Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (March, 2012), 20(1): 7-20
ISSN 0794-5698

Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats

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ABSTRACT: The antioxidant properties of methanolic extracts of six locally consumed plants in Nigeria were comparatively evaluated both *in vitro* and *in vivo*. The plants included *V. amygdalina* (bitter leaf); *C. rubens* (*'ebolo'*); *A. hybridus* (*'tete'*); *J. tanjorensis* (*jatropha*); *G. africana* (*'okazi'*) and *T. triangulare* (water leaf). The *in vitro* study indicate that the DPPH radical scavenging activity was highest ($p<0.05$) in *ebolo* and water leaf. The proanthocyanidin content was significantly higher ($p<0.05$) in *ebolo* and *tete*. *Jatropha* extract has the highest phenolic content. Flavonoid content is significantly high ($p<0.05$) in *jatropha* and water leaf extracts. *In vivo* study of the effect of the extracts, on nutritionally stressed male albino rats, show that the liver and kidney tissues of rats fed the protein deficient diet (PDD) had significantly lower ($p<0.05$) superoxide dismutase (SOD), catalase (CAT), vitamin E, vitamin C levels and higher lipid peroxidation levels when compared with the control. However, supplementation of the PDD diet with the various extracts resulted in significantly higher ($p<0.05$) levels of SOD, CAT, vitamin E, vitamin C and reduced lipid peroxidation relative to the PDD group. Likewise, feeding of normal rats with the extracts resulted in higher levels of these parameters when compared with the control. The results suggest that the plant leaves possess varied degrees of antioxidant activity both *in vitro* and *in vivo*.

Keywords: Nutritional oxidative stress, Reactive oxygen species, Antioxidant activity, Protein deficient diet, methanolic extract.

INTRODUCTION

For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicines. Besides, evidences from epidemiological studies suggest that high consumption of fruits and vegetables is linked to reduced risk of developing most oxidative stress – induced diseases (Dani *et al*., 2008; Wasson *et al*., 2008; Atrooz, 2009). Examples of such diseases include cancer, diabetes mellitus, protein energy malnutrition (PEM), cataract, infections and other degenerative diseases of aging (Dani *et al*., 2008; Wasson *et al*., 2008; Atrooz, 2009; Omoregie and Osagie, 2011; Dhanasekaran and Ganapathy, 2011).

Previous studies have shown that increased production of reactive oxygen species (ROS) may be one of the underlying causes of these diseases (Bender, 2006; Adesegun *et al.,* 2008; Wasson *et al*., 2008; Martin and Appel, 2010). Nevertheless, ROS can be generated during normal metabolism in the body; and if not removed may lead to any of the diseases. The long term effects of increased ROS level include damages to important cellular components especially proteins, nucleic acids and polyunsaturated fatty acids in cell membranes and plasma lipoproteins (Bender, 2006; Kasote *et al*., 2011).

Reports abound on the antioxidant activities of phytochemical constituents of medicinal plants (e.g. polyphenols, carotenoids, flavonoids, phenolics, vitamins C and E). These phytochemicals act as antioxidants by preventing damages to cell membrane due to cellular oxidative processes that may result in diseases (Oboh and Rocha, 2008; Wasson *et al*., 2008; Ebrahimzadeh *et al*., 2009; Atrooz, 2009; Omoregie and Osagie, 2011; Kasote *et al*., 2011). For instance, natural polyphenols from plant vegetables have been found to exert their beneficial effect by removing free radicals, chelating metal catalyst, activating antioxidant enzymes, etc (Atrooz *et al*., 2009; Oboh *et al*., 2009).

Gnetum africanum is a dioecious forest perennial liana, a member of Gnetaceae family, commonly called eru (English), *okazi* (Igbo). The leaves are edible and used in the treatment of enlarged spleen, sore throat, pain at child birth, snake poisoning, diabetes mellitus, and worm expeller (Ekpo, 2007; Orwa *et al*., 2009). *Amaranthus hybridus* is an annual herbaceous plant which belongs to the Amaranthaceae family. Its common names include "Amaranth or pigweed" (English), *tete* (Yoruba), etc.

The leaves are used for soup and medicinally as a blood booster (Oguntona, 1998; Ekpo, 2007). *Crassocephalum rubens* is a member of the Asteraceae family and commonly called ragleaf, thickweed, *ebolo* (Yoruba), etc. Its mucilaginous leaves are used in dried or fresh forms in varieties of dishes and medicinally as laxatives, to relief stomach ache, colitis, etc (Oguntona, 1998; Grubben, 2004).

Vernonia amygdalina, a member of Compositae family, is commonly known as bitterleaf. It possess characteristic astringent bitter taste and the leaves are employed as vegetable in soups, in treatment of malaria, diabetes mellitus, venereal disease, wounds, hepatitis and cancer (Kambizi and Afolayan, 2001; Hamill *et al*., 2003; Erasto *et al*., 2007). *Jatropha tanjorensis* is a perennial herb, a member of the Euphorbiaceae family, commonly called 'hospital too far', catholic vegetable, *Jatropha*. It is used locally in soups as well as medicinally in treating anaemia, skin disease, malaria fever (Oduola *et al*., 2005; Omoregie and Osagie, 2011). *Talium triangulare* (Portulaceae family) is popularly known as waterleaf, gbure (Yoruba), it serves as soup and it's a rich source of vitamins and minerals. It is employed as a laxative and purgative based on its preparation (Oguntona, 1998).

In recent times, antioxidants from plant sources have received a lot of attention and are preferred to synthetic ones. This is especially due to their potential health benefits, availability, affordability and in many cases, reduced toxicity (Oyewole *et al*., 2007; Ayoola *et al.*, 2008; Ebrahimzadeh *et al*., 2009**;** Omoregie and Osagie, 2011). This study therefore reports the *in vitro* antioxidant activities of six locally consumed vegetables (*Vernonia amygdalina*, *Crassocephalum rubens*, *Jatropha tanjorensis*, *Amaranthus hybridus*, *Talinum triangulare* and *Gnetum africana*). *In vivo* antioxidant activities of these plants were also examined in rats exposed to nutritional oxidative stress using a low protein diet. The plant leaves were selected based on their popular use as food and medicine from south-western Nigeria.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh leaves of *J. tanjorensis, T. triangulare, G. Africana, A. hybridus* and *V. amygdalina* were collected during the rainy season (between April – May, 2009) from a cultivated farm in Ugbowo, Edo State, Nigeria. The *Crassocephalum rubens* leaves were however collected freshly from a farmland at Ikare, Ekiti State, Nigeria. All the plant leaves were identified and authenticated by a Botanist from the Department of Botany, University of Benin, Benin City, Nigeria. Voucher specimen of each plant was thereafter deposited in the herbarium of the Department of Pharmacognosy of the same institution.

