Original Research Article

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

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ABSTRACT

Dennettia tripetala fruit is a popular Nigerian fruit from the family of plant known as Annonaceae. The whole fruit (flesh and seed) is usually consumed as snacks and it is oftentimes consumed with local gin (ufofop in Ibibio or kaikai in Igbo) or added to dishes as spice due to its peculiar strong pepperish taste and sweet aroma. The present study is aimed at evaluating the antioxidant potentials of ethanol extract of ripe and unripe *D. tripetala* (DT) fruit *in vitro.* 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.Unripe and ripe DT fruits, as well as vitamin C showed a competitive inhibition of DPPH and H_2O_2 free radicals. As concentration of the extracts increased from 20 to100 µg/mL, the % scavenging activity for vitamin C increased from 87.86 ± 0.11 to 90.66 ± 0.07 and for ripe DT fruits from 15.15 ± 0.24 to 25.52 ± 0.23, while for unripe, fruits values increased from 12.09 ± 0.35 to 23.06 ± 0.12. The IC_{50} values was highest in unripe (549.23) followed by ripe (276.63) and lowest in vitamin C (12.92) indicating that vitamin C was the best scavenger of DPPH radical. Similar trend was obtained for H_2O_2 scavenging activity as well as reducing power. Unripe DT fruit extract was more potent at chelating metal ions (IC_{50} was 95.38), followed by the standard ascorbic acid with IC_{50} of 97.03 and was lowest in ripe DT fruit extract with IC_{50} value of 124.66. Unripe and ripe DT are potent antioxidants in nature and may be used to supplement our diets as rich sources of natural antioxidants for health protection.

Key words: 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(Kasote *et al*.[1]. It is documented that two-thirds of the World's plant species have medicinal value [2] and almost all of the plants have potent antioxidant potentials [3] 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 and superoxide dismutase; while non-enzymatic antioxidant include glutathione, vitamins A, E and C [4] These antioxidants in plants play dual functions as they help in the performance of their normal physiological functions and protection from microbes, as well as help plants cope with environmental stress [5]

Plant phenolics perform antioxidant roles by acting as electron donors. 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 [6] and the ability to act as signaling molecules in cells through the modulation of protein kinase and lipid kinase signaling pathways [7,8]. In Phyto research, *in vitro* investigations are often made for two reasons namely; to screen and attribute antioxidant potential to plants as their secondary metabolites and to understand the possible mechanism of action of plant antioxidants [9]. 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, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS, ferric reducing potentials etc.

Pepper fruit (*Dennettia tripetala* Baker f.) is widely cultivated by the people of Cameroon, Ivory Coast and Southern Nigeria. In Nigeria, it is called mmimi in Igbo, Ako in Edo, Ata Igbere in Yoruba and nkarika in Ibibio.

Figure 1: (A) *D. tripetala tree with leaves and unripe fruits.* ***(B)*** *Ripe (red) and unripe (green) D. tripetala fruit [28]*

Pepper fruits are green when unripe and red when ripe. Phytochemical screening of the fruits revealed the presence of tannins, flavonoids, terpenes, alkaloids, cardiac glycosides etc. It is traditionally used for the treatment of cough, toothache, fever, diabetes and nausea during pregnancy. Its antioxidant, antihyperglycemic, anti-inflammatory and antinociceptive effects have been established *in vivo* [10,11,12]. The aim of this study was to evaluate the antioxidant potentials of ethanol extract of unripe and ripe DT fruits.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals used in the study were ascorbic acid, butylated hydroxy toluene(BHT), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ferric chloride, sodium phosphate, ammonium molybdate, ferrous ammonium sulphate, ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide(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.

2.2 Collection and Preparation of Plant Sample

Ripe and unripe fruits of *D. tripetala* were obtained between March to April 2018 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. Unripe fruits of *D. tripetala* were ground and dried at room temperature. The same was done for the ripe fruits of *D. tripetala*. The ground sample was stored in an air-tight container until required for use.

