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Effect of Methanol Leaf Extract of *Pterocarpus santalidoides* L' Herit ex DC on the Antioxidant and Lipid Profile of Wistar Rats

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ABSTRACT

The leaf of *Pterocarpus santalinoides* (Family: *Fabaceae*) is used in Nigerian folklore medicine to relieve symptoms of diabetes, treat diarrhea and facilitate wound healing. The aim of this study was to investigate the methanol leaf extract of *Pterocarpus santalinoides* (MEPS) for antioxidant and hypolipidemic potentials. Fifty-six (56) mature Wistar rats were assigned to four groups (n=14) and fed for 90 days with rat chow supplemented with varying concentrations of MEPS (2.5, 5.0 and 10.0 mg/kg feed). On the 30th, 60th and 90th days blood samples were obtained from the retro-orbital plexus of four rats randomly selected from each experimental group and subjected to antioxidant assay and lipid profile, following standard methods. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay was adopted for *in vitro* antioxidant evaluation of MEPS. The level of malondialdehyde was significantly ($p < 0.05$) decreased while catalase and superoxide dismutase were increased in the MEPS-treated rats by the different concentrations of MEPS. In the DPPH assay, MEPS (400 µg/mL) caused significant ($p < 0.05$) antioxidant activity (95.13%), comparable to 94.97% evoked by same concentration of ascorbic acid used as the reference drug. These results show that the methanol extract of *P. santalinoides* possesses significant antioxidant and hypolipidemic potentials.

***Keywords*:** Antioxidant, Lipid profile, Malondialdehyde, *Pterocarpus santalinoides,* Spectrophotometer.

Introduction

The antioxidant system of the body is made up of endogenous enzymes such as: catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase, as well as non-enzymatic (dietary) antioxidants which include: ascorbic acid (vitamin C), selenium, tocopherols and tocotrienols (vitamin E), carotenoids and polyphenols.[1] Antioxidant enzymes increase the rate of free radical quenching reactions while dietary antioxidants help to stabilize free radicals.[2,3] These actions of antioxidants protect cells from the deleterious effect of free radicals.[1,4] However, when free radicals or reactive oxygen species are produced excessively by the body (a condition known as oxidative stress) they cause damage to cell membranes, proteins, lipids and DNA.[5] It has been established that oxidative stress plays an important function in the pathophysiology of many diseases like: diabetes, cancer, ischemic perfusion injury, neurodegenerative and liver related diseases.[6-9]
Hyperlipidemia is one of the major causes of cardiovascular diseases.[10] Its prevalence is on the increase both in developed and developing countries.[11] According to literature, a positive correlation exists between oxidative stress and hyperlipidemia.[10] In view of the cost and side effects of chemically synthesized antioxidants and anti-dyslipidemic drugs which often result in patients' non-compliance, alternative remedies especially, herbal preparations are thus being explored.[12]

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According to Razack[1] and Rahman,[12] food supplements rich in bioactives of plant origin are potent recipes against oxidative stress and hyperlipidemia. This claim is supported by results of ethnopharmacological surveys which have shown that many commonly consumed herbs possess good hypolipidemic and antioxidant properties.[13]
The plant *Pterocarpus santalinoides* is a culinary vegetable that grows in Nigeria and some other West African countries.[14] Common names of the plant in some ethnic groups in Nigeria include: nturukpa (Igbo), gbengbe (Yoruba), gunduru (Hausa), nja (Efik), maganchi (Nupe), ikyarakwa or kereke (Tiv), and piegwu or uturukpa (Igede).[15] Among the rural natives in Nigeria, *P. santalinoides* leaf is used for treatment of stomach ache, diarrhea, diabetes mellitus as well as enhancement of wound healing.[16] This study sought to investigate the antioxidant and hypolipidemic potentials *P. santalinoides,* using albino rat model.

