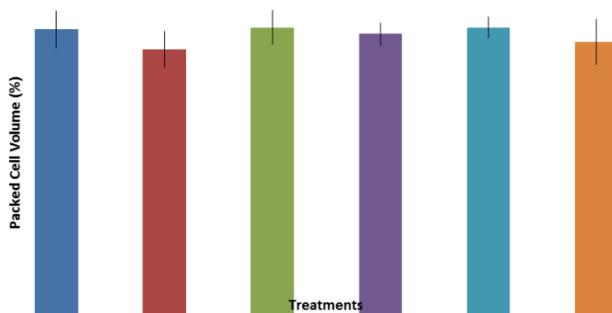


Fig. 3: Packed cell volume levels of rats undergoing 72 hourly CCl_4 intoxication (0.3 mL/kg) with concomitant daily administration of methanolic extract of the root of *Parinari curatellifolia* (2.5 mg/kg) for twelve days

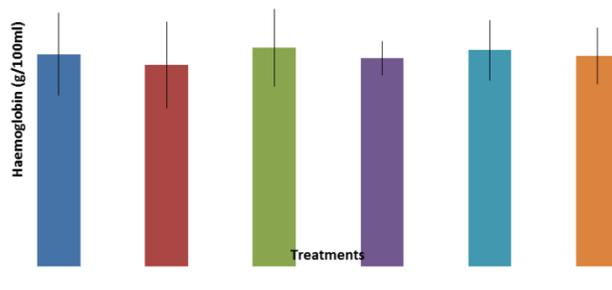


Fig. 4: Hemoglobin levels of rats undergoing 72 hourly CCl_4 intoxication (0.3 mL/kg) with concomitant daily administration of the methanolic extract of the root of *Parinari curatellifolia* (2.5 mg/kg) for twelve days

compared to the CCl_4 control are indicative of the protective effects of *P. curatellifolia* methanolic extract on membrane lipids and hence the integrity of red blood cell membrane.

The ability of the extract to exert antioxidant and hepatoprotective effects may be related to its polyphenol composition, particularly its content of flavonoids as is the case with other medicinal plants (Yang *et al.*, 2001; Yeh *et al.*, 2004; Atawodi *et al.*, 2009a, b; Atawodi *et al.*, 2010; Vauzour, *et al.*, 2010; Atawodi *et al.*, 2011a, b; Zia-UI-Haq *et al.*, 2011a, b; Asuku *et al.*, 2012; Zia-UI-Haq 2012a, b, c). Different mechanisms have been suggested to contribute to the reduction of lipid peroxidation by polyphenols, especially flavonoids. Flavonoids recycle other chain-breaking antioxidants like α -tocopherol by donating a proton to the tocopheryl radical or directly scavenge radical species through chain breaking (Frankel *et al.*, 1993; Raj and Shalinikapoor, 1999; Azzi, 2007).

From the forgoing, it could be concluded that *P. curatellifolia* root is a promising plant for further investigation in the development of drugs for combating

diseases associated with oxidative stress, since it possess significant antioxidant and hepatoprotective effects.

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