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ANTIOXIDANT AND FREE RADICAL SCAVENGER EFFECTS OF METHANOL LEAF EXTRACT OF *LUPINUS ARBOREUS.*

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ABSTRACT
Objective: To investigate the antioxidant and free radical scavenger profiles of the leaf extract of *Lupinus arboreus.* **Methods:** The crude methanol extract (CME) of the dried leaves obtained by cold maceration for 72 h were analyzed by the methods of 1,1-diphenyl–2–picrylhydrazyl (DPPH), phosphomolybdenum reduction assay, metal ion chelating activity, reducing power assay as well as superoxide radical scavenging assay. **Results:** The methanol extract of *L. arboreus* recorded IC_{50} value of 37.50 µg/ml for DPPH assay; Phosphomolybdenum value was 525± 40.28 mg ascorbic acid equivalent (AAE)/g extract; chelating ability was 35.33± 1.05 mg/EDTA/g extract. The reducing power at varying concentrations (50 – 250) µg/ml were 0.070 to 0.310 absorbance at 700 nm. The superoxide radical scavenging assay showed the IC_{50} value of 235.05 ± 3.55 µg/ml. **Conclusion:** The extract of *L. arboreus* possessed therapeutic value for natural antioxidant and free radical scavenging effects.

KEYWORDS: *Lupinus arboreus,* antioxidant, free radicals, DPPH, AAE.

1. INTRODUCTION

A wide range of pathological tendencies are caused by free radicals produced by ultraviolet light, sunlight, ionizing radiation, metabolic processes and clinical reactions. These include cardiovascular diseases, hepatic disorders, aging, carcinogensis and neuro-degenerative diseases.[1] Oxygen no doubt, is among the most essential factors required to sustain life but Reactive Oxygen Species (ROS) or free radicals are deleterious to the physiological system. By creating oxidative stress due to generation of free radicals above the antioxidant available in physiologic system, ROS may lead to lipid peroxidation, protein denaturation, and disruption of membrane fluidity.[2,3] Hence, chemicals called antioxidants interact with free radicals and neutralize them thereby frustrating the chain reaction associated with damage of vital molecules and organs. Beta-carotene, vitamins C and E are the principal micronutrient antioxidants among several enzyme systems that scavenge free radicals within the physiological body. In addition, trace metal called selenium is essential for harmonious function of one of the antioxidants enzyme systems. Paradoxically, the body relies on exogenous dietary sources mainly for the micronutrients since it cannot synthesize them although it manufactures some of the antioxidant endogenously to neutralize free radical.[4] In cells, endogenous chemicals can be classified as non-enzymatic and enzymatic antioxidants. By reduction reaction, the first line of defense against free radicals is superoxide dismutases (SOD). It is known to catalyze the dismutation of O_2^- (Superoxide anion radical) into H_2O_2 (hydrogen peroxide) which is transformed into selenoprotein.

Other major antioxidant enzymes directly linked to the neutralization of ROS and RNS include: catalase (CAT), glutathione reductase (GR_X) and glutathione peroxidase (GP_X). The GP_X enzyme eliminates H_2O_2 by employing it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). With NADPH as a source of reducing power, a flavoprotein enzyme glutathione reductase, generates GSH from GSSG. In the case of non-enzymatic antioxidants, they are further divided into metabolic and nutrient antioxidants. The former referring to endogenous antioxidants such as lipoid acid, L-ariginine, glutathione, melatonin, bilirubin, uric acid, transferrine, metal-chelating proteins, and coenzyme Q10, which are produced by body metabolism; while the later belong to exogenous compounds which cannot be synthesized in the body hence provided via dietry supplements and include trace metals (zinc, manganese, selenium), flavonoids, vitamins C and E, carotenoids, omega -3 and -6 fatty acids.[5–7]

Because of consumer preference, natural antioxidants are in top demand as food additives, nutraceuticals, and bio-pharmaceuticals.[8] Preponderance of herbs and spices that have been reported to exhibit antioxidant properties are known to possess compounds such as flavonoids, flavones, isoflavones.[9,8] *L. arboreus* has been

documented to contain plethora of pytochemicals including flavones and flavonoids[10,9]; exerts antinociceptive and anti-inflammatory[9], antimicrobial[11], antipyretic and antiemetic[12] effects. In the present investigation, the antioxidant and free radical scavenger activities of *L. arboreus* have been assessed.

