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RESEARCH ARTICLE

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Evaluation of Antioxidant Potential of *Melanthera scandens*

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Abstract
A methanol extract of dried leaves of *Melanthera scandens* was examined for antioxidant activities using a variety of assays, including 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, ferrous chelating, and ferric thiocyanate methods with ascorbic acid and EDTA as positive controls. The extract showed noticeable activities in most of these *in vitro* tests. The amount of phenolic compounds in the extract expressed in gallic acid equivalent was found to be 52.8 mg/g. The extract demonstrated inhibition of linoleic acid lipid peroxidation, active reducing power, and DPPH radical scavenging activities which were less than that of the positive controls. The extract also showed weaker iron chelating effect when compared with the EDTA positive control. The present results showed that *M. scandens* leaf extract possessed antioxidant properties and this plant is a potential useful source of natural antioxidants.

1. Introduction

Oxidation and reduction reactions are essential processes in all living organisms, but intermediates known collectively as reactive oxygen species, such as superoxide radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2) are also generated during some reactions [1,2]. These species can react with biological substrates, such as DNA and proteins, leading to degenerative diseases, ageing, cancer, arthritis, and atherogenesis [3,4]. Antioxidants are compounds that function as free radical scavengers, complexers of prooxidant metals, reducing agents, and quenchers of singlet-oxygen formation. They are regarded as protection agents minimizing reactive oxygen species-related oxidative damage in the body and reducing chronic diseases and lipid peroxidation [5–7]. There is increasing interest in compounds with antioxidant activity that could be used as supplements in humans and, thus, naturally occurring antioxidants, especially of plant origin, are a major area of scientific research [8–10]. *Melanthera scandens* (Schum. & Thonn.) Roberty (family Asteraceae) is a scrambling or scandent herb of waste thickets commonly dispersed in forest areas and widely distributed across tropical Africa, providing forage for all stock in thicket edges around villages and being a favorite food of hares in southern Nigeria. In the Ivory Coast, the leaves are used as a purgative and an antidote against poisoning, and the leaf decoction is used as a soothing cough mixture and for sore throat. In Nigeria, pulped, decocted, or macerated

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leaves are used as hemostatic preparations, applied to cuts and wounds to promote healing and curb inflammation [11]. Six saponins have been reported to have been isolated from *M. scandens* leaves and their structures elucidated by two-dimensional nuclear magnetic resonance techniques, including rotating frame nuclear Overhauser effect Spectroscopy. They are 3-O-beta-D-glucuronopyranosyl-oleanolic acid, 3-O-[beta-D-xylopyranosyl(1–>4) beta-D-glucuronopyranosyl]-oleanolic acid, 3-O-([beta-D-glucopyranosyl(1–>2)][beta-D-xylopyranosyl-(1–>4)]beta-D-glucuronopyranosyl)-oleanolic acid, and the corresponding 28-O-beta-D-glucosides [12]. *In vitro* antiplasmodial activity of a *M. scandens* extract against a chloroquine-resistant FcB1 strain of *Plasmodium falciparum* has revealed weak antimalarial activity [13]. Studies in our laboratory revealed the presence of phenolic compounds in the leaf extract. The relationship between phenolic compounds and antioxidant activity is well documented [14]; thus it was considered rational to determine the antioxidant potential of the extract.

2. Materials and Methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent, linoleic acid, ammonium thiocyanate, [4,4′-[3-(2-pyridinyl-1,2,4-triazine-5,6-diyl]bisbenzenesulfonic acid] (ferrozine), 90% 1,1-diphenyl-2-picrylhydrazyl (DPPH), $FeCl_2$ tetrahydrate, gallic acid, anhydrous sodium carbonate, anhydrous ferric chloride, potassium ferricyanide, and trichloroacetic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ascorbic acid and all other chemicals were of analytical grade from BDH Chemical Laboratory (England, UK).

