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PROXIMATE COMPOSITION AND ANTIOXIDANT ACTIVITY OF ETHANOL LEAF EXTRACT OF *EUCALYPTUS TERETICORNIS*

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ABSTRACT

This study evaluated the proximate composition and antioxidant activity in the ethanol leaf extract of *Eucalyptus tereticornis* using standard biochemical methods. The proximate analysis revealed that the leaf contains carbohydrate (40.90%), crude protein (12.6%), crude fiber (35.40%), crude fat (2.80%), ash (7.92%) and moisture (0.38%). The antioxidant potentials were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, iron reducing power, inhibition of lipid peroxidation and nitric oxide scavenging antioxidant systems. The antioxidant activities of the plant extract increased with increase in concentration. The extract showed high potency in DPPH and nitric oxide free radical scavenging activity compared with the known vitamin C antioxidant potentials. A high reducing power indicative of a high proton (H+) donating potential with a high inhibition of lipid peroxidation portrays a high potent antioxidant activity that protects cells from deleterious effects of free radicals which accounts for its medicinal use.

KEYWORDS: Eucalyptus leaf, proximate composition, Antioxidant activity

INTRODUCTION

The use of plants by man for food and medicinal needs is as old as time. Eucalyptus, a medicinal plant of myrtles and myrtaceae that is found in Australia, Tasmania, and now is extensively cultivated in many other countries including Nigeria (Garcia *et al*., 2004). It is known for its essential oils that are widely employed in the perfumery and fragrance industries and in treatment of muscle pain (Naveen *et al*., 2014), while the leaf has antimicrobial and anti-hyperglycemic action in in-vivo models (Shahraki and Shahraki, 2013; Jain *et al*., 2010).

The nutritional and therapeutic uses of this plant are anchored in the active components therein. These active components that have protective function are the phytochemicals, vitamins and minerals. (Okwu and Ekeke, 2003). Knowledge of their bioactive compounds is actualized in the synthesis of pharmacological products with reduced toxicity and side effects (Gupta *et al*., 2012). Besides, several organic compounds, trace elements and minerals found in this plant also play a vital role in general well-being as well as in the cure of diseases (Prasad, 2004).

Vitamins with varied biochemical functions, such as hormone-like functions; for instance, vitamin D has regulatory role in mineral metabolism, or regulators of cell and tissue growth and differentiation. Others function as antioxidants (vitamins A, E and C), while the B vitamins function as precursors for enzyme cofactors.

Bioactive compounds in medicinal plants provide health benefits in treating and managing debilitating diseases. Beta-carotene, a member of the carotenoid family found in yellow, orange and red colored fruits and vegetables (Holden *et al*., 1999) is easily converted to vitamin A which is a fat soluble vitamin. Lycopene is said to be the most potent oxygen quencher in the carotenoid family and functions to prevent lipid peroxidation, programmed cell death and DNA damage (Chauhan *et al*., 2011).

Flavonoids, like other antioxidants, functions within the body by mopping up cell damaging free radicals and metallic ions. Flavonoids and phenols are the largest group of phytochemicals that account for antioxidant activity in plants. These antioxidants are capable of slowing or preventing the oxidation of other molecules. The uncontrolled production of free radicals is involved in the onset of numerous diseases like cancer, rheumatoid arthritis, as well as in the degenerative processes associated with aging, such as Parkinson's and Alzheimer's diseases (Ali, *et al*., 2008; Di Matteo and Esposito, 2003).

However, cells are equipped with several defense systems against free radical damage; including oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or compounds such as a-tocopherol, ascorbic acid, carotenoids, polyphenolic compounds and glutathione (Niki *et al*., 1994). Naturally, there's equilibrium between the amount of free radicals generated within the system and antioxidants to scavenge or quench them, to guard the body against their harmful effects (Udedi *et al*., 2012).

