Original Research Article

In Vitro Antioxidant Potentials of Ethanol Extract of Ripe and Unripe *Dennetia tripetala* Fruits.

ABSTRACT

Introduction: *Dennettia tripetala* G baker fruit (pepper fruit) is a popular Nigerian plant from the family of plant known as Annonaceae. The whole fruit (flesh and seed) is usually consumed as snacks and it is oftentimes accompanied by alcohol (ufofop) or added to dishes as spice due to its peculiar strong pepperish taste and sweet aroma. It is traditionally used for the treatment of cough, toothache, fever, diabetes and nausea in pregnancy. Its antioxidant, anti-hyperglycemic, anti-inflammatory and anti-inociceptive effects has been established *in vivo.* **Aim**: The present study is aimed at evaluating the antioxidant potentials of ethanol extract of ripe and unripe DT fruit *in vitro.* **Method**: the antioxidant activity of the ethanol extract of DT was evaluated spectrophotometrically using various *in vitro* models like 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and hydrogen peroxide scavenging activity; metal chelating activity and reducing power. Vitamin C was used as the standard antioxidant. **Results:** Vitamin C, ripe and unripe extract scavenged the free radicals in a dose dependent manner; displayed a competitive inhibition of DPPH, and H_2O_2 radical; chelate metal ions and reduced Fe^{3+}; however, the ripe extract was more potent at scavenging DPPH and H_2O_2 radicals; and had higher reducing power than the unripe DT extract; while the unripe DT extract was more potent at chelating metal ions even better than standard ascorbic acid. **Conclusion:** the antioxidant potentials of ethanol extracts of ripe and unripe DT were studied *in vitro* and results have shown that they are potent antioxidant in nature and can supplement our diets as a rich source of natural antioxidants for health protection. **Conclusion:** Non-invasive independent predictors for screening esophageal varices may decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability.

Keywords: Antioxidant, DPPH, hydrogen peroxide, metal chelating activity, reducing power

1. INTRODUCTION

Plants remain a common and readily available source of food and medicine either in the form of traditional concoction or pure active agents. Much of the observed therapeutic effects of plants have been linked to their potential antioxidant activity. It is documented that 2/3 of the worlds plant species have medicinal value [1] and almost all of the plants have potent antioxidant potentials [2] According to literature, plants have a very efficient complex enzymatic and non-enzymatic antioxidant defense system to prevent free radical mediated toxic effects. Enzymatic antioxidant defense system includes glutathione peroxidase, catalase, superoxide dismutase; while enzymatic antioxidant include glutathione, vitamin A, E, C [3] These antioxidants also play dual functions: (1) they help in the performance of their normal physiological functions and protection from microbes. 2) they help plants cope with environmental stress [4]

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Plants phenolics perform antioxidant roles by acting as an electron donor. This is due to the presence of multiple hydroxyl ions; they are capable of donating hydrogen ions to stabilize unstable free radicals. Phenolics also have reducing power, metal ion chelating activity [5] and the ability to act as signaling molecules in cells through the modulation of protein kinase and lipid kinase signaling pathways [6,7]. In phyto research, in vitro investigations are often made for 2 reasons (1) to screen and attribute antioxidant potential to plants as their secondary metabolites (2) to understand the possible mechanism of action of plants

antioxidant [8] Various assays are used to evaluate plants abilities as electron donor, reducing agents and metal chelators. Example of these assays include DPPH scavenging potential, metal chelating activity, ABTS, ferric reducing potentials etc.

Pepper fruit (*Dennettia tripetala*) is widely cultivated by the people of Cameroun, Ivory Coast and Southern Nigeria. It is called mmimi in Igbo, Ako in Edo, Ata Igbere in Yoruba, nkarika in Ibibio. Phytochemical screening of the fruits revealed the presence of tannins, flavonoids, terpenes, alkaloids, cardiac glycosides etc. Its traditional usage includes treatment of cough, diarrhea, common cold, diabetes, nausea in pregnancy [9,10,11]. The aim of this study is to evaluate the anitioxidant potentials of ethanol extract of ripe and unripe DT fruits.

