

Full Length Research Paper

## Antioxidant activities of *Parquetina nigrescens*

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The antioxidant activities of different extracts (aqueous, methanol and flavonoid) of *Parquetina nigrescens* and butylated hydroxyl anisole, as reference compound, were evaluated in terms of scavenging effect, reducing power and inhibition of Fe<sup>2+</sup>/ascorbate-induced mitochondrial lipid peroxidation in rat liver. Extracts of *P. nigrescens* scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH) generated radicals in the increasing order of flavonoid > methanol > aqueous > BHA at 1000 µg/ml. The reducing power followed a similar trend as observed with scavenging activities. Extracts of *P. nigrescens*, at 50 mg/ml, exhibited significant ( $P < 0.05$ ) inhibitory effects on Fe<sup>2+</sup>/ascorbate-induced lipid peroxidation in rat liver mitochondria. These results showed that *P. nigrescens* contains antioxidants that could be useful in attenuating reactions which generate free radicals in the body.

**Key words:** *Parquetina nigrescens*, antioxidant activities, lipid peroxidation.

### INTRODUCTION

Lipid peroxidation is one of the free radical related processes that may occur in living systems under enzymatic or non-enzymatic control. For instance, generation of lipid-derived inflammatory mediators or generation of reactive oxygen species (ROS) such as hydroxyl radical (OH<sup>•</sup>), superoxide anion (O<sub>2</sub><sup>•-</sup>) and singlet oxygen is associated with non-enzymatic control (Yagi, 1987; Francisco et al., 1998). It has been shown that increased lipid peroxidation resulting from alcohol consumption, drug metabolism and exposure to toxic chemicals is one of the factors that predisposes the liver to damage (Kaneko et al., 2004). In view of the damaging effects of ROS intermediates, which may be significant in the pathophysiological processes of diseases, e.g. in liver necrosis and cirrhosis, arteriosclerosis and cancer, it is therefore an important health issue to reduce lipid peroxidation in human and animal biological systems (Floyd,

1990; Estherbauer et al., 1992; Ronald et al., 2004). Antioxidants are substances that delay or prevent the oxidation of these oxidizable substrates. They scavenge reactive oxygen species, activate a battery of detoxifying enzymes or inhibit the generation of ROS (Halliwell, 1994). Therefore, augmenting antioxidative defense through dietary or pharmacological intake to reduce lipid peroxidation has been advocated.

Natural antioxidants contained in dietary plants have been reported to play an important role in the prevention of carcinogenesis (Cerrutti, 1994) and in extending the lifespan of animals (Cutlar, 1991; Rikans and Hornbook, 1997). Sources of natural antioxidants are generally plant phenols. They may occur in all parts of plants and they can also be found in fruits, vegetables, nuts, seeds, leaves, flowers, roots and bark (Osawa et al., 1990; Pratt and Hudson, 1990). Studies have shown that many dietary polyphenolic constituents derived from plants are more effective *in vitro* than vitamins E and C and this might contribute significantly to their *in vivo* protective effects (Rene et al., 2001). These antioxidants may offer effective protection against peroxidative damage, caused by free radicals in living systems (Diplock et al., 1998).

*Parquetina nigrescens* is commonly found in secondary forest and around villages in Senegal and Nigeria. It is a perennial plant with twining stems and a woody base,

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**Abbreviations:** ROS, Reactive oxygen species; DPPH, 2,2-diphenyl-1-picrylhydrazyl; BHA, butylated hydroxyanisole; PUFA, polyunsaturated fatty acid.

shortly tapering 10 to 15 cm long, 6 to 8 cm broad, smooth and long stem. The leaves and whole plant are usually used for the treatment of liver disease, gonorrhoea, rickets and asthma (Imaga et al., 2008) by traditional healers in Nigeria. This study investigates this plant for possible antioxidant activities.

## MATERIALS AND METHODS

Leaves and stems of *P. nigrescens* were used for this study. This plant species was identified by a plant taxonomist and confirmed at the herbarium of the Forestry Research Institute of Nigeria, Ibadan.

### Phytochemical screening

The standard procedure of Trease and Evans (1998) was used to screen *P. nigrescens* extract for the presence of phenolic compounds, tannins, saponins and glycosides

### Preparation of extracts

#### Aqueous extract

Two hundred and fifty grams of powdered shade dried leaves of *P. nigrescens* was soaked in 2 L of distilled water for 72 h with intermittent agitation. After 72 h, the supernatant was decanted and evaporated and allowed to dry in a vacuum at 40°C. The crude aqueous extract (167 g) was stored at 4°C throughout the experiment.