2. Materials and methods

2.1. Plant materials

From Owerri, Imo State, Nigeria, the fresh leaves of *L. arboreus* were collected and officially identified at the Department of Pharmacognosy, Madonna University, Elele, Nigeria, where a voucher specimen (M/PC/199/10) has been deposited in the herbarium. The leaves were air-dried for 28 days at room temperature.

Fine powder (2 kg) of the ground leaves were extracted using absolute methanol (Sigma Aldrich, Germany) for 48 h. The crude methanol extract obtained after filteration was concentrated using a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany) and further oven-dried and stored in a refrigerator.

2.2. DPPH Radical Scavenging Assay

The sample (1 ml) was diluted to 50 ml with distilled water. Five different concentrations (40 – 200 μl) were taken from the diluted sample, and 2.5 ml of 0.1 mN methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. At 517 nm, the absorbance of the sample was measured against the blank (methanol). Percentage radical scavenging activity of the sample was calculated using the following relationship:

% DPPH radical scavenging activity =

$$\frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

The analysis was performed in triplicate.[13]

The concentration of the sample providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3. Phosphomolybdenum Reduction Assay

An aliquot of 0.1 ml sample solution was combined in a 4 ml vial with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank (methanol).[14] The results reported are mean values expressed as milligram of ascorbic acid equivalent/gram extract.

2.4. Metal ion chelating activity

Extract (400 μl) was added to 50µl of solution of 2 Mn $FeCl_2$. The reaction was initiated by the addition of 200µl of 5 Mm ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water)[15]. The results reported in the chelating of ferrous ions of extract are mean values expressed as mg EDTA/g extract.

2.5 Reduction power

Extract (40 – 200 μg) was taken in 1 ml of phosphate buffer and 5 ml of 0.2 M phosphate buffer (pH 6.6) was added. To this, 5 ml of 1% potassium ferricyanide solution was added and the mixture was incubated at 50°C for 20 min. After the incubation, 5 ml of 10% TCA was added. The content was then centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 ml) was mixed with 5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance of the reaction mixture was read spectroscopically at 700 nm.[16]

2.6 Superoxide Radical Scavenging Assay

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentration (50 – 250 μg) of sample extracts. The assay was based on the capacity of the sample to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. The absorbance was measured at 590 nm immediately after illumination. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as:

% superoxide radical scavenging activity =

$$\frac{(\text{control OD} - \text{sample OD})}{\text{Control OD}} \times 100$$

The analysis was performed in triplicate.[17]

The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.7 Statistical Analysis

The results were statistically analyzed and expressed as mean ± standard deviation.

3. RESULTS

The evaluation of antioxidant and free radical scavenger activity of methanol extract of *L. arboreus* using DPPH radical scavenging assay showed IC_{50} value of 37.50µ g/ml calculated from the graph (Fig. I). The phosphomolybdenum value was 525±40.28 mgAAE/g extract. The maximum chelating ability was 35.33±1.05 mg/EDTA/g extract (Table I). The reducing power assay at varying concentrations (50 – 250) µg/ml recorded 0.070 to 0.310 absorbance at 700 nm (Table II). The IC_{50}

value of superoxide radical scavenging assay showed 235.05±3.55 μg/ml calculated from the graph (Fig. II).

Table 1: Phosphomolybdenum and metal ion chelating activity of *L. arboreus*.

Phosphomolybdenum (mgAAE/g extract)	Metal ion chelating (mgEDTA/g extract)
525±40.28	35.33±1.05

Table II: Reduction power activity of *L. arboreus* extract.

Concentration (μg/ml)	Absorbance at 700 nm
50	0.070
100	0.140
150	0.200
200	0.290
250	0.310

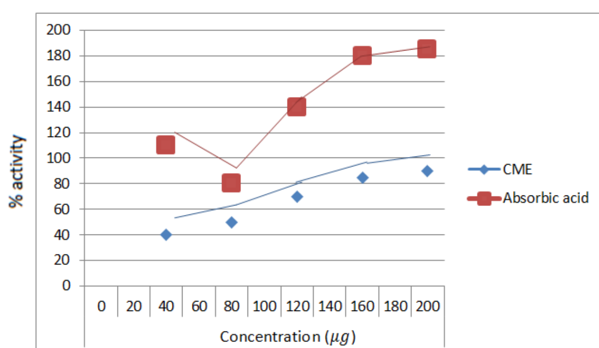

Figure I: DPPH radical scavenging activity of *L. arboreus* extract.