Preparation of Plant Extracts

One hundred grammes (100g) of washed, air-dried powdered leaves of the plants were extracted with methanol at room temperature for 48 hours with stirring at interval. The methanolic extracts obtained were concentrated to dryness at 40^{o}C using a rotary evaporator under reduced pressure (Ayoola *et al*., 2008). The dried extracts were weighed and then stored at 4^{o}C for subsequent analysis.

Estimation of Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the plant extracts against 1,1–diphenyl–2–picrylhydrazyl (DPPH) free radical was determined by a modified method of Szabo *et al* (2007).

Estimation of Total Phenolic Content

Total phenolic content of the leaves was estimated based on the Folin – Ciocalteau's method as modified by Singleton *et al* (1999) and Ayoola *et al* (2008). The total phenolic contents were expressed as gallic acid equivalents (GAE).

Estimation of Proanthocyanidin Content

Proanthocyanidin content of the leaves was estimated according to the method of Ayoola *et al* (2008). The final results were expressed as catechin equivalent.

Determination of Flavonoid Content

The total flavonoid content of the leaves was determined according to methods of Miliauskas *et al* (2004) and Ayoola *et al* (2008). The total flavonoid contents of the extracts were calculated as rutin equivalents.

In vivo Animal Study

Animals and Preparation of Experimental Diet

Eighty four (84) male albino rats of the Wister strain weighing between 100-120g were obtained from the animal breeding unit, Department of Biochemistry, University of Lagos, Nigeria. The experimental diet was locally sourced and prepared as previously reported by Omoregie and Osagie (2011). *Garri* served as a source of carbohydrate, processed soya beans served as protein source and fat and oil were obtained from groundnut oil. The mineral and

vitamins mixture were from ABC Plus® multivitamin capsule, a product of Hollard and Barrett Ltd, Nuneaton, Warwickshire, USA (see Table 1 for preparation of diet).

Table 1: Composition of the Experimental Diet (%)

Dietary Components	ND	PDD
Carbohydrate (*garri*)[1]	63	82
Protein (defatted soya beans) [1]	21	2
Fat (palm oil) [1]	8	8
Vitamin mix[2]	4	4
Mineral salt mix[2]	4	4

[1]Obtained from New Benin market, a local market in Benin City, Nigeria

[2] Contained all the vitamins and minerals (in recommended daily allowance). The vitamin and mineral mixture was obtained from ABC Plus® Capsule, a product of Hollard and Barrett Ltd., Nuneaton, Warwickshire, USA.

ND = Normal diet containing 20% protein;

PDD = Protein deficient diet containing 2% protein.

Source: Omoregie and Osagie, 2011

Feeding Pattern

The experimental animals were divided into two (2) groups with each group having seven (7) subgroups containing six (6) animals each. The first group was induced with nutritional oxidative stress, for six (6) weeks, using protein deficient diet (PDD) containing only 2% protein. Animals in this group were treated with PDD diet (serving as the negative control), PDD + plant extracts (PDD + *Ebolo*, PDD + *Jatropha*, PDD + *Tete*, PDD + Waterleaf, PDD + Bitterleaf and PDD + *Okazi* leaves extracts). The second group of animals was placed on normal diet (ND – positive control) with adequate protein (20%) as well as normal diet + plant extract (ND + Ebolo, ND + *Jatropha*, ND + *Tete*, ND + Waterleaf, ND + Bitter leaf and ND + *Okazi* extracts) as before. The extracts were administered to each animal orally and once daily at a safe dose of 300mg/kg body weight. The feeding experiment lasted six (6) weeks during which the animals were allowed access to food and water *ad libitum*. Food intake, faecal output and body weights were recorded weekly.

All experimental protocols were performed within internationally accepted guidelines for animals use and care (according to NIN guide for Laboratory Animals Welfare) with the approval of the Local Ethics Committee of the University of Benin, Benin City, Nigeria. At the completion of the feeding period, animals were fasted overnight and sacrificed by decapitation. Blood was collected and the sera obtained from the whole blood were stored at 4^0C. The tissues (liver and kidney) were removed, blotted dry, weighed and stored as before.

Preparation of Liver and Kidney Homogenates

One gramme (1g) of the liver and kidney tissues were homogenized in 10ml of ice-cold physiological saline to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 5,000g for 10min and the supernatants obtained were used for determination of superoxide dismutase (SOD), catalase (CAT), vitamin C, vitamin E and lipid peroxidation level.

Biochemical Assays

Superoxide dismutase (SOD) activity was assayed in the tissues based on the rapid auto-oxidation of adrenaline due to the presence of superoxide anions (Misra and Fridovich, 1972). This is measured spetrophotometrically at 420nm and SOD concentration expressed as Units / g tissue. Catalase activity in the tissues was determined as residual H_2O_2 after incubation with the enzyme (Kaplan and Groves, 1992). Estimation of lipid peroxidation in the tissues involved the determination of thiobarbituric acid reactive substances (TBARS), which are indicators of membrane lipid peroxidation. Values for TBARS were reported as malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5×10^5 M $cm^{-}1$ and expressed as mmole MDA g^{-1} of tissue (Gutteridge and Wilkins, 1982). The vitamin C and E contents of the tissues were determined as previously reported (Roe and Kuether, 1943; Desai, 1984). The total protein was determined by the Biuret method (Mokady *et al*., 1989).

Statistical Analysis

Data were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significant differences between the means were determined by Duncan's multiple range test and P values < 0.05 were regarded as significant (Sokal and Rohlf, 1995).

RESULTS

The DPPH scavenging activities of the methanolic extracts are presented in Figure 1. The DPPH scavenging activities of the extracts were comparable to that of vitamin E. However, *ebolo* and waterleaf extracts showed the highest radical scavenging ability with percentage inhibition of 77.6 and 69.5%, respectively, which was even higher than that of the standard vitamin E (67.2%). The DPPH scavenging capacities of all the extracts were dose-dependent.