2. MATERIALS AND METHODS

All materials and reagents used in this work were of analytical standards and grades.

2.1 Collection/Preparation of Plant Sample

The fruits of *Denettia tripetala* (both ripe and unripe) were obtained from Marian Market, Calabar, Cross River State. The samples were authenticated by a Botanist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State.

2.2 Extraction of *Dennettia tripetala* fruits

Denettia tripetala fruits (both ripe and unripe) were ground and dried under room temperature. The powder was stored in an airtight container until further use. Ground fruits of *Dennettia tripetala* (500 g) were extracted by macerating in 80% ethanol for 72 hr and filtered. The filtrate was concentrated in a water-bath at 45ºC to dryness. It was weighed and stored in a refrigerator at -4ºC until required for use.

2.3 Chemicals

Chemicals used in the study were ascorbic acid, butylated hydroxyl toluene, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ferric chloride, sodium phosphate, ammonium molybdate, ferrous ammonium sulphate, ethylenediaminetetraacetic acid (EDTA), dimethyl sulphoxide(DMSO), ammonium acetate, glacial acetic acid, trichloroacetic acid, sodium dihydrogen phosphate, disodium hydrogen phosphate and ferrozine. All the chemicals were purchased from British Drug House (BDH) England and all solvents used were of analytical grade.

2.4 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) Determination

Free radical scavenging activities of ethanol extract of *Dennettia tripetala* fruit and vitamin C were prepared at concentrations of 20, 40, 60, 80 and 200 µg/mL and were determined in accordance with the Shimada[12] method, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. DPPH was added to the solutions prepared and stirred vigorously. The mixtures were kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank [12]. The ability to scavenge the DPPH radical was calculated using the following equation:

% inhibition of DPPH radical = $[A_0 – A_1] / A_0) \times 100]$ where
A_0 is the absorbance of the control reaction and A_1=absorbance in the presence of the sample of ripe and unripe DT.

2.5 Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al.* [13] A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). 0.4ml hydrogen peroxide was added to ethanol extract (20-100 µg/ml) and absorbance value of the reaction mixture was recorded at 230 nm after 10 mins. Blank solution contained phosphate buffer without H_2O_2. The percentage of hydrogen peroxide scavenging was calculated thus:

% scavenging (H_2O_2) = $[(A_i – A_t) / A_i] \times 100$ where
A_i is the absorbance of control and A_t is the absorbance of test.

2.7 Reducing Power Assay

The reducing power ability of the extracts of ripe and unripe DT fruits was evaluated by the method described of Oyaizu [14]. The reaction mixture contained 1.0 mL of various concentrations of extracts (20–100 µg/mL), 2.5 mL of 1% potassium

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ferricyanide and 2.5 mL of 0.2 mol/L of 0.2 M sodium phosphate buffer. The mixture was incubated at 50◦C for 20 mins, cooled rapidly and the reaction ended by the addition of 2.5 mL of 10% trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 2.5 mL of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. After 10 min, the absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. Vitamin C was used as standard.

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2.6 Chelating Ability on Ferrous Ions

The ferrous ion chelating potential of the extracts were evaluated by Dinis *et al.* [15] method with slight modifications. The reaction mixture contained 2.0 mL of various concentrations of the extracts (20–100 µg/mL) and 0.1 mL of 2 mM/L $FeCl_3$. The reaction was initiated by the addition of 0.2 mL of 5 mM/L ferrozine, shaken vigorously and allowed at room temperature for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher Fe^{2+} chelating ability. The control contained all the reagents except sample. Ascorbic acid was used as standard. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

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% inhibition = [1-(Absorbance of test/Absorbance of control)] x 100

2.7 Statistical Analysis

Data were expressed as Mean ± Standard error of mean (SEM)(n = 3). Values differ significantly at P < .05 were inspected using SPSS software. The 50% inhibitory concentration (IC_{50}) of ethanol exract of ripe and unripe DT fruits were estimated from regression analysis of concentration-inhibition curves using EXCEL.