2.3 Extraction of *Dennettia tripetala* fruits

Ground fruits of ripe and unripe *D. tripetala* (500 g) were extracted by macerating in 2125 mL of 80% ethanol for 72 h and filtered. The filtrate was concentrated in a water-bath at 45 ºC to dryness. It was weighed and stored in a refrigerator at -4 ºC until required for use.

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

Free radical scavenging activities of ethanol extract of *D. 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 *et al.* [13] 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 [13]. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity(%) = $[A_0 – A_1] / A_0) \times 100]$ 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.* [14] A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). 0.4 mL 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 min. Blank solution contained phosphate buffer without H_2O_2. The percentage of hydrogen peroxide scavenging was calculated thus:

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

2.6 Reducing Power Assay

The reducing power ability of the extracts of ripe and unripe DT fruits were evaluated by the method described by Oyaizu [15]. The reaction mixture contained 1.0 mL of various concentrations of extracts (20–100 µg/mL), 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 0.2 mol/L of 0.2 M sodium phosphate buffer. The mixture was incubated at 50 ºC for 20 min, cooled rapidly and the reaction ended by the addition of 2.5 mL of 10% trichloroacetic acid, followed by centrifugation at 3000 rpm ~~min~~, for 10 min. Then, 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 the absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicated increased reducing power of the sample. Vitamin C was used as standard.

2.7 Chelating Ability on Iron (III) Ions

The iron (III) ion(Fe^{3+}) chelating potential of the extracts were evaluated by the method of Dinis *et al.* [16] 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 $FeCl_3$. The reaction was initiated by the addition of 0.2 mL of 5 mM 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 iron (III) ion (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:

Ferrous ion (Fe^{2+}) chelating activity (%) = [1-(Absorbance of test/Absorbance of control)] x 100

2.8 Determination of IC_{50} Value

The inhibitor concentration against the percentage activity was plotted ([I]-activity % graph). Using the linear (y=mx+c) equation on the graph, for y=50 value, x point became the IC_{50} value.

2.9 Statistical Analysis

Data were expressed as Mean ± Standard error of mean (SEM) (n = 3). The 50% inhibitory concentration (IC_{50}) of ethanol extract of unripe and ripe DT fruits were estimated from regression analysis of concentration-inhibition curves using EXCEL.

3. RESULTS AND DISCUSSION

The results of the *in vitro* antioxidant potentials of ethanol extracts of unripe and ripe pepper fruits are present in tables 1-4. Results showed that both fruits scavenged DPPH and H_2O_2 free radicals; chelated iron metals and reduced Fe^{3+} in a concentration-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 unripe and ripe fruit extracts. Notably, the values of the IC_{50} were inversely proportional to the potency of the plant material.

Free radical scavenging activities are important 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 unripe and ripe DT fruits on 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

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

Data presented as (Mean ± SEM), n=3. UDT-unripe D. tripetala, RDT-ripe D. tripetala

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

Conc. (µg/ml)	UDT (%)	RDT (%)	Vitamin C (%)
20	8.18 ± 1.82	24.74 ± 0.58	24.06 ± 0.28
40	17.73 ± 1.55	27. 47 ± 0.73	26.45 ± 0.34
60	22.01 ± 0.98	28.15 ± 1.16	27.46 ± 1.29
80	23.04 ± 0.10	27.48 ± 0.38	28.15 ± 0.94
100	28.16 ± 0.28	29.18 ± 0.36	30.03 ± 0.72
IC_{50}	205.69	179.06	167.79

Data presented as (Mean ± SEM) n=3. UDT-unripe D. tripetala, RDT-ripe D. tripetala

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

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

Data presented as (Mean ± SEM), n = 3. UDT-unripe D. tripetala, RDT-ripe D. tripetala

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

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

Data presented as (Mean ± SEM), n = 3. UDT-unripe D. tripetala, RDT-ripe D. tripetala