Figure 2a shows the Proanthocyanidin content of the extracts against standard catechin. Among the extracts, Ebolo and tete had considerably higher level of Proanthocyanidin (0.49 ± 0.021mg/ml and 0.382 ± 0.0019 mg/ml, respectively) than the standard catechin (0.314 ± 0.014mg/ml). However, the proanthocyanidin content of the remaining extracts decreased in the order waterleaf > okazi > Jatropha > bitter leaf. The phenolic content of the extracts as represented in figure 2b was considerably low when compared with the standard gallic acid. However, *Jatropha* recorded the highest phenolic content (1.966 ± 0.070mg/g dry extract) among the extracts. The total flavonoid content of the extracts (Figure 2c) was particularly high for ebolo (1.905 ± 0.0013mg/ml), waterleaf (1.704 ± 0.0136mg/ml) and *Jatropha* (1.616 ± 0.0038mg/ml) extracts as against the other extracts but was close to rutin (2.351 ± 0.081mg/ml).

Figure 3 depicts the effects of the extracts on food intake, faecal output and weight gain / loss on the treated rats. The protein deficient diet (PDD) fed rats showed preliminary signs of protein energy malnutrition (PEM) with significantly lower ($p<0.05$) food intake (anorexia), faecal output and higher weight loss. The degree of anorexia was however reduced in the PDD + extracts treated rats except for the *okazi* extract treated rats which showed significantly higher weight loss ($p < 0.05$) when compared with the other groups. The normal diet + extracts fed rats had normal appetite for food as observed in the significantly higher ($p<0.05$) food intake, faecal output and weight gain when compared with control (ND).

Catalase activity was significantly lower ($p<0.05$) in the PDD fed rats more in the liver than the kidney when compared with the control. However, oral supplementation of the PDD diet with the various extracts resulted in significantly higher ($p < 0.05$) catalase level when compared with the PDD treated rats. The only exception was the *tete* (PDD+TE) extract fed group in which the hepatic catalase activity remained as low as that of the PDD group irrespective of the treatment with the extract. But the catalase activity of the normal rats treated with the extracts remained normal (Figure 4).

Figure 5 presents the effect of the leaves extracts on superoxide dismutase (SOD) activity in the liver and kidney. The result show significantly lower ($P<0.05$) SOD level, especially in the liver of the PDD (10.4 units/g tissue), PDD + Bitterleaf (7.4 units/g tissue) and PDD + Waterleaf (9.4 units/g tissue) treated rats relative to the control (39.1 units/g tissue). In contrast, the SOD levels in the kidney were normal in all the groups studied. The only exception was the PDD fed rats which showed reduced level of this enzyme.

The hepatic and kidney malonaldehyde (MDA) level of the rats are shown in Figure 6. The MDA level was significantly lower ($p<0.05$) in all the extracts treated rats as against the control. The PDD group showed characteristic evidence of lipid peroxidation with high MDA level relative to the control.

Figure 7 presents hepatic and kidney vitamin E level in the extract treated rats. The results show significantly higher ($p<0.05$) vitamin E level in extracts fed malnourished rats, except for the PDD+WL group, which recorded relatively low vitamin E levels, when compared with the PDD fed rats. However, the vitamin E level still remained lower than that of the controls. On the other hand, treatment of the normal diet fed rats with the plant extracts resulted in significantly higher ($p<0.05$) vitamin E level when compared to the control values.

The vitamin C level was significantly lower ($p<0.05$) especially in the PDD treated rats. The extracts treated normal and the malnourished rats have significantly higher ($p<0.05$) vitamin C level when compared with the PDD group, but the vitamin C level was lower than that of control (Figure 8).

The protein concentration in the liver and plasma were significantly higher ($p<0.05$) in the extracts-treated malnourished rats relative to the PDD fed rats. A similar trend was seen when the normal diet fed rats were treated with the various extracts except for the *ebolo*, waterleaf, *okazi* and bitterleaf groups, which have significantly lower ($p<0.05$) plasma total protein levels (Figure 9).

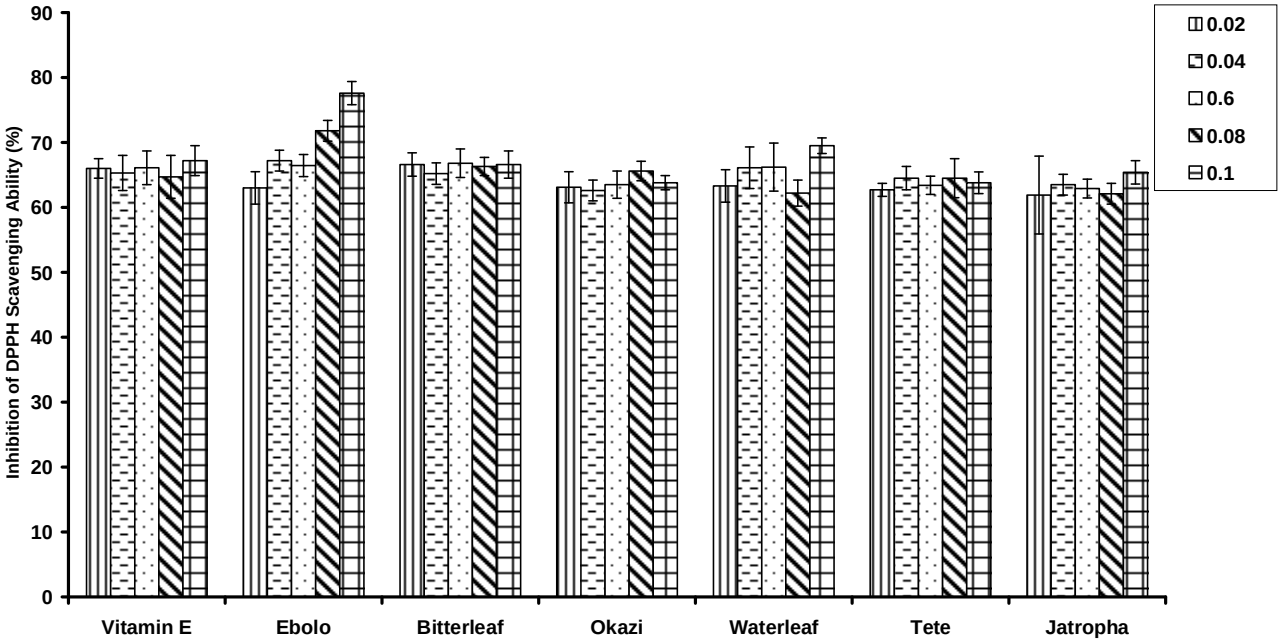

Figure 1: DPPH Scavenging Capacity of the Methanolic Extracts Compared with Vitamin E

All values represent the mean ± SD of triplicate readings (n = 3). The scavenging ability of the extracts was estimated in 1.5 x 10^{-7}M methanolic solution of DPPH at different concentrations of the plants extracts and % inhibition values were calculated from the absorbance values.