#### Methanol extract

Exactly 200 g of powdered shade dried leaves of *P. nigrescens* was Soxhlet extracted for 16 h and evaporated in vacuum at 40°C. The crude methanol extract (141 g) was stored at 4°C throughout the experiment.

#### Flavonoid extract

Part of the methanol extracts of *P. nigrescens* was fractionated on silica gel G-60 column chromatography with a mobile phase of ethyl acetate - formic acid (85:15 v/v) to yield the flavonoid fraction of the extract. The flavonoid was assessed using a spray system of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (1 mg/ml) (Tomaturo, 1994).

### Preparation of mitochondria

Mitochondria were isolated from rats by the methods of Hunter et al. (1959). Rats were killed by decapitation and the liver were quickly removed and chilled for 5 to 10 min in 0.25 M sucrose. Weighed portions were homogenized in ice cold 0.25 M sucrose of 6 to 8 volumes of the weight of the liver. The nuclear fraction was removed by centrifugation (1,500 xg) and mitochondria were obtained by centrifugation at 8,000 xg for 10 min. The mitochondria were washed twice with sucrose solution and centrifuged at 8,000 xg for 10 min each. The precipitate was finally washed in 25 mM Tris/1.75 mM KCl buffer. Mitochondria pellet equivalent to 1 g (wet weight) of liver was suspended in 1 ml of the 25 mM Tris/1.75 mM KCl buffer. All steps were carried out at 4°C.

### In vitro antioxidant test

#### Reducing power

This is the reduction of the Fe<sup>3+</sup>/ferricyanide complex to the ferrous form Fe<sup>2+</sup>. The Fe<sup>2+</sup> can be monitored as the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each extract. The reducing power was determined by the method of Oyaizu et al. (1986) as described by Yen and Chen (1995). Briefly, extracts (250 to 1000 µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2M, pH.6.6) and potassium ferricyanide (K<sub>3</sub>Fe (CN)<sub>6</sub> 2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700 nm.

#### Scavenging effect on DPPH radical

The effect of *P. nigrescens* extracts on DPPH radical was estimated according to the method of Hatano et al. (1988) as described by Yen and Chen (1995). Extracts (250 to 1000 µg) in 4 ml distilled water were added to a methanol solution of DPPH (1 mg/ml). The mixture was shaken and left to stand at room temperature for 30 min and the absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

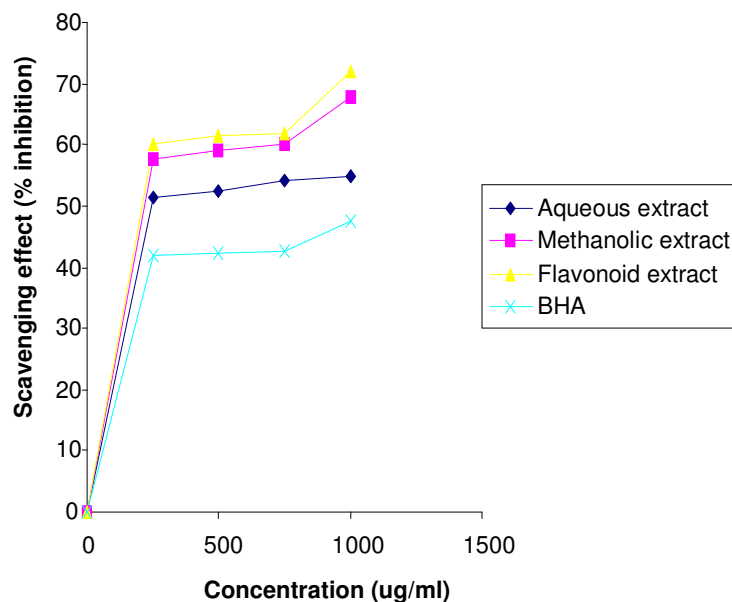
#### Ascorbic acid-induced lipid peroxidation of mitochondria

Lipid peroxidation induced by ascorbic acid was carried out by the method described by Shimada and Yashinda (1979). Briefly, mitochondria (2 mg protein/ml) were incubated with 200 µM ascorbic acid and 20 µM FeSO<sub>4</sub> in 25 mM Tris/175mM KCl buffer at room temperature. At designated times, 2 ml aliquot was withdrawn and mixed with 2 ml of 10% trichloroacetic acid and centrifuged. The resultant supernatant was assayed for malondialdehyde by thiobarbituric acid method described by Rice-Evans et al. (1986).

