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Original Article

Dennettia tripetala seeds inhibiting ferrous sulfate-induced oxidative stress in rat tissues *in vitro*

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ABSTRACT

Objectives: This study was designed to investigate the antioxidant activity of pepper fruit (*Dennettia tripetala*) seed extract. **Methods:** Phenol content, reducing activity, Fe(II)-chelating ability, and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical-scavenging ability of the extract were consequently examined whereas the antioxidant potentials and inhibitory effect of *D. tripetala* seed on $FeSO_4$-induced oxidative stress in some rat tissues were evaluated. **Results:** The seed had a high concentration of total phenol. Incubation of the liver and brain tissue homogenates in the presence of Fe^{2+} caused a major increase in the malondialdehyde (MDA) contents of the homogenates. However, the aqueous seed extract of *D. tripetala* caused a significant decrease in the MDA contents of the liver and brain in a dose-dependent manner. The aqueous extract of the seed had a considerably higher inhibitory effect on Fe^{2+}-induced lipid peroxidation in the rat brain than the liver homogenates. **Conclusion:** The high inhibitory effect of *D. tripetala* seed is attributable to its high phytochemical content, Fe^{2+}-chelating ability, DPPH scavenging ability, and reducing power. These results suggest that the seed extract of *D. tripetala* possesses body antioxidant pool-improving potentials which may contribute to the prevention of the incidence of neurodegenerative maladies.

KEY WORDS: Antioxidant pool, *Dennettia tripetala*, 1,1-diphenyl-2 picrylhydrazyl radical, malondialdehyde

INTRODUCTION

Oxidative stress plays a foremost role in the progression of chronic and degenerative ailments such as cancer, autoimmune maladies, rheumatoid inflammation, cataract, aging, and cardiac and neurodegenerative diseases [1,2]. However, consumption of diets rich in secondary metabolites could help combat degenerative diseases caused by oxidative stress by improving the body's antioxidant status [3].

High levels of iron play a crucial role in degenerative diseases by acting catalytically in the generation of reactive oxygen species (ROS) that has the potentials to wreck cellular lipids, nucleic acids, amino acids, and starch leading to an extensive range of injury in cellular function and integrity. ROS can directly attack the unsaturated fatty acids of the biomembranes and induce lipid peroxidation. Malondialdehyde (MDA) is the end product of lipid peroxidation, a process in which ROS degrades polyunsaturated fatty acids. The role of the antioxidative defense system such as superoxide dismutase, catalase (CAT), glutathione peroxidase, and various diet antioxidants has been recognized [4] in combating oxidative damage.

The need to explore natural supply of novel bioactive agents has increased in the past three decades [5]. Focus is shifting from synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole to the use of natural antioxidants as a result of toxicity [3] and instability of the synthetic ones at elevated temperature [6]. Endogenous antioxidants in plants may play vital roles in antioxidative defenses against oxidative injury [7], possibly by conserving the biological functions of cells. There is increasing interest in the protective biological role of natural antioxidants confined in dietary vegetation that are candidates for the deterrence of oxidative damage. Some of these antioxidants are polyphenol compounds that are found in all plant parts.

Dennettia tripetala G. Baker (*Annonaceae*), additionally called pepper fruit, is a well-known Nigerian spicy ethno-medicinal plant found within the tropical rain forest region of Nigeria and typically in Savanna areas. It is regionally referred to as "Nkarika" by the Efiks of Calabar. *D. tripetala* fruit has additionally been reportedly used as spice in flavoring food, and as seasoning added to prepared food such as meat, sausages, soups, and vegetables. The leaves, fruits, seeds, roots, and stem of the plant are used as food. Apart from nutrients supplied by spices, they contain several secondary metabolites with potential natural antioxidant properties [8].