2.2. Plant material

The aerial part of *M. scandens* was collected at Ibadan, in Oyo, Nigeria and authenticated by Mr. K.I. Odewo of the Forest Research Institute of Nigeria, Ibadan, Nigeria after comparison with the voucher specimen with number FHI 94215. The leaves were plugged out and dried at 30ºC and milled to produce fine powder.

2.3. Extraction

About 500 g of powdered plant material was extracted with 3 L of methanol using a Soxhlet apparatus for 48 hours and the resulting extract concentrated under reduced pressure using a rotary evaporator until a semisolid sticky mass was obtained. The yield was 12.63% weight in weight.

2.4. Determination of total phenolic content

The total phenolic content of the *M. scandens* extract was determined with Folin-Ciocalteu reagent [15]. Gallic acid equivalents (GAE, mg/g) were determined from a calibration concentration curve. The extract (100 mg/mL, 1.0 mL) was mixed thoroughly with 5 mL Folin-Ciocalteu reagent (diluted 10-fold) and after 5 minutes, 4.0 mL of sodium carbonate (0.7 M) was added and the mixture allowed to stand for 1 hour with intermittent shaking. The 765 nm absorbance of the resulting blue color was measured in a spectrophotometer, with all determinations in triplicate and the values for total phenolic content expressed as GAE.

2.5. Determination of DPPH radical scavenging activity

The free radical scavenging activities of *M. scandens* extract was measured using DPPH and the method of Yen and Chen [16] with modifications. A 1.0 mL volume of extract (0.2–5.0 mg/mL) was diluted to 20% of the original concentration with methanol, 1.0 mL of 1 mM methanolic DPPH added, and the mixture shaken vigorously and allowed to stand in dark for 30 minutes. The 517 nm absorbance was then measured by spectrophotometer, with ascorbic acid as a positive control and deionized water as a blank. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity.

2.6. Determination of iron chelating ability

The extract's ferrous ion chelating activity was estimated by a ferrozine assay [17], in which 1.0 mL of extract (0.2–5.0 mg/mL) was mixed with 3.7 mL of deionized water and added to 1.0 mL of 1 mM $FeCl_2$. The reaction was initiated by the addition 0.2 mL of 5 mM ferrozine and then the mixture shaken vigorously and left to stand for 20 minutes. The 562 nm absorbance was measured by spectrometer, with EDTA as a positive control. The results were expressed as percentage inhibition of ferrozine-Fe^{2+} complex formation, with the ability to chelate ferrous ion determined using the formula given below:

$$\text{Chelating effect } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the ferrozine-Fe^{2+} complex and A_{sample} the absorbance in the presence of extract or standard.

2.7. Determination of reducing ability

The reducing ability of the extract was determined by the method of Lai et al with modifications [6].

Different concentrations of extract (0.1–5.0 mg/mL) in 1 mL of distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6, and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid added, and then the mixture centrifuged at 1000 g for 10 minutes. The upper 2.5 mL layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and the 700 nm absorbance measured against a blank by spectrophotometer, with ascorbic acid as a positive control. Increased absorbance of the reaction mixture indicated increased reduction capability.

2.8. Determination of antioxidant activity

Antioxidant activity of the extract was determined according to a ferric thiocyanate method carried out in a linoleic acid emulsion [18]. Different concentrations of extract (0.5–8.0 mg/mL) were mixed with a 0.56% w/v linoleic acid emulsion, pH 7.0, and 2 mL of 0.2 M phosphate buffer, pH 7.0, and incubated at 60°C in the dark for 12 hours to accelerate oxidation. A 4.5 mL volume of 75% ethanol, 0.2 mL of 4 M ammonium thiocyanate solution, 0.1 mL of sample solution, and 0.2 mL of 10 mM ferrous chloride in 3.5% HCl were mixed in sequence and, after 3 minutes, the 500 nm absorbance for the resulting red color measured by spectrometer. High absorbance indicated high linoleic acid emulsion oxidation and the percentage inhibition of lipid peroxidation in linoleic acid emulsion calculated as follows:

$$\text{Inhibition } (\%) = [(A_o - A_1)/A_o] \times 100$$

where A_o was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample of extract or standard compounds.