3. RESULTS AND DISCUSSION

The study was carried out to evaluate the free radical scavenging potential of ripe and unripe DT fruits. The results in Table 1 -4 showed that both fruits scavenged DPPH, H_2O_2 free radicals, chelated iron metals and reduced Fe^{2+} in a dose dependent manner. The IC_{50} which is the concentration of extracts that is able to inhibit 50% of the free radicals generated was used to measure the potency of the ripe and unripe fruit extracts. Notably, the concentration of the IC_{50} is inversely proportional to the potency of a plant material.

Free radical scavenging activities are mandatory due to the detrimental roles of free radicals in foods and in biological systems. Various methods are currently used to evaluate the antioxidant activity of plant polyphenols. Most chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. DPPH and H_2O_2 radical scavenging methods are common spectrophotometric procedures for examining the antioxidant potency of plant extracts.

Table 1: Effect of ethanol extract of ripe and unripe DT fruit on 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical activity

Conc. (µg/ml)	RDT (%)	UDT (%)	Vitamin C (%)
20	15.15 ± 0.24	12.09 ± 0.35	87.86 ± 0.11
40	15.67 ± 0.32	14.32 ± 0.23	88.46 ± 0.11
60	23.66 ± 0.03	20.30 ± 0.34	89.46 ± 0.70
80	24.18 ± 0.40	22.31 ± 0.23	90.14 ± 0.04
100	25.52 ± 0.23	23.06 ± 0.12	90.66 ± 0.07
IC_{50}	276.63	549.23	12.92

Data presented as (Mean ± SEM), n=3

Table 2: Effect of ethanol extract of ripe and unripe DT fruit on hydrogen peroxide scavenging activity.

Conc. (µg/ml)	RDT (%)	UDT (%)	Vitamin C (%)

20	24.74 ± 0.58	8.18 ± 1.82	24.06 ± 0.28
40	27. 47 ± 0.73	17.73 ± 1.55	26.45 ± 0.34
60	28.15 ± 1.16	22.01 ± 0.98	27.46 ± 1.29
80	27.48 ± 0.38	23.04 ± 0.10	28.15 ± 0.94
100	29.18 ± 0.36	28.16 ± 0.28	30.03 ± 0.72
IC_{50}	179.06	205.69	167.79

Data presented as (Mean ± SEM) n=3

Table 3: Effect of ethanol extract of ripe and unripe DT fruit on reducing power

Conc. (µg/ml)	RDT (%)	UDT (%)	Vitamin C (%)
20	0.70 ± 0.00	0.73 ± 0.00	1.00 ± 0.00
40	0.71 ± 0.00	0.74 ± 0.00	1.00 ± 0.00
60	0.76 ± 0.00	0.76 ± 0.00	1.07 ± 0.00
80	0.84 ± 0.00	0.78 ± 0.00	1.14 ± 0.00
100	1.00 ± 0.00	0.82 ± 0.00	1.38 ± 0.00

Data presented as (Mean ± SEM), n = 3

Table 4: Effect of ethanol extract of ripe and unripe DT fruit on metal chelating activity

Conc. (µg/ml)	RDT (%)	UDT (%)	Vitamin C (%)
20	55.52 ± 0.42	24.00 ± 0.36	26.61 ± 0.36
40	57.02 ± 0.32	56.32 ± 0.91	35.74 ± 0.10
60	61.03 ± 0.32	59.34 ± 0.23	36.45 ± 0.30
80	64.16 ± 0.23	66.97 ± 0.42	40.97 ± 0.17
100	68. 67 ± 0.21	68.98 ± 0.23	47.29 ± 0.35
IC_{50}	124.66	95.38	97.03

Data presented as (Mean ± SEM), n = 3

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances [16] In the DPPH assay (table 1), the antioxidants in the ripe and unripe fruit extract were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine by hydrogen-donation to form non-radical form DPPH–H [8,17] Table 3 showed that the extracts IC_{50} values for DPPH scavenging activity were effective in the orders: unripe DT fruit < ripe DT fruit < Vitamin C. thus suggesting a more potent DPPH free radical scavenging action for ripe DT fruit compared to unripe DT fruit. This finding contradicts the findings of Adedayo *et al*. [18] who reported a higher DPPH scavenging activity for the unripe DT fruits.