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances [17]. In the DPPH assay (Table 1), the antioxidants in the unripe and ripe fruit extracts were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine by hydrogen-donation to form non-radical DPPH–H [9,18]. Table 1 showed that the ~~ext~~ IC_{50} values of the extracts for DPPH scavenging activity were effective in the orders: Vitamin C < ripe DT fruits < unripe DT fruits, thus suggesting a more potent DPPH free radical scavenging action for ripe DT fruit compared to unripe DT fruit. This finding does not corroborate the findings of Adedayo *et al*. [19] who reported a higher DPPH scavenging activity for the unripe DT fruits compared to the ripe 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 unripe and ripe DT fruits to scavenge hydrogen peroxide has been 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: Vitamin C < ripe DT fruits < unripe DT fruits. Hydrogen peroxide gives rise to hydroxyl radical ($OH^{\cdot}$)- a very reactive free radical which can damage the cell membrane by initiating lipid peroxidation[20]. 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^{3+} indicates the capacity of a plant extract to donate electrons, which is an important mechanism of plant polyphenol's antioxidant action [21, 22]. The results in Table 3 showed that the ripe extract had a better Fe^{3+} reducing power than the unripe, however, the standard vitamin C was the best. The antioxidant components present in the ethanol extracts of ripe and unripe DT fruit may have resulted in the reduction of Fe^{3+}/ferricyanide complex to its iron(II) ion form (Fe^{2+}) thus, demonstrating its reducing power ability. This work is in contrast with the work of Adedayo *et al*.[19] 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 *et al*. [23] 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: iron(II) ion (Fe^{2+}) or iron(III) ion (Fe^{3+}). Iron(III) ion is abundant in foods and has the ability to combine with vitamin C to form iron(II) ion. Iron(II) ion can in turn combine with oxygen to form hydrogen peroxide and hydroxyl ion free radicals which are capable of causing oxidative damage to biomolecules [24,25]. Table 4 shows the iron(II) ion (Fe^{2+}) chelating activities of ripe and unripe ethanol extracts of DT fruits. The IC_{50} values of the extract 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 iron(II) ion chelating effect of DT fruits may confer protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid peroxidation[26]. 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 [27] Absorbance of the plant extracts was inversely proportional to the metal chelating activity. Adedayo *et al*.[19] reported that the unripe fruits had higher metal chelating activity than the ripe DT fruit.

Bassey *et al.* [28], reported on the possible beneficial effect of DT in boosting spermatogenesis by reducing the concentration of prostate specific antigen (PSA) in male albino Wistar rats. This may have been due to DT's potent antioxidant potentials as observed in this study. Khaki *et al.* [29] reported that antioxidants can protect the DNA and vital molecules from oxidative damage; and can improve spermatogenesis thereby promoting fertility rate in men.

4. CONCLUSION

There is a surge of interest in the antioxidative properties of 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 unripe and ripe DT fruits exhibited potent

antioxidant activities. The extracts IC_{50} values were effective in the orders: % DPPH activity- unripe DT fruit < ripe DT fruit <Vitamin C; % H_2O_2 activity- unripe DT fruit < ripe DT fruit < vitamin C; reducing power- Vitamin C < unripe DT < ripe DT; % metal chelating activity-ripe DT fruit < vitamin C <unripe DT fruit. Therefore, the present data suggest that ethanol extract of ripe and unripe DT fruits may be used to supplement our diets as rich sources of natural antioxidants for health protection. In food Industries, it may be used to reduce or prevent lipid oxidation in food products, thereby promoting and maintaining nutritional quality, as well as extension of 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.