Peppers are a crucial agricultural crop, not solely due to their economic importance, but also for the nutritional value of its fruits, primarily because they are an excellent source of natural

colors and antioxidant compounds [9]. The intake of these compounds in diets is a crucial health-defensive factor. It was recognized as being beneficial for the prevention of prevalent human diseases, comprising cancer and cardiovascular diseases, when taken daily in adequate amounts. A wide spectrum of antioxidants present in pepper fruits includes vitamin C, phenol, and carotenoids [10]. Several studies have correlated the antioxidant activity of plant diets, comprising pepper, with their total phenol content [11-13].

D. tripetala seed has been supposedly utilized in traditional medicine for the management or prevention of diseases related to oxidative stress. In the recent years, there has been rummage around for natural antioxidants, thus this study seeks to evaluate the antioxidant potentials and properties of *D. tripetala* seed as typified by its total phenolic content, radical scavenging, and iron-chelating activities to substantiate the previously reported traditional use and to analyze doable mechanisms by which this effect is exerted.

MATERIALS AND METHODS

Sample Collection

Fresh samples of *D. tripetala* seed were purchased from a local market, in Ado-Ekiti metropolis, Nigeria, and authenticated at the Department of Plant Science, Ekiti state University, Ado-Ekiti, Nigeria.

Chemicals and Reagents

Chemicals were purchased from Sigma-Aldrich, reagents used were bought from BDH Chemicals. All other chemicals and reagents were of analytical grade.

Aqueous Extract Preparation

The sample was washed, dried at room temperature, and powdered. It was kept dry in air-tight containers prior to the analysis. One gram of the powdered sample was weighed into 20 ml of distilled water and was left for 24 h. It was filtered and the filtrate centrifuged for 10 min and the clear supernatant collected was used for subsequent analysis.

Determination of Total Phenol Content

The total phenolic content was determined using the method reported by Singleton *et al.* [14]. Appropriate dilutions of the extract were oxidized with 2.5 ml of 10% Folin–Ciocalteu reagent (v/v) and neutralized by 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was read at 765 nm. The total phenolic content was expressed as milligrams gallic acid equivalent (GAE)/g dry weight.

Assessment of Lipid Peroxidation

Production of thiobarbituric acid-reactive species (TBARS) from animal tissues was evaluated using the modified method of Ohkawa *et al.* [15]. The rats were sacrificed by cervical dislocation method. The liver and brain were removed and placed on ice. 1 g of the tissues was homogenized in cold 0.1 M tris buffer at pH 7.4 (1:10 w/v) with about 10 up and down strokes in a Teflon-glass homogenizer. The homogenates were centrifuged at 12,000 rpm for 5 min to yield a supernatant which was used for the assay and pellet that was discarded. The supernatant (100 μl) with or without 50 μl of the freshly prepared pro-oxidant (iron[II] sulfate), 100 μl of the plant extract at different concentrations (0.02-0.06 ml), and an approximate volume of distilled water which gives a total volume of 300 μl were incubated at 37°C for 1 h. The color reaction was carried out by adding 200, 500, and 500 μl of each of 8.1% sodium dodecyl sulfate (SDS), 1.33 M acetic acid (pH 3.4), and 0.6% TBA, respectively. The reaction mixture was incubated at 97°C for 1 h, and the absorbance was read in a spectrophotometer after cooling the tubes at a wavelength of 532 nm and expressed as MDA produced (% control) using MDA standard curve (0-0.035 mM).

1,1-Diphenyl-2 Picrylhydrazyl (DPPH) Free Radical Scavenging Activity

Radical scavenging ability of the extracts against DPPH radical was evaluated as portrayed by Liyana-Pathirana and Shahidi [16]. A solution of 0.135 mM DPPH was prepared in methanol. The solution (1 ml) was mixed with 1 ml of the extract (8.3-33.33 μl). The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

$$(Abs_{control} - Abs_{sample})/Abs_{control} \times 100\%$$

$Abs_{control}$: Absorbance of DPPH radical + methanol
Abs_{sample}: Absorbance of DPPH radical + sample extract/ standard.