2.9. Statistical analysis

Data were expressed as mean ± SD. Analysis of variance was performed by ANOVA procedure and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

The content of phenolic compounds in *M. scandens* extract measured by Folin-Ciocalteu reagents expressed in GAE was found to be 52.8 mg/g. Plant phenolics remain a prominent class of compounds with antioxidant or free radical scavenging properties; phenolics act as reducing agents and free radical quenchers by delocalization of gained electrons and stabilization due to the aromatic nucleus resonance effect, thus preventing the continuation of free radical chain reaction [19,20]. The high phenolic content of this leaf extract suggested that antioxidant activity of the extract may be related to its phenolics.

The DPPH method is commonly used to determine the antioxidant activity of phenolic compounds and plant extracts and was used here to determine the hydrogen atom or electron donating ability of *M. scandens* extract and the standard antioxidant ascorbic acid. A significant decrease ($p < 0.05$) in DPPH· concentration was observed due to the scavenging power of the extract and ascorbic acid (Figure 1). The radical scavenging effect of the extract, ascorbic acid, and butylated hydroxytoluene on DPPH· concentration decreased in the order: ascorbic acid > extract with EC_{50} values of 0.05 and 0.43 mg/mL, respectively, which suggested that the extract's radical scavenging ability played a role in its antioxidant property but the effect was significantly less ($p < 0.05$) than that of synthetic drugs used as positive controls. DPPH· is a stable free radical with an 517 nm absorption band due to its odd electron and it loses this absorption when reduced by an antioxidant or free radical species as the electron becomes paired, leading to a stoichiometric decolorization from purple to yellow depending on the number of electrons gained [21,22].

The ability of the extract and ascorbic acid to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the 700 nm absorbance after incubation. Reductants supply electrons to the reduced species and, in antioxidant substances, reductants cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, causing the test solution to change color from yellow to green or blue, based on the substance's reducing ability [23]. In this study, there was a steady increase in the reductive potential of the extract and ascorbic acid

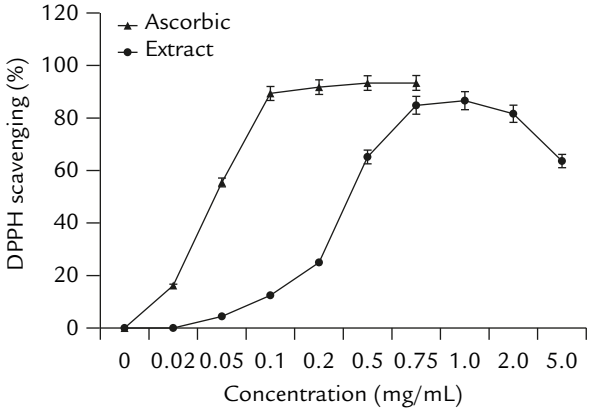

Figure 1 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effect of extract and ascorbic acid. Values presented as mean ± SD ($n = 3$).

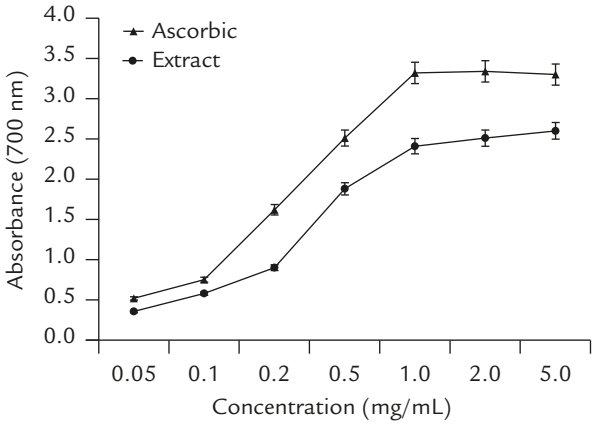

Figure 2 Reductive effect of extract and ascorbic acid. Values presented as mean±SD (n=3).