REFERENCES

1. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci, 2015;11(8):982.
2. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89: 217–33.
3. Szent-Giörgyi A. Lost in the twentieth century. Annu Rev Biochem, 1963;36:1–15.
4. Chand S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. Afr J Microbiol Res, 2009; 3: 981–96.
5. Kasote DM, Katyare SS, Hegde, MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci, 2015;11(8): 982.
6. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci, 1997;2: 152–59
7. Halliwell B, Rafter J, Jenner A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? Am J Clin Nutr, 2005; 81: 268S–76S.
8. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signaling molecules? Free Radic Biol Med, 2004; 36: 838-49.
9. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst, 2002; 127: 183–98.
10. Iseghohi S. A review of the uses and medicinal properties of *Dennettia tripetala* (Pepperfruit). Med Sci, 2015; 3(4): 104-111.
11. Oyemitan IA, Iwalewa EO, Akanmu M A, Olugbade TA. Antinociceptive and anti-inflammatory effects of essential oil of *Dennettia tripetala* G. Baker (Annonaceae) in rodents. Afr J Tradit Complement Altern Med, 2008; 5(4): 355-362.
12. Akpakpan EI, Bassey UE, Etim OE,Ekanemesang UM. The effect of ethanol extract of ripe *Denettia tripetala* fruit (Pepper Fruit) on indices of liver and kidney function in male albino Wistar rats. Int J Biochem Res Rev, 2017; *16*(3): 1-7.
13. Shimada K. Fujikawa K. Yahara K. Nakamura T.Antioxidative proper-ties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, J Agric Food Chem, 1992;(40): 945–948.
14. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10: 1003.
15. Oyaizu, M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn J. Nutr, 1986;(44) : 307–315
16. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch Biochem Biophys, 1994; 315:161.
17. OzcelikB, Lee JH, Min DB. Effects of light, oxygen and pH on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to evaluate antioxidants. J Food Sci, 2003; 68: 487
18. Soares JR, Dins TCP, Cunha AP, Almeida LM. Antioxidant activity of some extracts of *Thymus zygis*. Free Radic Res, 1997; 26: 469.
19. Adedayo BC, Oboh G, Akindahunsi AA. Changes in the total phenol content and antioxidant properties of pepperfruit (*Dennettia tripetala*) with ripening. Afr J Food Sci, 2010; 4(6): 403-409
20. Kappus, H. Lipid peroxidation-mechanism and biological relevance. In: Free Radical and Food Additives, Aruoma, OI, Halliwell B. Editors., pp. 59–75, Taylor & ,London, UK, 1999
21. Nabavi SM, EbrahimzadehMA,NabaviSF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran, Pharmacognosy Mag, 2009; 4 (18):123–127.
22. Meir J, Kanner B, Akiri SP, Hadar. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves, J Agric Food Chem, 1995; 43:1813–1817
23. Ihemeje A, Ojinnaka MC, Obi KC, Ekwe CC. Biochemical evaluation of pepper fruit (*Dennettia tripetala*) and its use as substitute for ginger in zobo drink production. Acad Res Int, 2013; 4(6): 513.

24. Kang MJ, Lee SS, Koh HC. Prooxidant properties of ascorbic acid in the nigrostriatal dopaminergic system of C57BL/6 mice. Toxicology, 2012; 294: 1-8.
25. Rietjens IM, Boersma MG, HaanmLd, Spenkelink B, Awad HM, Cnubben NH,et al. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ Toxicol Pharmacol, 2002;11: 321-33.
26. Elmastas M, GulcinI, OzturkL, Gokce I. Investigation of antioxidant properties of spearmint (*Mentha spicata* L.). Asian J Chem, 2005; 17:137.
27. Elmastas M, Turkekul I. Ozturk L, Gulcin I, Isıldak O, Aboul-Enein HY. The antioxidant activity of two wild edible mushrooms (*Morchella vulgaris* and *Morchella esculanta*). Comb Chem High Throughput Screen, 2006a; 6: 443
28. Bassey UE, Akpakpan E I, Etim OE, Akaka EUA., Ekanemesang UM. Effect of ethanol extract of ripe fruits of *Dennetia tripetala* on prostatic and testicular functions of male albino rats. J Med Res, 2018; *4*(2): 89-92.
29. Khaki A, Fathiazad F, Nouri M, Khaki AA, OzanciChelar C, Ghafari-Novin M, et al. The effects of Ginger on spermatogenesis and sperm parameters of rat. Iran J Reprod Med, 2009; 7: 7-12.