Iron(II) (Fe^{2+}) Chelation Assay

The Fe^{2+}-chelating ability of the extract was examined employing a changed protocol of Minotti and Aust [17] with a small modification proposed by Puntel *et al.* [18]. Freshly prepared 500 μM $FesSO_4$ (150 μl) was added to a reaction mixture containing 168 μl of 0.1 M tris-HCl (pH 7.4), 218 μl saline, and therefore the extract 100 μl (40-200 μl). Reaction mixture was incubated for 5 min, before 13 μl of 0.25% 1, 10-phenanthroline (w/v) was added. Afterward, the absorbance was read at 510 nm. Then, Fe(II)-chelating ability was calculated with respect to the control.

Percentage Fe^{2+}-chelating ability (%) = $(Abs_{control} - Abs_{sample})/Abs_{control} \times 100$

$Abs_{control}$: Absorbance of the control (reacting mixture without the test sample)
Abs_{sample}: Absorbance of reacting mixture with the test sample.

Determination of Reducing Property

Reducing potentials of the extract was employed by assessing the ability of the extract to scale back $FeCl_3$ solution as explained by modified method of Wu *et al.* [19]. In brief, 2.5 ml of sample was added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 25 min, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture and centrifuged for 10 min. Five milliliters of upper layer solution was mixed with 2 ml distilled water and 0.5 ml of 0.1% $FeCl_3$ (0.1%) in the tube. After 10 min, the solutions were read at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Distilled water was used as blank, ascorbic acid was the reference standard.

Data Analysis

The results of the replicates were pooled and expressed as mean ± standard deviation. Analysis of variance and Student's *t*-test were carried out [20]. Significance was accepted at $P < 0.05$. The extract concentration causing 50% (EC_{50}) inhibition of lipid peroxidation in the rat tissues' EC_{50} for pepper extracts was examined.

RESULTS

The *D. tripetala* seed extract presented a relatively high phenolic content with the level of 130.20 mg GAE/g. The ability of aqueous extract of *D. tripetala* to inhibit Fe^{2+}-induced lipid peroxidation was determined in the liver and brain homogenates, as shown in Tables 1 and 2. The result revealed that incubation of the liver homogenate in the presence of Fe^{2+} caused a statistically significant ($P < 0.05$) increase in the MDA content of the rat liver homogenate compared with the basal as shown in Table 1. However, the aqueous seed extract of *D. tripetala* inhibited MDA production in rat's liver in a dose-dependent manner (0.02-0.06 mg/ml). Nevertheless, judging by the EC_{50} values, the plant had a significantly high (EC_{50} = 0.094 mg/m, $P < 0.05$) inhibitory effect on Fe^{2+}-induced lipid peroxidation in the liver homogenate.

Incubation of rat brain tissue homogenates in the presence of Fe^{2+}-induced lipid peroxidation also caused a statistically significant increase ($P < 0.05$) in the rat's brain MDA content as shown in Table 2; however, the extract inhibited MDA production content in the tissue in a dose-dependent manner (0.02-0.06 mg/ml). On the other hand, the plant had a statistically significantly high (EC_{50} = 0.078 mg/ml, $P < 0.05$) inhibitory effect on Fe^{2+}-induced lipid peroxidation in the brain homogenate.

Figure 1 presents the DPPH radical scavenging ability of the *D. tripetala*. The results revealed that aqueous extract of *D. tripetala* scavenged DPPH radicals in a concentration-dependent manner (8.3-33.33 µl/l). Figure 2 displays the Fe^{2+}-chelating ability of the *D. tripetala*. The results revealed that *D. tripetala* can chelate Fe^{2+}. It also demonstrated that the extract possesses strong Fe(II)-chelating activity.