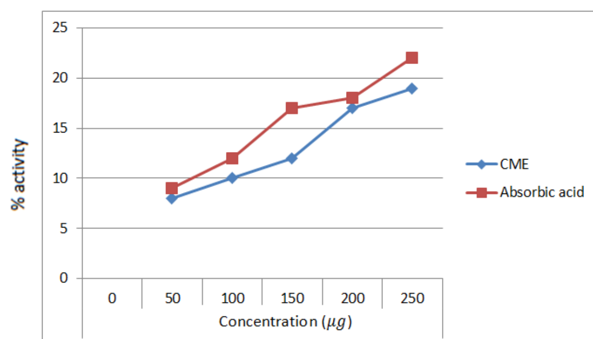

Figure II: Superoxide radical scavenging activity of *L. arboreus* extract.

4. DISCUSSION

4.1 DPPH radical scavenging effect

The result of the antioxidant and free radical scavenger activity of *L. arboreus* leaves extract was found to be IC_{50} value of 37.50μg/ml. The lower IC_{50} value represents the higher antioxidant activity of the extract. The DPPH scavenging capacity of *L. arboreus* may not be unrelated to the flavonoids and phenolic compounds present.[18,11,10, 9] This is in agreement with reports that spices and herbs exhibiting free radical scavenging effect contain phytochemicals such as polyphenols, flavonoids and phenolic compounds.[8] DPPH radical is a stable radical widely used to assess the ability of substances to act as free radical scavengers or hydrogen donors hence to evaluate the antioxidant activity. DPPH radical has a maximum absorption at 517 nm which can readily undergo reduction reaction by an antioxidant. The convenience and ease of this reaction confer its widespread use in assessment of free radical-scavenging activity.[19] It should be noted that as it expresses the quantity of antioxidant needed to reduce its radical concentration by 50%, IC_{50} values are negatively related to the antioxidant activity.

4.2 Phosphomolybdenum assay

The result showed the phosphomolybdenum value as 525±40.28 mg AAE/g extract (Table I). Higher phosphomolybdenum value represents higher antioxidants activity and this method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound with the formation of green phosphate/Mo (V) complex showing the maximal absorption at 695nm.[14]

4.3 Metal ion chelating activity

The result showed that the methanol leaf extract of *L. arboreus* has maximum chelating ability of 35.33±1.05 mg/EDTA/g extract (Table 1). This is in consonance with the iron chelating data that the extract can play protective role against oxidative damage by sequestering Fe(II) ions that may otherwise catalyzed Fenton type reactions or involve in metal-catalyzed hydroperoxide decomposition reactions.[20] Though an essential mineral for normal physiological function yet, an excess of iron may lead to cellular injury. Under Fenton reaction, reduced metals may form reactive hydroxyl radicals hence contributing to oxidative stress.[8] The ability to chelate or deactivate transition metals, which has the tendency to catalyze hydroperoxide decomposition and Fenton type reactions, is an important mechanism of antioxidant activity.

4.4 Reducing Power

The colour of the test solution turns to green and blue colours which depend on the reducing power of each compound. It was observed that the reductive capabilities increase with increasing of concentration in ascorbic acid and in extract (Table II). By measuring the formation of pearls Prussian blue, reducing power can be monitored at 700 nm, in which the presence of antioxidant causes the conversion of Fe^{3+}/ferricyanide complex into ferrous.[21] The compounds with reducing power means that they are election donors hence can act as primary and secondary antioxidants, so reducing power is closely blended with antioxidant activity[14]

4.5 Superoxide radical scavenging assay

The result showed that the extract possessed potent free radical scavenging activity compared to the standard (ascorbic acid) at low IC_{50}. The IC_{50} of *L. arboreus* extract was found to be 235.05±3.55μ g/ml. Graphically represented (Fig. II), it was noted that as concentration

rises, the % scavenging increases linearly for the extract and the standard (ascorbic acid), revealed by the regression analysis. The superoxide radical scavenging assay is based on generation of super oxide radical by auto oxidation in the presence of light of riboflavin. The super oxide radical reduces Nitro blue tetrazolium (NBT) to a blue coloured formazan that at 560 nm can be measured. Note, *in-vitro* super oxide radical scavenging activity is measured by riboflavin/light/Nitro blue tetrazolium reduction. Super oxide can form singlet oxygen and hydroxyl radical, over production of super oxide anion radical leads to redox imbalance which is associated with harmful physiological consequences.[22]

CONCLUSION

From the various *in-vitro* assays, it could be concluded that extract of *L. arboreus* may be a probable source of natural antioxidant and hence of pharmacological importance.

Conflict of interest: We have not declared any conflict of interest.

Source of support: Nil.

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