Materials and Methods

Plant collection and extraction
Leaves of *P. santalinoides* were harvested in May, 2014 from its habitat in Amaokwe Ugwu Nkpa in Abia State, Nigeria. Identification of the plant was done at the Bioresources Development and Conservation Program, Nsukka. A representative specimen with voucher number: MOUAU/VPP/2014/017 was deposited in the institution's herbarium. The leaves were air-dried and pulverized using an electric blender. The coarse powdered leaf sample (600 g) was extracted with 80% v/v methanol (1.8 L), by cold maceration method. The extract was oven-dried (40°C) after passing through a rotary evaporator, and stored as methanol extract of *Pterocarpus santalinoides* (MEPS) at 4°C until time of use.[17]

Animals
Mature (10-weeks old) Wistar rats bred in the Laboratory Animal Unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were procured for the study. The rats weighed

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116.6 ± 3.02 g. Stainless steel rat cages were used to house them, while pelleted feed (Vital feed®, Nigeria) and clean drinking water were served *ad libitum* to the rats. The experimental procedures were approved by the institution's Ethics Committee for use of Laboratory Animals (Approval No. MOUAU/CVM/REC/19011), and the rats were managed according to NIH Guidelines for Care and Use of Laboratory Animals.[18]

Preparation of experimental feed

The extract (MEPS) was incorporated in feed at graded concentrations (2.5, 5.0 and 10.0 mg/kg feed) by separately dissolving in 20 mL of water the quantity of MEPS required to formulate each concentration (based on the treatment duration, mean weight and standard feed consumption rate of rats). Afterwards, 810 mL of water per kilogram of feed was added to the reconstituted extracts, mixed thoroughly, pelleted and dried in a hot air oven (40°C). The experimental feed was stored in clean, moisture-free containers throughout the experiment.[19]

Determination of the in vitro antioxidant activity of MEPS

The *in vitro* antioxidant activity of MEPS was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay.[20] Different concentrations (50, 100, 200 and 400 µg/mL) of MEPS (2 mL) were mixed with 0.5 mM DPPH (in 1 mL of methanol) in a cuvette. One milliliter of methanol plus 2 mL of the extract was used as the blank while 1 mL of the 0.5 mM DPPH solution plus 2 mL of methanol served as the negative control. Ascorbic acid was used as reference standard.[9,21] The concentrations were prepared in triplicates and incubated for 30 minutes in the dark at room temperature (22-25°C). The absorbance was read at 517 nm and the percentage antioxidant activity calculated as follows: $\%\ antioxidant\ activity$

$$= \frac{100 - (\text{absorbance of sample} - \text{absorbance of blank})\ x\ 100}{\text{absorbance of control}}$$

In vivo antioxidant activity and lipid profile

Fifty-six mature Wistar rats were randomly assigned into 4 groups (n=14), with male and female rats housed in separate cages to prevent breeding. Group 1 rats served as control and received feed without extract. Rats in groups 2-4 were treated with MEPS at 2.5, 5.0 and 10.0 mg/kg feed respectively. All the rats were allowed free access to drinking water, but fed with 10 g feed/100 g body weight/day, which is the normal feed consumption rate for rats.[19] On days 30, 60 and 90, four rats were randomly selected from each group and weighed. Blood was collected from the retro-orbital plexus into plain sample bottles and allowed to stand for 30 minutes after which they were centrifuged at 2500 rpm for 10 minutes. Sera were harvested and stored at -18 °C until time of use.[22]

Antioxidant assay

The method of Draper and Hadley,[23] with some modifications was followed to estimate the serum malondialdehyde (MDA) level. The serum sample (0.1 mL) was mixed with 0.9 mL of distilled water in a test tube. A 0.5 mL of 25% Trichloroacetic acid (TCA) was added to the test-tube and centrifuged to obtain the protein-free supernatant, to which was added 0.5 mL of 1% thiobarbituric acid (TBA). The mixture was incubated for 1 hour at 95º C to allow the free MDA in the supernatant react with TBA and generate an MDA-TBA adduct, which was read colorimetrically at 532 nm.