### In vivo antioxidant effect

Animals were divided into 4 groups with six rats in each group. Groups A and B served as normal and CCl<sub>4</sub> intoxicated controls, respectively, and received 1 ml/kg 0.9% normal saline, orally, on a daily basis. Group C was treated (orally) with 50 mg/kg/day crude flavonoid extract of *P. nigrescens*, dissolved in corn oil for 9 days. Group D, served as standard and received 50 mg/kg/day butylated hydroxyl anisole dissolved in corn oil for 9 days. Thirty minutes after treatment on days 7 and 8, groups B, C and D were treated intraperitoneally with CCl<sub>4</sub> (1.195 g/kg of body weight/day) in corn oil, while group A received 1 ml/kg/day in corn oil. All the animals were killed 24 h after the final dose of CCl<sub>4</sub>. Blood collected from the heart and serum were separated by centrifugation at 3000 rpm for 10 min. The liver was quickly removed, washed in 1.15% KCl, homogenized in 0.25 M sucrose and centrifuged (3000 rpm) for 15 min at 4°C. The supernatant was separated and used for the analysis.

The method of Misra and Fridovich (1972) was used for the estimation of superoxide dismutase, while catalase activity was determined by the procedure of Sinha (1968). Tissue and serum lipid peroxidation and glutathione were measured as thiobarbituric acid reactive substance (Rice-Evans et al., 1986) and non-protein sulfhydryl group (Sedlak and Lindsay, 1968).



**Figure 1.** Scavenging effects of various extracts of *P. nigrescens* on DPPH radical.

#### Protein determination

The method of Lowry et al. (1950) was used for the determination of protein.

#### Statistical analysis

Statistical analysis was performed using Student "t-test". Significant value was set at  $p < 0.05$ .

### RESULTS

Phytochemical analysis showed the presence of flavonoids, saponins, glycosides, cardiac glycosides, tannins, anthraquinones, phlobatannins and oils. The free radical scavenging activities of different extracts of *P. nigrescens* on DPPH radical are shown in Figure 1. The lowest antioxidant activity was observed with butylated hydroxyanisole (BHA), while flavonoid had the strongest effect. At 1000  $\mu\text{g}$  concentration, the activities were in the increasing order of BHA < aqueous < methanol < flavonoids.

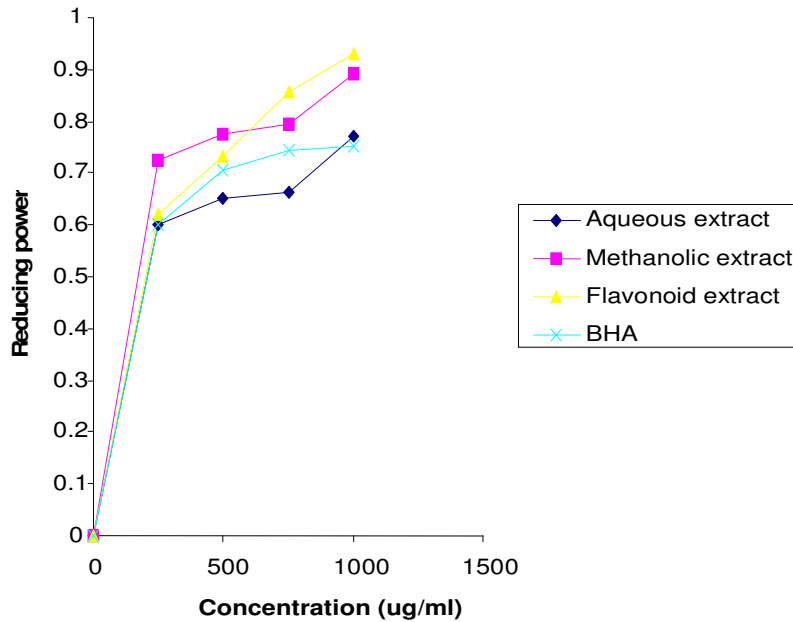
Figure 2 depicts the reducing power of extracts of *P. nigrescens* as a function of concentration. The presence of antioxidants in these extracts caused the reduction of  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form. At 1000  $\mu\text{g}/\text{ml}$ , the reducing powers were in the increasing order BHA < aqueous < methanol < flavonoids. Figure 3 shows the inhibition of 200  $\mu\text{M}$  ascorbate/20  $\mu\text{M}$   $\text{Fe}^{2+}$  induced lipid peroxidation by different extracts (50 mg) of *P. nigrescens* between 0 and 60 min. Different extracts of *P. nigrescens* retarded lipid peroxidation in the mitochondria.