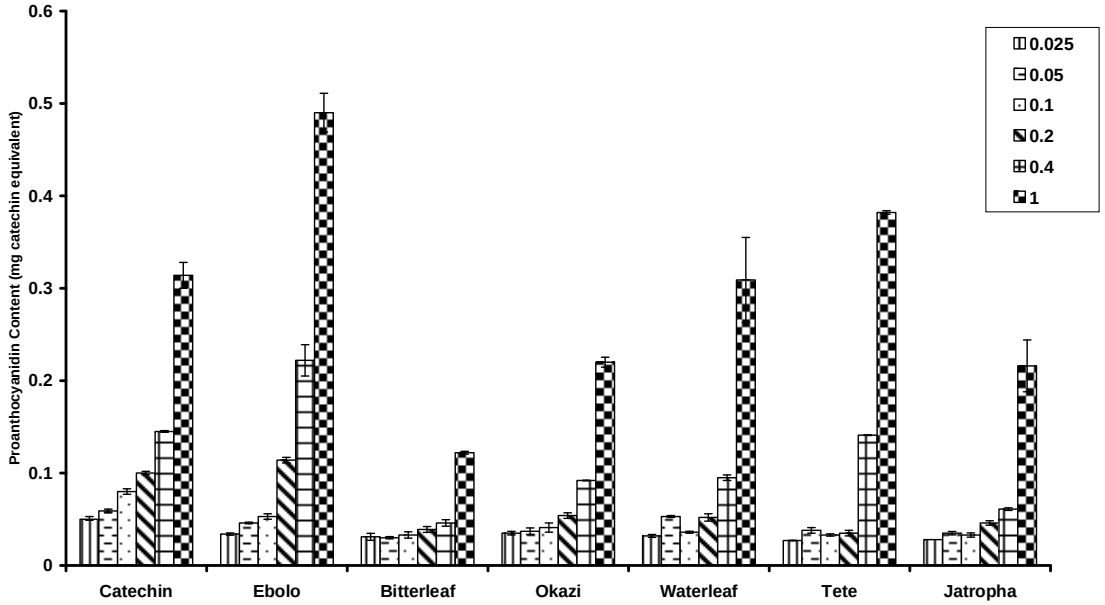

Figure 2a: Proanthocyanidin Content of the Methanolic Extracts Compared with Catechin

Values represent the mean ± SEM of triplicate readings (n = 3). Proanthocyanidin content was expressed as catechin equivalent (CE). The proanthocyanidin content of the leaves was estimated by preparing different concentrations of the extracts and standard catechin in methanol. 0.5ml of HCL was added to each solution and absorbance at 500nm was measured.

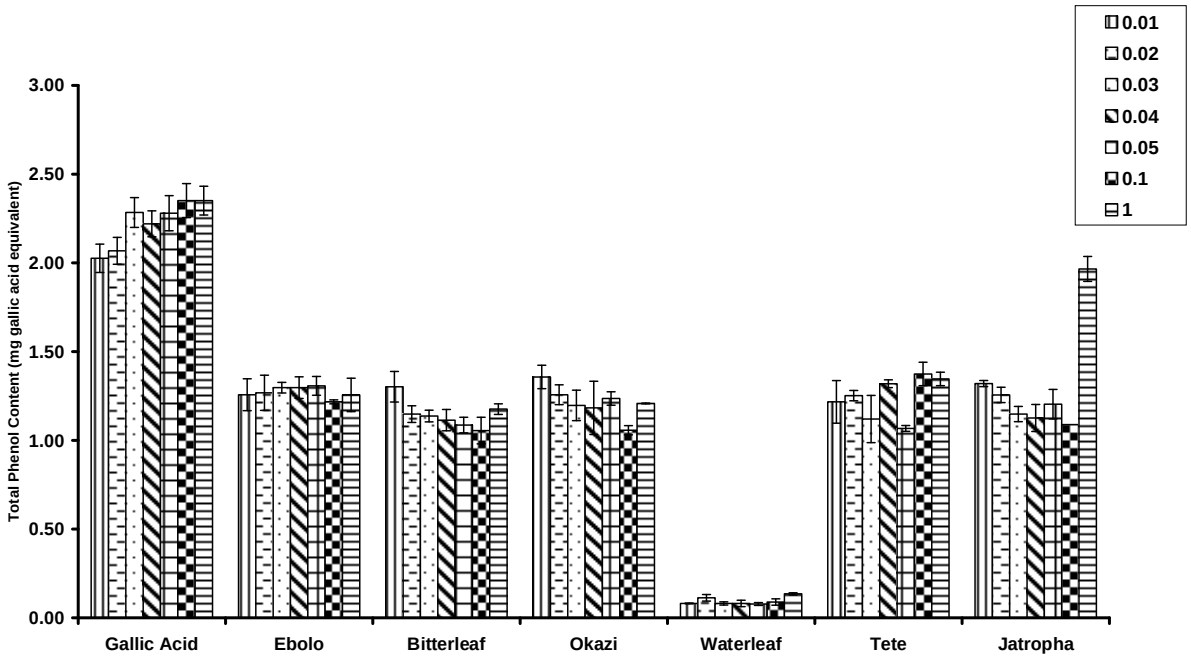

Figure 2b: Total Phenol Content of the Methanolic Extracts Compared with Gallic Acid

Values represent the mean ± SD of triplicate readings (n = 3). Total phenol content was expressed as gallic acid equivalent (GAE).

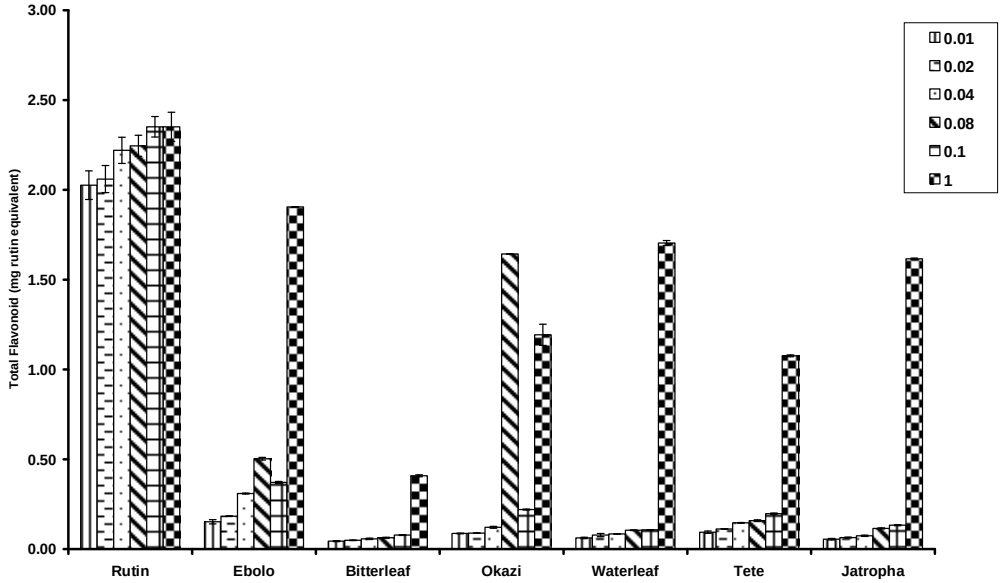

Figure 2c: Total Flavonoid Content of the Methanolic Extracts Compared with Rutin