A modified method of Atawodi [24] was used to determine the catalase activity. To a test-tube containing 2.8 mL of 50 mM Potassium phosphate buffer (pH 7.0), 10 µL of the serum sample was first added, followed by 0.1 mL of freshly prepared 30 M hydrogen peroxide. The decomposition rate of hydrogen peroxide was estimated after 5 minutes at 240 nm on a spectrophotometer.

ElabScience® SOD assay kit (ElabScience Biotechnology Company Ltd, South Africa) was used to determine superoxide dismutase (SOD) activity. The kit is based on the hydroxylamine method which relies on xanthine oxidase to estimate the activity of SOD, with a Chem5V3 semi-automated clinical Chemistry analyzer, (Erba Diagnostics, Germany) set at 550 nm.[25]

Lipid profile

The manufacturer's instructions outlined in Randox® test kits were followed to determine the serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL).[26-29]

Statistical analysis

The data obtained from the study are presented as Mean ± S.E.M. and analyzed by means of One Way Analysis of Variance, using SPSS, version 20. Separation of the variant means was done by the Least Significance Difference of the various groups. Values of $p < 0.05$ were accepted as significant.

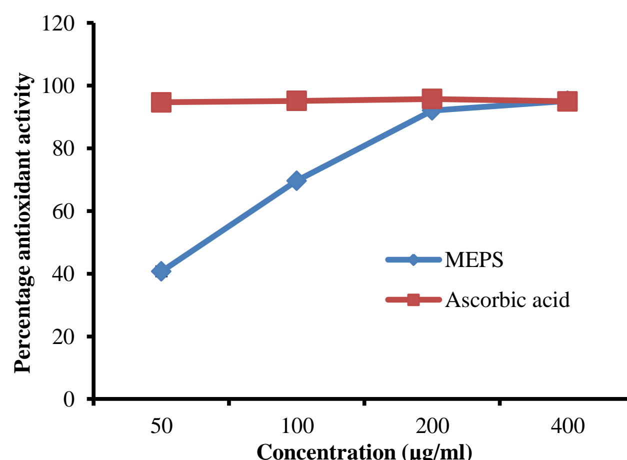
Results and Discussion

In vitro antioxidant assay

The DPPH photometric assay tested the free radical scavenging potential of MEPS *in vitro*. The extract exhibited significant ($p < 0.05$), concentration-dependent effect, with the highest concentration of MEPS (400 µg/mL) causing an antioxidant activity of 95.13%, which compares to 94.97% evoked by same concentration of ascorbic acid (Fig. 1). This result suggests that MEPS contains bioactive compound capable of donating hydrogen ion to a free radical thereby removing the odd electron which accounts for the radical's reactivity.[30]

In vivo antioxidant activity

Malondialdehyde is a direct product of lipid peroxidation and its concentration in the serum is usually elevated in conditions associated with oxidative stress.[31] Hence, determination of MDA level is employed for testing substances with antioxidant potential. In this experiment, MEPS significantly ($p < 0.05$) reduced the level of MDA in the treated groups, when compared to the control (Fig. 2). This signifies the ability of MEPS to effectively ameliorate lipid peroxidation, which is a common cause of cell damage via disruption of the cell membrane.[32] Catalase is a hemoprotein involved in minimizing oxidative injury to cells through hydrogen peroxide detoxification.[33,34] Catalase activity in this study was significantly ($p < 0.05$) increased in the test groups with respect to the control rats, by the varying doses of MEPS throughout the duration of the experiment (Table 1). Superoxide dismutase is an important antioxidant enzyme which catalyzes the dismutation of superoxide, thus prevents the reaction of nitric oxide with superoxide anion to yield reactive peroxynitrite .[35,36] The activity of SOD in the rats treated with 2.5 mg/kg MEPS did not differ significantly ($p > 0.05$) from that of the control on day 30, however, higher doses of the MEPS (5.0 and 10.0 mg/kg feed) caused significant ($p > 0.05$) increase in the activity of the enzyme.