Data on the effect of *P. nigrescens* crude flavonoid extracts on serum antioxidant enzymes (superoxide dismutase and catalase), lipid peroxidation and glutathione are shown in Table 1. Superoxide dismutase activity did not change significantly ( $P > 0.05$ ) with  $\text{CCl}_4$  intoxication (Group B) when compared with the normal control. However, significant differences were observed in *P. nigrescens* treated group C and BHA treated group D ( $P < 0.05$ ) with increases of 37 and 64%, respectively. Significantly ( $P < 0.05$ ) reduced catalase activity was observed in  $\text{CCl}_4$  intoxicated control (group B) when compared with values in the normal control (group A), groups C (50 mg/kg PN +  $\text{CCl}_4$ ) and group D (50 mg/kg BHA +  $\text{CCl}_4$ ). Administration of *P. nigrescens* crude flavonoid extract and BHA caused reductions in lipid peroxidation products and glutathione that were elevated in group B ( $\text{CCl}_4$  intoxicated control group).

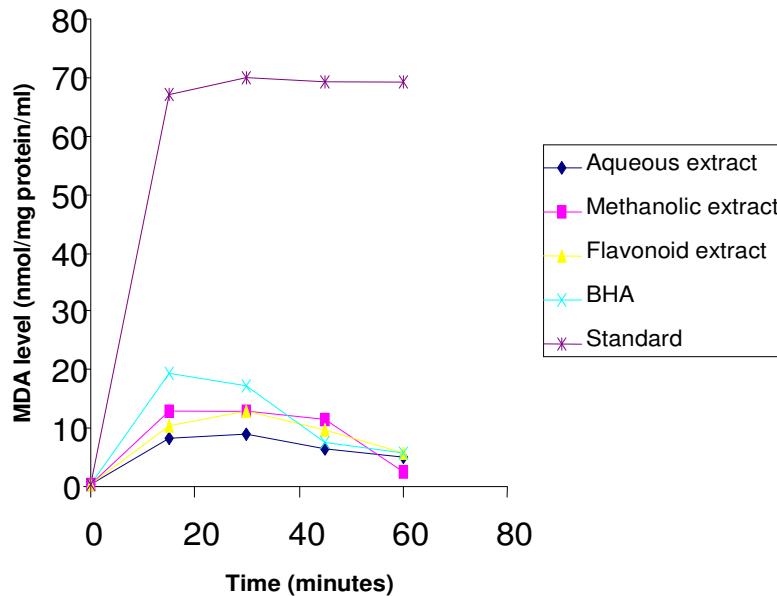
Table 2 depicts the effect of *P. nigrescens* crude flavonoid extract and BHA on the tissue glutathione, catalase, lipid peroxidation and superoxide dismutase of  $\text{CCl}_4$  intoxicated animals. Treatment with both extracts caused effects similar to those observed in the serum of  $\text{CCl}_4$  intoxicated rats.

### DISCUSSION

The present study showed that different extracts of *P. nigrescens* exhibited radical scavenging activity that could play an important role in attenuating superoxide generated reactions (Nuutila et al., 2003; Amarowicz et al., 2004). The extracts of *P. nigrescens* scavenged stable



**Figure 2.** Reducing power of various extracts of *P. nigrescens*.



**Figure 3.** Inhibition of Fe<sup>2+</sup>/ascorbate-induced lipid peroxidation in rat by 50 mg/ml extract.

free radical DPPH more than BHA and in the decreasing order of flavonoid > methanol > aqueous > BHA. Polyphenols in plants donate electrons or hydrogen atoms to terminate radical chain reactions by converting free radicals to more stable products, therefore exhibiting strong antioxidant activity. Exposure to chemicals, drugs and infection has been known to increase the generation of reactive oxygen radicals, which in turn affects the

antioxidant/oxidant homeostasis in the biological system (Caro and Cedderbaum, 2004). In the liver for example, free radicals cause cell death either through necrosis or apoptosis (Gressner and Bachem, 1995). Subsequently, dysfunction in the synthetic and metabolic activities of this organ, which can affect other biological processes such as decrease in the synthesis of vitamin C, could lower antioxidant status. The implication of health benefits

**Table 1.** Effects of *P. nigrescens* crude flavonoid extracts on serum antioxidant enzymes (superoxide dismutase and catalase), glutathione and lipid peroxidation products (TBARS) in CCl<sub>4</sub> intoxicated rats.