MATERIALS AND METHODS

Collection and Identification of sample

The fresh leaves of *Eucalyptus tereticornis* were collected from the Botanical garden of Federal University of Technology, Owerri, Imo State, Nigeria.

Sample Preparation and Extraction

The leaves were washed and shade-dried under room temperature for two weeks. The dried leaves were then pulverized into powder using an electric grinding machine. Two hundred grams (200g) of powdered leaves were measured using electronic weighing balance (Model: Adam AFP800L) and soaked with 80% Ethanol (1000ml) for 72hrs and intermittently stirred with a spatula. The mixture was then filtered into a conical flask with Whatman no 1 filter paper and the filtrate evaporated to dryness in a water bath at 500C. It was then stored in an air tight container for further use.

Proximate analysis

The nutritional composition (Moisture content, crude Protein, crude fat, ash and crude fiber) of the leaf extract was analyzed using the official method of Association of Analytical Chemist (AOAC, 2000). The carbohydrate was determined by difference method as reported by (Onyeike *et al*., 1995). Thus: % Carbohydrate = 100–(% moisture + %crude fiber + %ash + % crude fat + % crude protein). The total energy content was determined by multiplying the values of crude protein, crude fat and total carbohydrates by the water factors; 4, 9 and 4, respectively. The sum of the products is expressed in kilocalories per 100 g sample as reported by (Onyeike and Ehirim, 2001).

Thus:

Total energy (Kcal) = 4 x (Protein + carbohydrate) + 9 x (lipid)

ANTIOXIDANT ASSAY

DPPH Scavenging Activity

This was assayed with the method of (Ebrahimzadem *et al*., 2009) Stable 2, 2-diphenyl-1-picryl hydrazylradical (DPPH) was used for the determination of free radical scavenging activity by measuring the decrease in DPPH radical absorption after exposure to radical scavengers. Different concentrations of the extract ((0 – 500 and or 0 – 1200μg/ml; 0.3ml) were mixed with 2.5ml of methanolic solution of DPPH (100μM) in test tube and the absorbance was taken after 1hr at a wavelength of 517nm using ascorbic acid standard. The percentage scavenging activity was calculated using the formula: %RSA = (ADPPH-Abs)/ADPPH) ×100.

Where Abs is the absorbance of the test solution with the sample; ADPPH is the absorbance of DPPH solution. The inhibitory concentration (IC_{50}) of sample at 50% RSA was calculated from the graph of %RSA against the sample concentration.

Inhibition of Lipid Peroxidation using TBA (Thiobarbituric acid) Reactive Substance

This was determined by the method of Barros *et al*., 2007. A homogenate of brain of a goat was used to determine the extent of inhibition of lipid peroxidation because it is rich in polyunsaturated fatty acid (PUFA). The homogenate was centrifuged at 3000g for 10min and supernatant incubated with 0.2ml of sample at various concentrations (0 – 500 and or 0 – 1200μg/ml) in the presence of 0.1ml of 10μM Ferrosulphate and 0.1ml of 0.1mM ascorbic acid at 37°C for 1hr. The reaction was stopped by the addition of 0.5ml of 28% TAC and 0.38ml of 2% TBA and the mixture was heated at 80°C for 20mins, centrifuged at 3000g for 10mins to remove the precipitated protein. The absorbance of Malondialdehyde (MDA) - TBA complex in the supernatant was read at a wavelength of 532nM. The inhibition ratio (%) was calculated using the following formula; Inhibition ratio (%) = [(A-B)]/A) ×100%; where A and B were the absorbance of the control and the compound solution respectively. The extract concentration providing 50% lipid peroxidation inhibition (IC_{50}) was calculated from the graph of antioxidant activity percentage against the extract concentrations using ascorbic acid as the standard.