The total flavonoid content of the leaves was determined by adding 2ml of $AlCl_3$ (2% in ethanol) to 2ml of the extract and standard rutin at varied concentrations. The absorbance values were measured at 420nm after one hour of incubation at room temperature. Data represent the mean ± SEM of triplicate readings (n = 3). Total flavonoid content was expressed as rutin equivalent (RE)

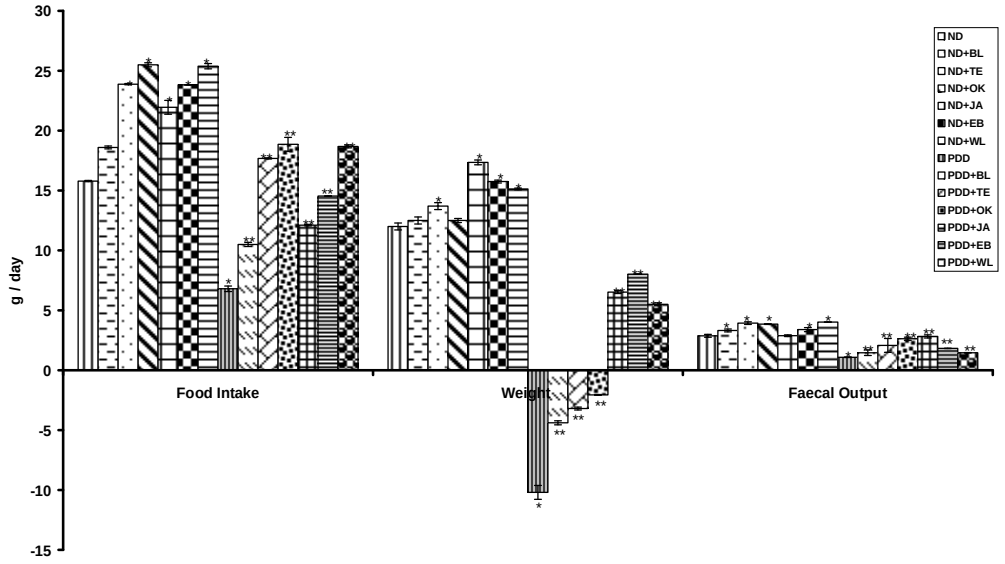

Figure 3: Effect of Methanolic Extracts on Food Intake, Weight Gain / Loss and Faecal Output in Rats

All values represent mean ± SEM (n = 6). Differences between means were analyzed by one - way ANOVA and significant differences obtained by Duncan's multiple range test ($P < 0.05$). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. * Statistically significant ($P < 0.05$) relative to control (ND) group.

** Statistically significant ($P < 0.05$) when compared with protein deficient diet (PDD) fed group.

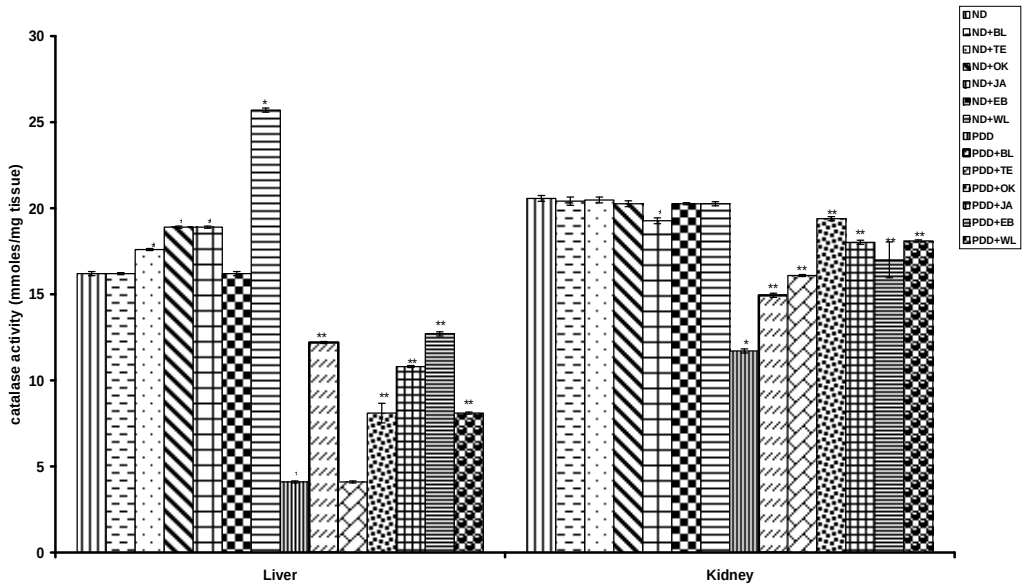

Figure 4: Effect of the Methanolic Extracts on Catalase Activity in Rats

Values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test ($P < 0.05$). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. Catalase activities are expressed as mmoles / mg tissue. * Statistically significant ($P < 0.05$) relative to control (ND) group. ** Statistically significant ($P < 0.05$) when compared with protein deficient diet (PDD) fed group.