Test	Group A	Group B	Group C	Group D
SOD (units)	1.30±0.004	1.34±0.007	2.19±0.00	3.82±0.01*
CAT (µmol H <sub>2</sub> O <sub>2</sub> decomposed/min)	16.42±1.22	8.60±0.02**	16.50±0.12	15.00±0.00*
TBARS (nmol/mg protein)	3.55±0.004	10.46±0.01**	7.13±0.02*	1.50±0.04*
GSH (µg/mg protein)	2.12±0.02	3.44±0.03**	2.11±0.02*	1.50±0.03*

Values are expressed as mean ±SD for 6 rats in each group. Group A = Normal control; Group B = CCl<sub>4</sub> control; Group C = 50 mg/kg *P. nigrescens* + CCl<sub>4</sub>; Group D = 50 mg/kg BHA + CCl<sub>4</sub>; \*\* significantly different (P < 0.05) from normal control; \* significantly different (P < 0.05) from the CCl<sub>4</sub> control; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; lipid peroxidation products (TBARS).

**Table 2.** Effects of *P. nigrescens* crude flavonoid extract on antioxidant enzymes (superoxide dismutase and catalase), glutathione and lipid peroxidation products (TBARS) in liver homogenate CCl<sub>4</sub> intoxicated rats

Test	Group A	Group B	Group C	Group D
SOD (units)	1.60±0.005	1.29±0.022**	5.20±0.013*	1.60±0.006
CAT (µmol H <sub>2</sub> O <sub>2</sub> decomposed/min)	18.94±0.02	13.90±0.03**	16.84±0.14*	13.33±0.03
TBARS (nmol/mg protein)	4.43±0.01	8.56±0.02**	2.50±0.09*	3.31±0.002*
GSH (µg/mg protein)	5.50±0.003	7.60±0.017**	5.83±0.001*	5.33±0.002*

Values are expressed as mean ±SD for 6 rats in each group. Group A = Normal control; Group B = CCl<sub>4</sub> control; Group C = 50 mg/kg *P. nigrescens* + CCl<sub>4</sub>; Group D = 50 mg/kg BHA + CCl<sub>4</sub>; \*\*significantly different (P < 0.05) from normal control; \*significantly different (P < 0.05) from the CCl<sub>4</sub> control.

of polyphenols has increased due to their confirmed antioxidant (Usain et al., 1997), regulatory (Rushmore and Pickett, 1990) and scavenging abilities (Robak and Gryglewski, 1983).

Flavonoids for example, in conjunction with vitamins C and E have been shown to increase the inhibition of lipid peroxidation in polyunsaturated fatty acid (PUFA) caused by reactive oxygen species (Julie and Kussum, 2002). Flavonoids modulate enzyme activities, inhibit cellular proliferation and act as potential anti-inflammatory agents (Bravo, 1998). In addition to these, some flavonoids can chelate transition metal ions responsible for generation of reactive oxygen species and therefore inhibit the synthesis of lipooxygenase (McAnlis et al., 1997).

The flavonoid extracts of the plant extract under study reduced liver damage probably through induction of superoxide dismutase and catalase with simultaneous utilization of reduced glutathione (GSH). These eventually caused reduction in lipid peroxidation in *P. nigrescens* treated animals, thus confirming the observed *in vitro* antioxidant properties of extracts of *P. nigrescens*. Similarly, antioxidant activities have been exhibited through protection and enhancement of endogenous antioxidant by acting on the antioxidant response elements in the enhancer region at the promoter site of the gene that codes for the enzyme (Rushmore et al., 1991). The antioxidant properties shown by extracts of *P. nigrescens* seem to suggest that the extracts may have health benefits for regular consumers or its acclaimed medicinal use in the treatment of liver disease.

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