DISCUSSION

Phenolics, which are secondary metabolites in plants [21], and mycofungi [5] have received much attention in the recent years. *D. tripetala* seed extract presented a relatively high phenolic content compared to some other Nigerian spices [6] and some wild mushrooms [13]. The high phenolic content of

Table 1: Antioxidant activity of aqueous extract of *D. tripetala* seeds on iron sulfate-induced lipid peroxidation in rat liver homogenates

Treatment groups	*D. tripetala* concentration (mg/ml)	MDA (µM/g tissue)
Normal	-	2.71 ± 0.64^a
Control	-	20.36 ± 2.01^b
Iron+seed extract	0.02	1.9 ± 0.2^c
Iron+seed extract	0.03	1.77 ± 0.2^c
Iron+seed extract	0.04	1.56 ± 0.2^d
Iron+seed extract	0.05	1.2 ± 0.26^e
Iron+seed extract	0.06	0.86 ± 0.12^f

Results are expressed as means of three experiments in duplicate±standard deviations; values with different superscript letters differ significantly ($P<0.05$) by Duncan's multiple range test. *D. tripetala: Dennettia tripetala,* MDA: Malondialdehyde

Table 2: Antioxidant activity of aqueous extract of *D. tripetala* seeds on iron sulfate-induced lipid peroxidation in rat brain homogenates

Treatment groups	*D. tripetala* concentration (mg/ml)	MDA (µM/g tissue)
Normal	-	1.62 ± 1.11^a
Control	-	18.49 ± 0.78^b
Iron+seed extract	0.02	0.82 ± 0.22^c
Iron+seed extract	0.03	0.74 ± 0.22^c
Iron+seed extract	0.04	0.58 ± 0.23^d
Iron+seed extract	0.05	0.46 ± 0.19^d
Iron+seed extract	0.06	0.26 ± 0.21^e

Results are expressed as means of three experiments in duplicate±standard deviations; values with different superscript letters differ significantly ($P<0.05$) by Duncan's multiple range test. *D. tripetala: Dennettia tripetala,* MDA: Malondialdehyde

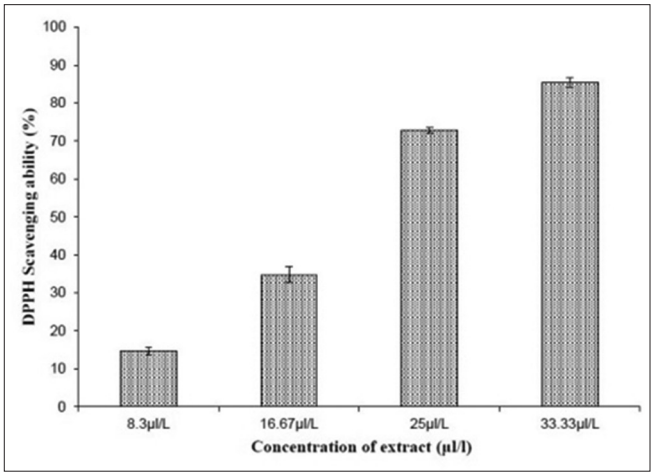

Figure 1: 1,1-diphenyl-2 picrylhydrazyl radical scavenging activity of aqueous extract of *Dennettia tripetela* seeds

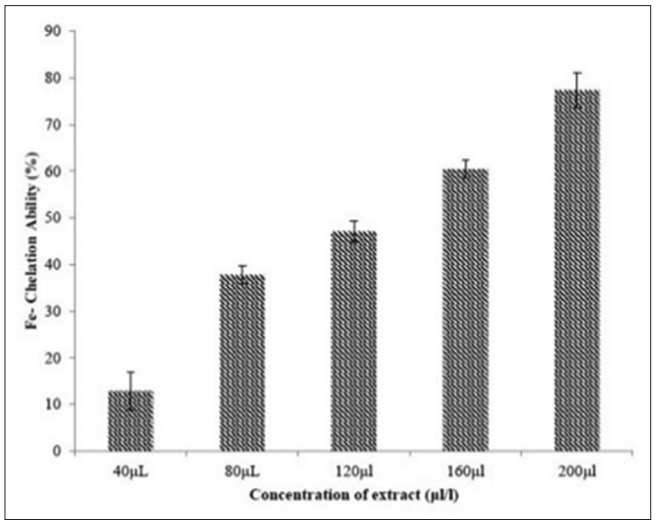

Figure 2: Iron-chelating ability of aqueous extract of *Dennettia tripetela* seeds

D. tripetala seed extract corroborates the reports of Lu *et al.* [22] that the main active component in spices includes phenolics, flavonoids, and essential oils. Polyphenolic compounds have shown inhibitory effects on mutagenesis and carcinogenesis in humans on ingestion of up to 1 g of diets rich in fruits and vegetables daily [23].