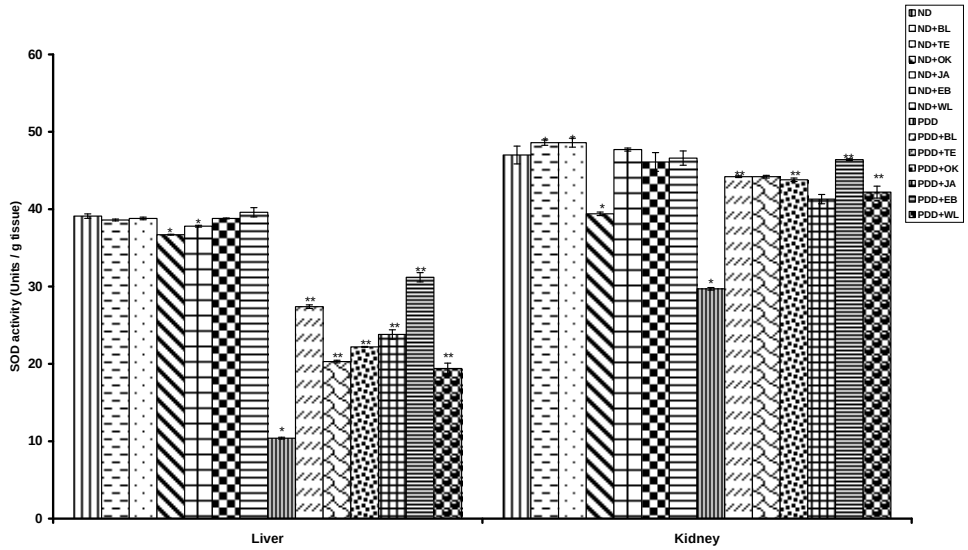

Figure 5: Effect of the Methanolic Extracts on Superoxide Dismutase (SOD) Activity in Rats

Values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test (P < 0.05). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. SOD activities are expressed as units / g tissue. * Statistically significant (P < 0.05) relative to control (ND) group. ** Statistically significant (P < 0.05) when compared with protein deficient diet (PDD) fed group.

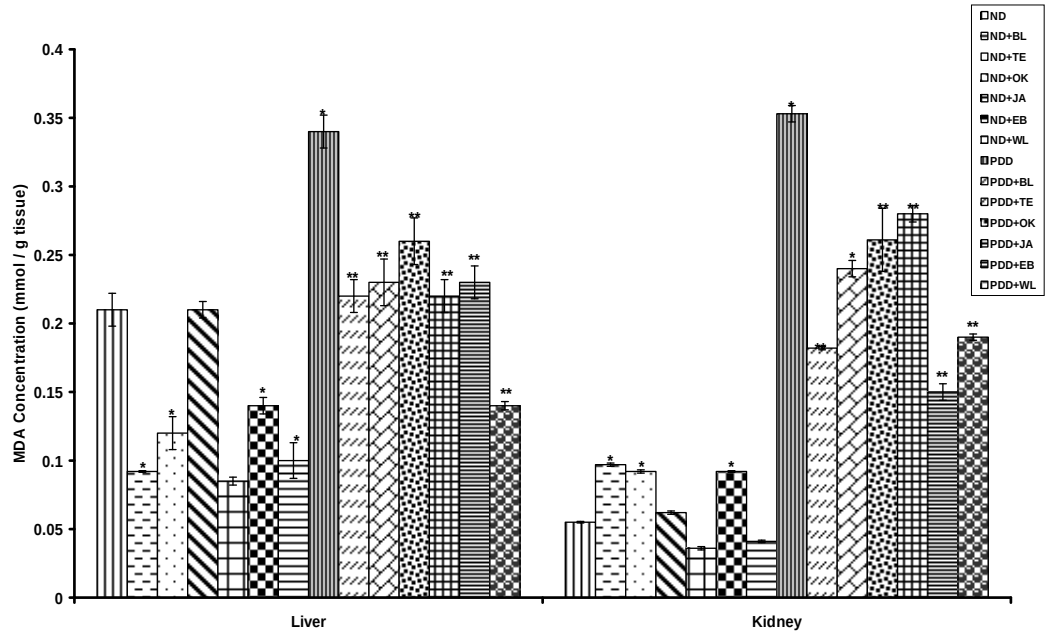

Figure 6: Effect of the Methanolic Extracts on Membrane Lipid Peroxidation in Rats

Values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test (P < 0.05). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. Lipid peroxidation levels are expressed as mmole MDA / g tissue.* Statistically significant (P < 0.05) relative to control (ND) group. ** Statistically significant (P < 0.05) when compared with protein deficient diet (PDD) fed group.

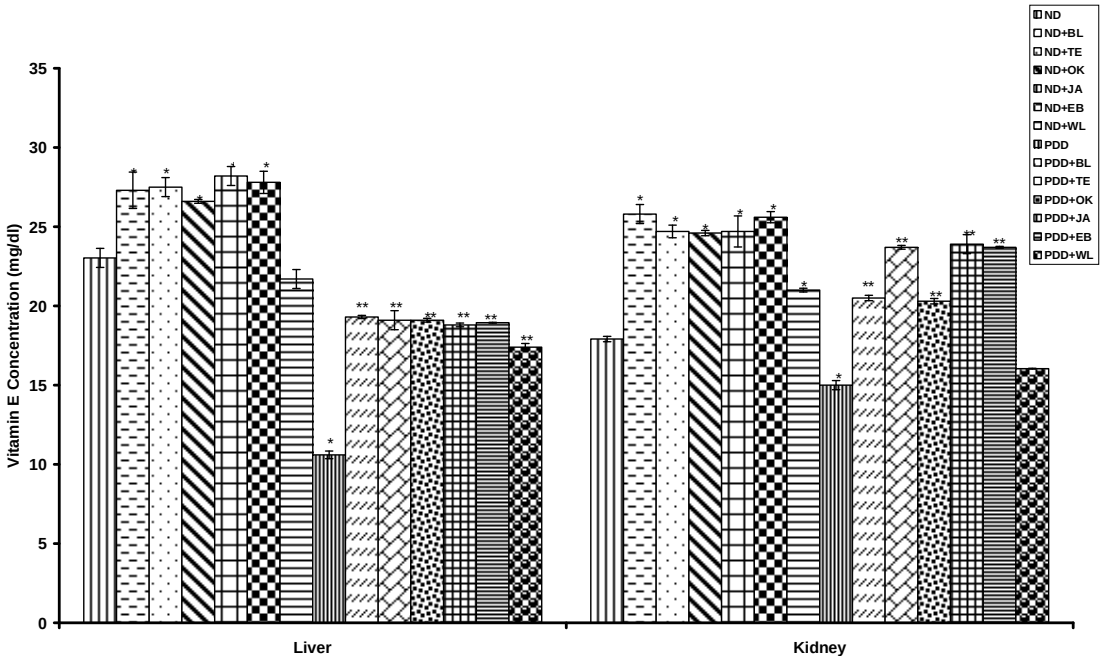

Figure 7: Effect of the Methanolic Extracts on Vitamin E Concentration in Rats

Values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test ($P < 0.05$). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. * Statistically significant ($P < 0.05$) relative to control (ND) group. ** Statistically significant ($P < 0.05$) when compared with protein deficient diet (PDD) fed group.