MEPS: Methanol extract of *Pterocarpus santalinoides*

Figure 1: *In vivo* antioxidant activity of *P. santalinoides*, using the DPPH spectrophotometric assay.

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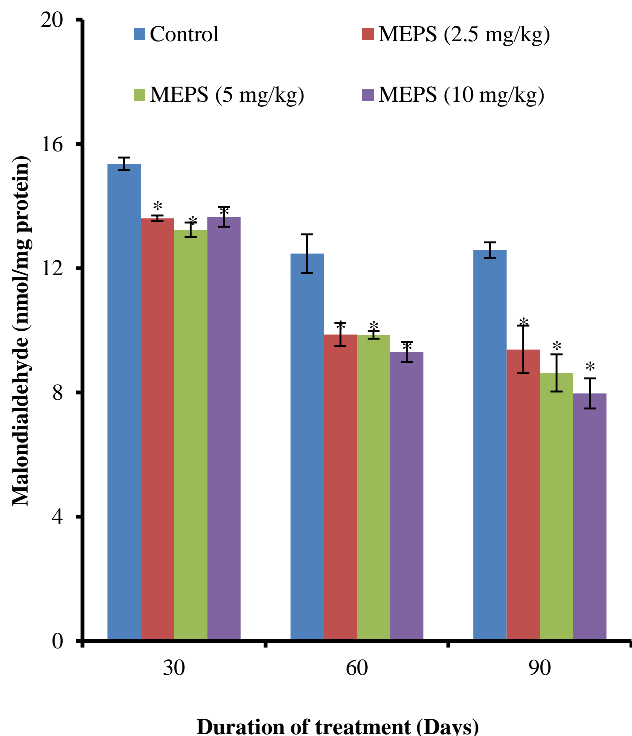

Figure 2: Malondialdehyde levels of rats fed with MEPS for 90 days. *p < 0.05 when compared to control. MEPS: Methanol extract of *Pterocarpus santalinoides*

On the 60th and 90th days, MEPS at all the doses tested significantly ($p < 0.05$) increased the level of SOD in the extract-treated groups when compared to the control (Table 1). The significant increase in SOD activity in the treated rats by MEPS further suggests effective antioxidant potential of MEPS. The *in vivo* antioxidant activity of MEPS in this study agrees with Ihedioha *et al.*,[25] who reported significant decrease in serum MDA levels and elevation of SOD and CAT by *P. santalinoides* in carbon tetrachloride-intoxicated rats. It is therefore likely that consumption of *P. santalinoides* leaf will enhance the antioxidant defense system of the body, and consequently help to prevent and/or manage oxidative stress-related diseases.

Lipid profile

The serum total cholesterol and triglycerides levels in the MEPS-treated groups did not vary significantly ($p > 0.05$) from their values in the control rats on day 30, however on days 60 and 90 there were significant ($p < 0.05$) decreases in total cholesterol and triglycerides levels in the MEPS-treated rats when compared with the control (Table 2). Also, on day 30, there was no significant ($p > 0.05$) difference in HDL between groups treated with MEPS and the untreated (control) group, but on days 60 and 90, MEPS induced significant ($p < 0.05$) increase in HDL levels in the treated groups when compared to the control (Table 2). Throughout the duration of the study, the level of LDL was significantly ($p < 0.05$) reduced by all doses of MEPS in the treated groups when compared with the control (Table 3). The lipid-modulatory effect of MEPS suggests that the extract may limit cholesterol biosynthesis and boost endogenous production LDL receptors whose role is to incorporate LDL into hepatocytes, resulting in decreased LDL/HDL ratio observed in the MEPS-treated rats.[37] This hypolipidemic effect of MEPS indicates that *P. santalinoides* may be of benefit in the management or treatment of health conditions linked with hyperlipidemia such as diabetes and cardiovascular diseases predisposed or exacerbated by atherosclerosis.[38]

Table 1: Effect of 90 days administration of MEPS on catalase and superoxide dismutase activities in Wistar rats