Reducing Power Assay

The method of Barros *et al*. (2007) was used to determine the reducing power. It uses the principle that increase in the absorbance of the reaction mixture is an indicative of increase in the antioxidant activity. Different concentrations of the test sample were mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50° C for 20 min followed addition of 2.5ml of 10% Trichloroacetic acid. The mixture was centrifuged at 1000rpm for 8mins and the supernatant mixed with 5 ml of deionised water and 1 ml of 0.1% of ferric chloride and the absorbance was read at 700 nm. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

Nitric Oxide Scavenging Activity

The method of Rozina *et al*. (2013) was used in the determination. In aqueous solution, sodium nitroprusside decomposes at physiological pH7.2 to produce nitric oxide (NO) which reacts with oxygen to produce stable products nitrate (NO_3^-) and nitrite (NO_2^-) radicals. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric ions. In this research, 2.7ml of sodium nitroprusside (10mM) in phosphate buffer was mixed with various concentrations of the sample and incubated at 30°C for 2hours. The control had the same reaction mixture without the extract. The absorbance of the chromophore that formed during diazotization was read after incubation at 550nm. Inhibition of nitrite formation by the plant extract and standard antioxidant ascorbic acid were calculated relative to control. IC_{50} which is an inhibitory concentration (IC_{50}) of each extract required to reduce 50% of nitric oxide formation was deduced.

Statistical Analysis

The data was analyzed using One Way Analysis of Variance (ANOVA). Differences at ($p < 0.05$) were considered significant.

RESULT

Proximate composition

Table 1 shows the proximate composition of the ethanol leaf extract of *E. tereticornis*. The leaf has high calorie of 239.20 Kcal/100g with high carbohydrate content and very little ash.

Table 1: Proximate composition of ethanol leaf extract *E. tereticornis*

Parameter	**Composition**
Carbohydrate	40.90±0.02
Crude protein	12.60±0.01
Crude fibre	35.40±0.10
Fat	2.80±0.30
Total ash	7.92±0.21
Total moisture	0.38±0.01
Total energy	239.20 Kcal/100g

ANTIOXIDANT ACTIVITY

DPPH scavenging activity

The Radical Scavenging Activity (RSA) of ethanol leaf extract of *E. tereticornis* using vitamin C as standard is presented in Fig. 1. The scavenging activity of the plant extract increased relative to the standard, vitamin C. At peak, the RSA for the extract was 93% while that of Vitamin C was 96%. IC_{50} values of *E. tereticornis* and Vitamin C interpolated from the graph of DPPH radical scavenging ability at 50% RSA shows that the *E. tereticornis* has a higher DPPH scavenging ability when compared with the standard; Vitamin C in Table 1.

Table 1: IC_{50} values of *E. tereticornis* and Vitamin C interpolated from the graph of DPPH radical scavenging ability.

Extract	**IC_{50}ug/ml**
E.tereticornis	**280**
Vitamin C	**80**

Inhibition of lipid peroxidation assay

Fig. 2 presents the percent inhibitory ability of the extract on lipid peroxidation with vitamin C as standard. The inhibition increased with increase in concentration with both samples with the plant extract having a lower inhibition than that of the standard and their IC_{50} values shown in Table 2 interpolated from the graph in Fig. 2.

Fig. 2: Inhibition of Lipid Peroxidation by ethanol extract of E. tereticornis and Vitamin C.

Table 2: IC_{50} values of *E. tereticornis* and Vitamin C inhibition of lipid peroxidation ability.

Extract	IC_{50} (ug/ml)
E. tereticornis	450
Vitamin C	150

Reducing power assay

The reducing power activity of the ethanol leaf extract of *E. tereticornis* with Vitamin C standard is presented in Fig. 3. The extract exhibited a high reducing power which paralleled the concentration. The extract and vitamin C attained IC_{50} at 300μg/ml and interlocked and increased with respect to concentration.

Fig. 3: Reducing power of ethanol extract of *E. tereticornis* and Vitamin C.

Table 3: IC_{50} values of ethanol extract of E. tereticornis and Vitamin C reducing power capacity.