Hydrogen peroxide has strong oxidizing properties. It can be produced endogenously *by* many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize lipids and proteins. The ability of ethanol extract of ripe and unripe DT fruits to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* [19] as shown in Table 2 and compared with that of vitamin C as standards. These results showed that the ripe DT fruit extract had an effective hydrogen peroxide scavenging activity than the unripe fruit. The IC_{50} of the H_2O_2 activity decreased as follows: unripe DT fruit < ripe DT fruit < vitamin C. Hydrogen peroxide gives rise to hydroxyl radical ($OH^{\cdot}$)- a very reactive free radical which can damage the cell membrane by initiating lipid peroxidation[19]. Therefore, the ability of ripe and unripe extract to scavenge hydrogen peroxide is very important mechanism for cell protection from damage by xenobiotics.

The ability to reduce Fe^{2+} indicates the capacity of a plant extract to donate electrons, which is an important mechanism of plant polyphenol's antioxidant action [20, 21]. The results in table 3 showed that the ripe extract had a better Fe^{2+} reducing power than the unripe, however, the standard vitamin C superceeded them all. The antioxidant components present in the ethanol extracts of ripe and unripe DT fruit caused the reduction of Fe^{3+}/ferricyanide complex to its ferrous form (Fe^{2+}) thus, demonstrating its reducing power ability. This work is in contrast with the work of Adedayo *et al*. [18] who reported a higher reducing power for

unripe DT fruits. There is a positive relationship between reducing power and the presence of phenolics and flavonoids. Ihemeje and colleagues [22] reported that both ripe and unripe DT fruits contain flavonoids; this may have been responsible for their reducing power.

In nature iron is found in two states: ferrous ion (Fe^{2+}) or ferric ion (Fe^{3+}), ferric ion is abundant in foods and has the ability to combine with vitamin C to form ferric ion. Ferric ion can in turn combine with oxygen to hydrogen peroxide and hydroxyl ion free radicals which are capable of causing oxidative damage to biomolecules [23,24]. Table 4 shows the ferrous ion (Fe^{2+}) chelating activities of ripe and unripe ethanol extracts of DT fruits. The extracts IC_{50} values for metal chelating activity were effective in the orders: unripe DT fruit < vitamin C < unripe DT fruit. This implies that the unripe fruit was a better Fe^{2+} chelator than the standard vitamin C. The ferric chelating effect of DT fruits may confer protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid peroxidation [25]. In this reaction, ferrozine quantitatively formed complexes with Fe^{2+}; in the presence of chelating agents the complex formation was interrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction, allowed the estimation of the metal chelating activity of the chelator [26] Absorbance of a plant extracts was inversely proportional to the metal chelating activity. Adedayo *et al*. [18] reported that the unripe fruits had higher metal chelating activity than the ripe DT fruit. His findings corroborate the result of the present study; however; the difference is that the researcher used aqueous DT fruit extract.

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4. CONCLUSION

There is a surge of interest in the antioxidative properties of the plants due to their possible uses as natural agents to replace artificial ones. The results of the free radical scavenging potential of ethanol extract of ripe and unripe DT fruits exhibited potent antioxidant activity. The extracts IC_{50} values were effective in the orders: % metal chelating activity-ripe DT fruit < vitamin C <unripe DT fruit; % H_2O_2 activity- unripe DT fruit < ripe DT fruit < vitamin C; % DPPH activity- unripe DT fruit < ripe DT fruit <Vitamin C; and reducing power- Vitamin C < unripe DT < ripe DT. Therefore, the present data suggest that ethanol extract of ripe and unripe DT fruits can supplement our diets as a rich source of natural antioxidants for health protection. In food industries, it be used for minimizing or preventing lipid oxidation in food products, inhibit the production of toxic oxidation products thereby promoting and maintaining nutritional quality and extending the shelf life of foods and pharmaceuticals. Further studies should be done to isolate bioactive compounds in order to establish their pharmacological potentials.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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