Treatment	Day 30		Day 60		Day 90	
	CAT	SOD	CAT	SOD	CAT	SOD
Control	0.37 ± 0.02	37.1 ± 1.0	0.41 ± 0.02	36.7 ± 0.8	0.36 ± 0.05	38.3 ± 1.2
MEPS (2.5 mg/kg)	0.39 ± 0.01*	38.5 ± 0.3	0.49 ± 0.04*	42.4 ± 0.4*	0.43 ± 0.03*	45.5 ± 0.6*
MEPS (5.0 mg/kg)	0.39 ± 0.03*	41.3 ± 0.4*	0.50 ± 0.05*	47.2 ± 0.4*	0.45 ± 0.08*	49.9 ± 1.3*
MEPS (10 mg/kg)	0.40 ± 0.01*	47.9 ± 0.8*	0.56 ± 0.02*	49.8 ± 0.5*	0.54 ± 0.01*	49.9 ± 0.7*

CAT = Catalase (μmol/mg protein); SOD = (IU/mg protein); MEPS = Methanol extract of *Pterocarpus santalinoides* * $p < 0.05$ when compared with control

Table 2: Effect of MEPS on total cholesterol (TC) and triglycerides (TG) of Wistar rats

Treatment	Day 30		Day 60		Day 90	
	TC	TG	TC	TG	TC	TG
Control (No extract)	73.9 ± 2.1	78.9 ± 2.1	89.9 ± 1.1	64.6 ± 0.3	73.4 ± 1.0*	71.2 ± 1.8
MEPS (2.5 mg/kg)	72.0 ± 1.1	67.3 ± 12.5	86.0 ± 0.5*	61.2 ± 0.3*	63.8 ± 0.8*	63.8 ± 0.9*
MEPS (5.0 mg/kg)	75.6 ± 1.6	62.7 ± 0.6	83.7 ± 1.0*	61.4 ± 0.1*	65.1 ± 0.5*	62.5 ± 1.1*
MEPS (10.0 mg/kg)	73.1 ± 0.3	78.8 ± 1.5	77.4 ± 0.5*	51.0 ± 0.3*	65.1 ± 0.1*	60.3 ± 0.9*

TC = Total cholesterol (mg/dl); TG = Triglycerides (mg/dl); MEPS = Methanol extract of *Pterocarpus santalinoides*
* $p < 0.05$ when compared with control

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Table 3: Effect of MEPS on serum HDL and LDL levels of Wistar rats

Treatment	Day 30		Day 60		Day 90	
	HDL	LDL	HDL	LDL	HDL	LDL
Control (No extract)	47.8 ± 1.4	15.8 ± 0.4	37.5 ± 0.7	13.9 ± 0.3	34.5 ± 2.1	13.2 ± 0.6
MEPS (2.5 mg/kg)	48.2 ± 9.4	09.9 ± 0.3*	46.4 ± 0.4*	10.2 ± 0.3*	42.6 ± 0.1*	10.8 ± 0.5*
MEPS (5.0 mg/kg)	50.9 ± 1.5	12.5 ± 0.1*	41.7 ± 0.2*	11.1 ± 0.2*	42.6 ± 0.7*	10.3 ± 0.6*
MEPS (10.0 mg/kg)	48.2 ± 1.7	12.8 ± 0.3*	44.9 ± 0.3*	08.2 ± 0.2*	44.7 ± 1.3*	10.1 ± 0.8*

HDL = High density lipoprotein (mg/dl); LDL = Low density lipoprotein (mg/dl); MEPS = Methanol extract of *Pterocarpus santalinoides*
* $p < 0.05$ when compared with control

Conclusion

The results of this study reveal that methanol leaf extract of *Pterocarpus santalinoides* possess significant antioxidant and hypolipidemic activities. Further studies may be needed to demonstrate the importance of *P. santalinoides* in the treatment of diseases associated with oxidative stress and hyperlipidemia.

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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