Extract	**IC_{50}ug/ml**

E. tereticornis	**300**
Vitamin C	**300**

Nitric oxide scavenging activity

The nitric oxide (NO) radical scavenging ability of the leaf extract and Vitamin C as standard is presented in Fig 4. Inhibition of nitrite formation by the *E. tereticornis* extracts increased with increase in concentration relative to the standard vitamin C and IC_{50} interpolated from the graph as shown in Table 4.

Fig. 4: Nitric oxide scavenging activity of ethanol extract of *E. tereticornis* compared to the standard (Vitamin C).

Table 4: IC_{50} values of *E. tereticornis* and Vitamin C.

Extract	IC_{50}ug/ml
E. tereticornis	900
Vitamin C	100

DISCUSSION

From the result of proximate analysis, the leaf of *E. tereticornis* could serve as a high source of energy considering the high carbohydrate content of 40.90 ± 0.02% and a crude protein concentration of 12.60 ± 0.01%. Though plant protein is of lower quality in comparison to animal protein, but their combination provides adequate nutritional value (Pamela *et al*., 2005). Furthermore, any plant food that provides more than 12% protein is considered a good source of protein (Hassan and Umar, 2006). The leaf is rich in crude fiber of 35.40 ± 0.10% and its consumption is of health benefit as it aids digestion and absorption of glucose and fat. It also reduces the chances of occurrence of digestive disorders and some diseases such as cardiovascular diseases, colon cancer, diabetes, hypertension and obesity (Food and Agricultural Organization, 1990). Although crude fiber enhances digestibility, its presence in high level can cause gastro-intestinal disturbances and decreased nutrient usage (Oladiji and Mih, 2005) because of high content of cellulose and a little lignin which is indigestible in human (Onwuka, 2005). The crude fat content of *E. tereticornis* leaf extract was low, 2.80 ± 0.30%, indicating a poor source of lipids.

This is of dietary importance as it provides 1–2 % of caloric energy essential for cardiovascular function (Kris-Etherton *et al*., 2002). Lipid is also a good source of energy, aids in transport of fat-soluble vitamins, contributes to important cell processes, insulates and protects internal tissues (Pamela *et al*., 2005; Jones, 1985). Ash which is the leftover after all the moisture and the organic materials (fat, protein, carbohydrates, vitamins, organic acid) have been removed was 7.92 ± 0.21%. Water is an essential compound of many foods, (Datta *et al*., 2019) and 20% of the total water consumption is through food moisture (FNB, 2005). The moisture content of the leaf was low, an indication of stability and low susceptibility to microbial growth when stored for long periods. The energy (calorific) value was high, 239.20Kcal/100g, an indication of good dietary supplement (Datta *et al*., 2019) which is comparable with reported values of some medicinal plants which ranged from 261.33 to 485.70Kcal/100g (Ullah *et al*., 2013).

For the determination of antioxidant activity, diphenyl-1-picrylhydrazyl (DPPH) radicals are model systems widely used to determine the scavenging activity of several natural bioactive compounds (DiMascio *et al*., 1989).

The DPPH radical scavenging activity of plant extract at different concentrations is shown in Figure 1. The activity increased with increase in concentration when compared with that of the standard; vitamin C. The result in this study indicates that the plant is potentially active in scavenging free radicals as it has a percentage RSA of 93% when compared to the standard which showed 96%.

IC_{50} which is a measure of inhibitory concentration is inversely related to the activity and a lower value is an indicative of greater antioxidant activity of the extract. It is the concentration of the extract that can quench 50% of DPPH in the solution under the experimental conditions. The extract however, showed IC_{50} value of 280µg/ml which is less active than the standard vitamin C with IC_{50} value of 80µg/ml as shown in table 1.