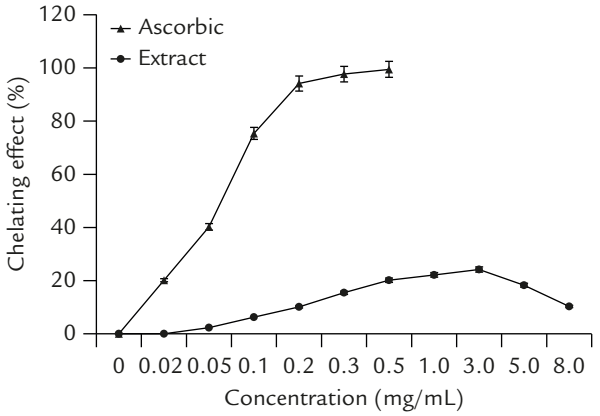

Figure 3 Iron chelating effect of extract and ascorbic acid. Values presented as mean±SD (n=3).

with increased concentration (Figure 2), but the extract showed lower activities than the synthetic antioxidant. The extract may have acted as a scavenger by neutralizing free radicals through donating electrons and rendering the radicals stable, thus also quenching the free radical chain [24].

Transition metals such as iron have been implicated in the conversion of $\cdot O_2^-$ and H_2O_2 into highly reactive OH that could lead to serious damage to membranes, proteins, and DNA. This could result in cellular death, carcinogenesis, or mutagenesis [25,26]. Ferrous chelating activities of the extract, ascorbic acid, and butylated hydroxytoluene are shown in Figure 3. Ferrozine complexes with Fe^{2+} quantitatively and is thus commonly used for estimation of ferrous chelating capacity of compounds. In this study, the extract and EDTA inhibited the formation of ferrous and ferrozine complexes and led to fading of test solutions' red color, which can be measured to assess the chelating ability of compounds [27]. The chelating activity here was found to increase with increased concentrations, but the extract demonstrated a moderate metal chelating ability which was significantly less ($p<0.05$) than EDTA, a known metal ion chelator. This suggested that the iron binding ability of the extract played a small role in its antioxidant activity.

The ferric thiocyanate method in a linoleic acid system is commonly employed in assessing the antioxidant activity of compounds, measuring the amount of peroxide produced [18]. The extract and the positive control ascorbic acid demonstrated a dose dependent activity, increasing with increasing concentrations. The percentage inhibition of lipid peroxidation by ascorbic acid and *M. scandens* extract was 46.4% and 22.5%, respectively, at a dose of 5.0 mg/mL, suggesting that the lipid peroxidation protection ability of the extract was less than that of ascorbic acid (Figure 4). The inhibition of lipid peroxidation may also be involved in the antioxidant activities of the extract.

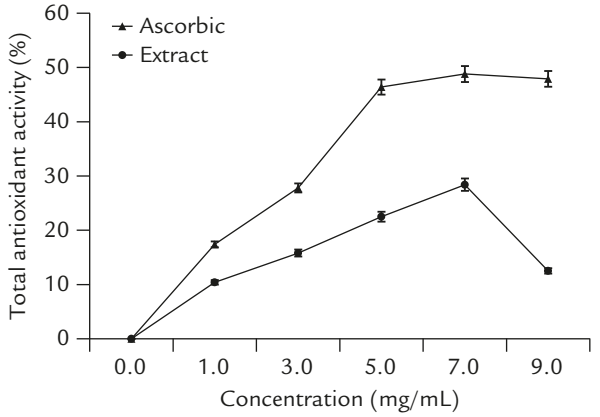

Figure 4 Effects of extract and ascorbic acid on linoleic acid peroxidation. Values presented as mean±SD (n=3).

4. Conclusions

In this study, *M. scandens* leaf extract was found to be an effective antioxidant using different assay techniques, such as reducing power assessment, ferric thiocyanate, and DPPH scavenging methods. Its activities were however less than the ascorbic acid positive control. The extract is a potential source of natural antioxidants.

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