Countless studies have shown that healthful plants with antioxidative potentials would possibly act as radical scavengers, reducing mediators, chelating mediators for transition metals, quenchers of singlet oxygen molecules, and activators of antioxidative defense enzymes to suppress radical injury in biological system [5,13,24].

The discovery that Fe^{2+} caused a considerable increase in the MDA content of the liver and brain homogenates is in agreement with earlier report that revealed Fe^{2+} to be a powerful originator of lipid peroxidation [25]. Increased lipid peroxidation in the presence of Fe^{2+} may be well attributed to the fact that Fe^{2+} will catalyse one-electron transfer reactions that produce ROS like the highly reactive hydroxyl radical, that is made from H_2O_2 through the Fenton reaction [26]. Therefore, doable depletion of iron might decrease oxidative stress throughout the entire body [27].

The ability of the water-extractable secondary metabolites from *D. tripetala* seed to inhibit Fe^{2+}-evoked lipid peroxidation within the liver homogenate is shown in Table 1. The decrease in the Fe^{2+}-elicited lipid peroxidation in the rat tissue homogenates in the presence of the extract may be as a result of the capability of the extracts to scavenge radicals made by the Fe^{2+}-catalysed production of ROS within the liver homogenate. Elevated Fe^{2+} content within the brain had been connected to a bunch of neurodegenerative diseases. Although the water-extractable secondary metabolites from the spices caused a concentration-dependent momentous decrease within the MDA content of the Fe^{2+}-stressed brain homogenate and *D. tripetala* vital smothered MDA production within the rat brain tissue homogenates [Table 2], the decrease in the Fe^{2+}-induced lipid peroxidation within the rat brain homogenates in the presence of the extracts might be as result of the ability of the extracts to chelate iron (Fe^{2+}) [28].

D. tripetala aqueous seed extracts at totally different concentrations (8.3-33.33 µl/l) revealed considerable DPPH scavenging activity. This assay validates the radical scavenging activity of the aqueous seed extract. DPPH radicals are often scavenged by substances capable of donating atom resulting in a reduced form of DPPH, resulting in decoloration of the violet DPPH solution. The degree of decoloration is a sign of the novel scavenging potentials of the substance which may be thought of as antioxidants and radical scavengers [29]. The antiradical scavenging activity of the *D. tripetala* aqueous seed extracts mentioned in this study is owing to the presence of phenols that usually possesess high metal-chelating activity. This result correlates with the reports of Jazek *et al.* [30], Sadi *et al.*, [31] and Ojo *et al.* [32,33].

Figure 2 reveals the iron-chelating ability of the aqueous extract of *D. tripetala* seed. Fe^{2+} chelation was found to be concentration dependent. This result agrees with those of Fe^{2+}-elicited lipid peroxidation [Tables 1 and 2]. This is often not sudden as the high phenolic content of the seed extract which already suggested its potential antioxidant properties. This suggest that metal chelation may be one of the possible mechanisms through which antioxidant secondary metabolites from *D. tripetala* seed forestall lipid peroxidation in tissues by forming a complex with metal, so preventing the initiation of lipid peroxidation.

In conclusion, aqueous extracts of *D. tripetala* contains giant amounts of phenolic compounds, exhibiting elevated antioxidant and radical scavenging activities. The extract additionally chelates metal and own reducing activity. These assays specify that *D. tripetala* extract is an important supply of antioxidant, which could be helpful in thwarting the development of various oxidative stress. The scavenging activities discovered against DPPH and hydroxyl radicals, in addition to the protective activities against lipid peroxidation, led us to propose *D. tripetala* extract as a promising natural supply of antioxidants appropriate for application in nutritional or pharmaceutical fields within the prevention of radical-mediated diseases.

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