Oxidative degradation of polyunsaturated fatty acids in the cell membrane produces malonaldehyde (MDA) which is degradable. This process is called lipid peroxidation and found to cause the destruction of cell membrane and cell damage in bio-systems (Gordon, 1990). Several pathological disorders such as atherosclerosis, inflammation and liver injury are associated with lipid peroxidation of cell membranes (Kubow, 1992). MDA, one of the major products of lipid peroxidation, has been extensively used as an index for lipid peroxidation and as a marker for oxidative stress (Singh *et al*., 2012).

The reaction of MDA with thiobarbituric acid (TBA) has been used widely as a sensitive assay method for lipid peroxidation (Ajila *et al*., 2007). The generation of Fe^{2+} ascorbate in the brain homogenate of goat was inhibited by *E. tereticornis* extract as shown in table 2. The percentage inhibition activity increased with increase in concentration but non-significantly ($p>0.05$) when compared with the standard. However, the standard exhibited more potent inhibition activity with an IC_{50} of 150µg/ml as against the extract with an IC_{50} of 450µg/ml. Therefore, *E. tereticornis* is capable of inhibiting the process of lipid peroxidation and this could be attributed to the bioactive compounds present in the extract (Ani *et al*., 2020). From the studies, it could be suggested that phenolic compounds have the ability to suppress lipid peroxidation either through free radical quenching, electron transfer, radical addition or radical recombination (Ohkawa *et al*., 1978). This effect will forestall the oxidation of biomolecules and extent of oxidative stress that would give rise to physiological dysfunctions (Scarfiotti, 1997).

The plant extract competes with oxygen to scavenge for the nitrite radical generated in aqueous environment. The extract removed nitrite radicals as there was increase in activity at higher concentrations with respect to the standard. The nitric oxide radical scavenging potency (IC_{50}) as shown in table 4 was interpolated from Fig 4. The plant extract with an IC_{50} value of 900µg/ml greater than vitamin C which has 100µg/ml is comparatively of a lower potency in free radical scavenging activity. However, the observed high free radical scavenging activity of the plant extract with respect to vitamin C standard could be attributed to high content of phenol as phenols have been demonstrated to possess the ability to scavenge free radicals through proton donation or electron withdrawing (Sharma and Vig, 2013) and this supports its application as a natural antioxidant.

The reducing power assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species (ROS). The reducing power assay is based on the principle that substances with reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. This is merely a reduction of Fe^{3+} to Fe^{2+} and increase in absorbance is an indicative of an increase in reductive ability which is an indicative of its antioxidant potential ability (Shahidi and Wanasundara, 1992). In this study, the increase in reducing power of the extract paralleled that of the standard with an equimolar concentrations of IC_{50} of 300 µg/ml as interpolated from Fig. 3.The extract contains reductones which exert antioxidant activity by proton donation; a demonstration of its reducing capacity is indicative of its potential antioxidant properties (Abbasi *et al*., 2013; Duh *et al*., 1999).

CONCLUSION

From the result of the analysis, *Eucalyptus tereticornis* has antioxidant activity against excited oxygen species as it is rich in bioactive compounds; a justification for its medicinal value.

REFERENCES

Abbasi, M.A, Saleem, H., Aziz-ur-Rehman, Riaz T. and Ajai, M. Determination of Antioxidant Activity and Phytoconstituent Screening of Euphorbia heterophylla Linn. *British Journal of Pharmaceutical Research*, 2013; **3**(2): 202-216.

Ajila, C.M, Naidu, K.A, Bhat, S.G. and Prasada, Rao UJS. Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 2007; **105**: 982–988.

Ali, S.S., Kasoju, A., Luthra, A., Singh, H. and Sharanabasava. Indian medicinal herbs as sources of antioxidants. *Food Res. Int*., 2008; **41**(1): 1-15.