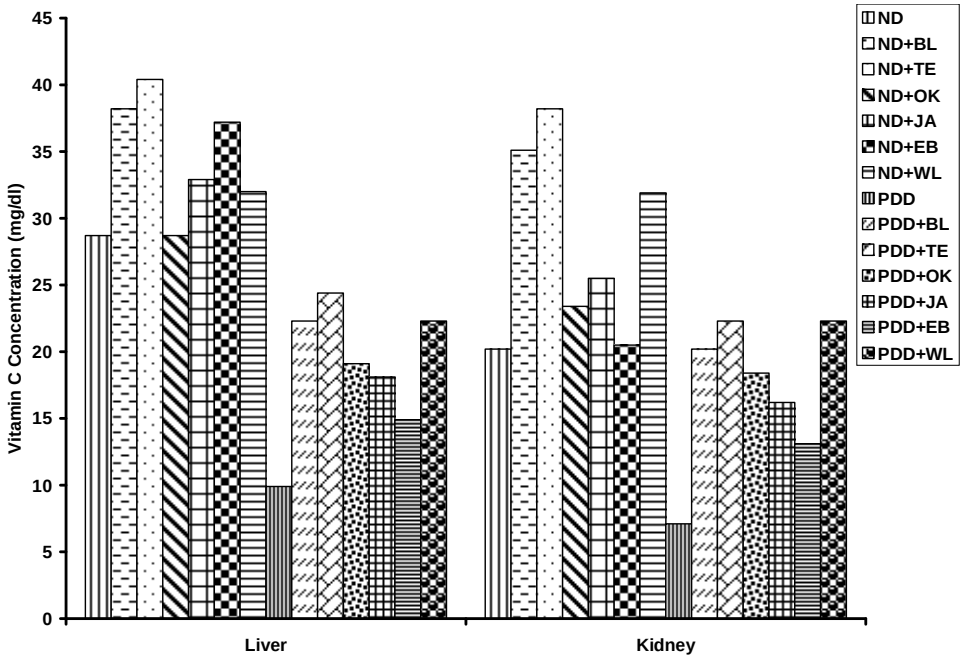

Figure 8: Effect of the Methanolic Extracts on Vitamin C Concentration in Rats

Values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test ($P < 0.05$). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. * Statistically significant ($P < 0.05$) relative to control (ND) group. ** Statistically significant ($P < 0.05$) when compared with protein deficient diet (PDD) fed group.

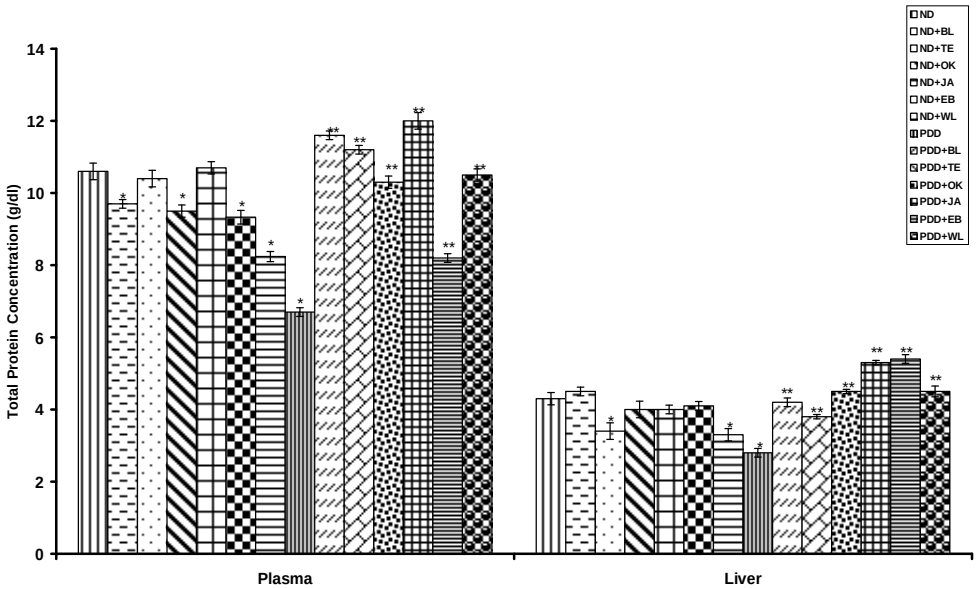

Figure 9: Effect of the Methanolic Extracts on Total Protein Concentration in Rats

All values represent mean ± SEM (n = 6). Differences between means were determined by one - way ANOVA and significant differences obtained by Duncan's multiple range test ($P < 0.05$). ND = Normal Diet; ND+BL = ND + Bitterleaf; ND+TE = ND + Tete; ND+OK = ND + Okazi leaf; ND+JA = ND + *Jatropha* leaf; ND+EB = ND + Ebolo leaf; ND+WL = ND + Waterleaf; PDD = Protein Deficient Diet; PDD+BL = PDD + Bitterleaf; PDD+TE = PDD + Tete; PDD+OK = PDD + Okazi leaf; PDD+JA = LPD+J = PDD + *Jatropha* leaf; PDD+EB = PDD + Ebolo leaf; PDD+WL = PDD + Waterleaf. * Statistically significant ($P < 0.05$) relative to control (ND) group.

** Statistically significant ($P < 0.05$) when compared with protein deficient diet (PDD) fed group.

DISCUSSION

In this study, six (6) commonly consumed plants in Nigeria were evaluated for their *in vitro* antioxidant activities. The antioxidant activities of the various extracts were also assessed *in vivo* in rats exposed to nutritionally challenged diets for six (6) weeks.

In vitro Antioxidant Study

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Due to its odd electron, 2,2–diphenyl–picryl–hydrazyl (DPPH) radical gives a strong absorption band at 517nm in visible spectroscopy (deep violet colour). As the electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes; the resulting decolourisation is stoichiometric with respect to the number of electrons taken up (Ayoola *et al*., 2008). Thus, the efficacies of antioxidants are often associated with their ability to scavenge stable, highly reactive, free radicals (Atrooz, 2009; Krishnaraju *et al*., 2009). This may be useful in treatment of radical related pathological damage. These highly reactive free radicals have been implicated in the pathology of different diseases in humans such as diabetes mellitus, artherosclerosis, cancer, Parkinson's disease and other neurodegenerative disorders (Krishnaraju *et al*., 2009; Aiyegoro and Okoh, 2009). It is also reported that antioxidant compounds such as phenols and other phytochemicals play a vital role in removing free radicals and in inhibition of lipid peroxidation (Atrooz, 2009; Krishnaraju *et al*., 2009; Aiyegoro and Okoh, 2009).

In the present study, all the plants extracts exhibited comparable DPPH free radical scavenging ability in a dose-dependent manner. The scavenging ability of the extracts may be a reflection of the total activities of various components present in these extracts (Wang *et al*., 2008; Rached *et al*., 2010). Indeed, several studies have reported that the antioxidant activity of most plants with therapeutic properties may be due to the presence of natural substances mainly phenolic compounds (Atrooz, 2009; Rached *et al*., 2010).

Plant phenolics, which occur widely in the plant kingdom, especially in fruits and vegetables, constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Maisuthisakul *et al*., 2007; Ayoola *et al*., 2008; Ebrahimzadeh *et al*., 2009). There is a strong relationship between total phenolic content and total antioxidant activity in selected fruits, vegetables and grain products (Demiray *et al*., 2009). The antioxidant capacity of phenolic compounds is mainly attributed to their redox properties, which allow them to act as

reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Demiray *et al*., 2009; Premanath and Lakshmidevi, 2010). In this study, the phenolic content of the extracts was varied and lower than that of the standard gallic acid.

The reduced phenolic content as found in the extracts, when compared with that of the standard gallic acid, may be due to the fact that phenolic compounds, depending on the number of phenolic groups, respond differently to the Folin-Ciocalteau reagent (Singleton *et al*., 1999; Wong *et al*., 2006). Nevertheless, the antioxidant properties of a single compound within a group can vary remarkably, so that the same levels of phenolics do not necessarily correspond to the same antioxidant response (Parejo *et al*., 2002; Demiray *et al*., 2009).

Flavonoids may account for part of the benefits associated with the consumption of fruits and vegetables. They have been reported to interfere with the activities of the enzymes involved in ROS generation, quenching of free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction (Wong *et al*., 2006; Aiyegoro and Okoh, 2009).

Proanthocyanidins are polymeric flavan-3-ols. They are the second most abundant group of natural phenolics (Prior, 2003). They are also effective antioxidants which provide several health benefits, including the prevention of cancer, urinary tract infection and cardiovascular diseases as well as the inhibition of LDL oxidation and platelet aggregation (Howard *et al*., 2000; Bors *et al*., 2000). The results from this study showed that all the extracts had considerably increased proanthocyanidin content with the highest and least values observed in *ebolo* and bitter leaf, respectively.

***In vivo* Animal Study**

Early physical features of PEM were observed particularly in the PDD fed rats, which include reduced food intake (anorexia), weight loss, reduced faecal output, hair loss and failure to thrive. These features were however less obvious in the PDD + extracts treated rats. These findings agree with previous literature on induction of PEM using a low protein diet (Omoregie and Osagie, 2011). Nutritional oxidative stress in PEM describes an inbalance between the pro-oxidant load and the antioxidant defense system in the body (Sies *et al.*, 2005).

In the malnourished state, there is a characteristic depression of the free radical defense mechanism including alterations in the activities of antioxidant enzymes, essential polyunsaturated fatty acids, vitamins and minerals elements are compromised, exposing the body's own antioxidant defense system to damage by ROS (Omoregie and Osagie, 2011). Malonaldehyde is the major oxidation product of peroxidized PUFAs and increased MDA level is an important indicator of lipid peroxidation. Catalase on the other hand, is an enzymatic antioxidant widely distributed in all animal tissues including the red blood cell and liver. Catalase decomposes H_2O_2 and helps protect the tissues from highly reactive hydroxyl radicals. SOD, another antioxidant enzyme, removes superoxide radical by converting it to H_2O_2 (Krishnaraju *et al*., 2009).

Similarly, in this study, the animals placed on the PDD diet for six (6) weeks showed appreciably reduced activities of the antioxidant enzymes SOD, CAT, vitamins E and C as well as high level of malonaldehyde (an index of lipid peroxidation) in the liver and the kidney. However, the extracts treated rats showed normal levels of these parameters probably due to the bioactive principles inherent in them. In this study, the reduced total protein in the PDD fed rats may be associated with the malnourished state of these animals. Moreover, past reports have shown that low level of protein is one of the important factors of anaemia in PEM (Omoregie and Osagie, 2007; Omoregie and Osagie, 2011). They affect the bone marrow erythroid activity and decrease haemoglobin content (Omoregie and Osagie, 2007). There was reversal of this effect in the extract treated rats.

The present study involved a comparative evaluation of the antioxidative activity of methanolic extracts from various plants both *in vitro* and *in vivo*. The overall antioxidative capacity of the extracts suggest a positive correlation as well as synergistic effects with respect to the DPPH scavenging activity, total phenol, flavonoid and proanthocyanindin components of the extracts. This indicates that the components are more likely to contribute to the antioxidant potential of the extracts (Tarawneh *et al*., 2010). But there were wide degrees of variations, between the plant extracts especially in their effectiveness as antioxidants. This was particularly obvious in *okazi* and bitterleaf extracts, which showed no correlation between their *in vitro* and *in vivo* antioxidant activities. It is possible that the *in vivo* antioxidant capacity observed in these extracts was not solely from the phenolic contents alone, but could be due to the presence of some other phytochemicals such as

ascorbic acid, tocopherol and pigments or the synergistic effects among them (Sengul *et al.*, 2009).

Furthermore, other factors may be responsible for this variation such as, the extracting solvent, the isolation procedures employed, the purity of active compounds, seasonal changes and the test system employed. All these factors may influence the synthetic pathways of the active compounds in the plant extracts (Gardeli *et al*., 2008; Tsai *et al*., 2008; Demiray *et al*., 2009; Rached *et al*., 2010). Extracts from *Jatropha tanjorensis, Crassocephylum rubens and Talinum triangulare* exhibited the most potent antioxidant activity both *in vitro* and *in vivo.*

The plants leaves may be considered as potential sources of natural antioxidants for therapeutic or industrial purpose and as alternative for the synthetic products, which are known for their multiple disadvantages. This study therefore confirms local claims on the efficacy of the plants leaves and may provide effective intervention for free radical mediated diseases. Indeed, our ancestors made soups and medicine from these plants leaves and fed them to vulnerable groups in the population including lactating mothers and feverish individuals.

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