Ani, O.N., Ani, O., Asogwa, K. K., Onyishi, C. K., Ujah, I. I. and Ebulue, M.M. Nutritional profile, bioactive compound content and antioxidant activity of ethanol leaf extract of *Eucalyptus tereticornis* , *European Journal of Biomedical and Pharmaceutical sciences* , 2020; **7**(5): 61 – 73.

AOAC. Official method of Analysis 16th Edition, Association of official Analytical Chemists, Washington D.C US. 2000; 200-210.

Barros, L., Baptista, P., Correia, D.M., Morais, J.S. and Ferreira, I.C.F.R. Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, 2007; **55**: 4781-4788.

Chauhan, K., Sharma, S., Agarwal, N. and Chauhan, B. Lycopene of tomato fame: its role in health and disease. *International Journal of Pharmaceutical Sciences and Research* IJPSR, 2011; **10**: 99–115.

Datta, S., Sinha, B.K., Bhattacharjee, S. and Seal, T. Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. *Heliyon*, 2019; **5**(3): e01431.

Di Matteo, V. and Esposito, E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Curr Drug Targets CNS Neurol Disord*, 2003; **2**(2): 95-107.

DiMascio, P., Kaiser, S. and Sies, H. Lycopene as the most effective biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys*, 1989; **274**: 532-8

Duh, P.D., Tu, Y.Y. and Yen, G.C. Antioxidant activity of the aqueous extract of harn jyur (Chrysanthemum morifolium Ramat). *Lebensmittel-Wissenschaft and Technologie*, 1999; **32**: 269-277.

Ebrahimzadem, M.A., Jashidi, M., Shabani, E. and Hashemi, Z. Evaluation of three methods of the Extraction of Antioxidants from leaf and aerial parts of Lythrum salicaria L. (Lythraceae). *International food research Journal*, 2009; **21**(2): 783-788.

FNB. Food and Nutrition Board Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and

Food and Agriculture Organization. Roots, Tubers, Plantains and Bananas in Human Nutrition. FAO Corporate Document Repository, Rome, 1990. https://www.fao.org/docrep/t0207e/T020 7Eo8.httm.

Garcia, M.D., Fernandez, M.A., Alvarez, A. and Saenz, M.T. Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of Pimentaracemosavar. ozua (Myrtaceae). *J Ethnopharmacol*, 2004; **91**(1): 69-73.

Gordon, M.H. The mechanism of antioxidant action in vitro. In: BJF Hudson (Ed.), Food antioxidants. *Elsevier Applied Science, London*, 1990; 1–18.

Gupta S, Kumar MNS, Duraiswamy B Chhajed, M Chhajed A, In-vitroAntioxidant and Free Radical Scavenging Activities of Ocimum Sanctum. *World Journal of Pharmaceutical Research*, 2012; **1**: 78- 94.

Hassan LG, Umar KJ. Nutritional value of Balsam Apple (Momordicabalsamina L.) leaves. Pak. *J. Nutr*., 2006; **5**(6): 522-529.

Holden JM, Eldridge AL, Beecher GR., Buzzard IM, Bhagwat S, Davis CS, Douglass LW, Gebhardt S, Haytowitz D, Schake SI. Carotenoid content of U.S. foods: An update of the database. *J. Food Compos Anal*, 1999; **12**: 1696–196.

Jones MM, Johnson DO, Netterville JT, Wood JI, Joesten M. Chemistry and Society 5th ed. Sanders college publishers U.S.A., 1985; 521-577.

Kris-Etherton PM., Hecker KD., Bonanome A, Coval SM., Binkoski AE., Hilpert KF., Griel AE., Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. PubMed. 2002; **9**: 71–88.

Kubow S. Routes of formation of toxic consequences of lipid oxidation products in foods. *Free Radical Biological Medicines*, 1992; **12**: 63–81.

Niki E, Shimaski H, Mino M. Antioxidantism-Studies on products of browning reaction: antioxidative activity of products of browning reaction. *Japan Journal of Nutrition*, 1994; **40**: 307–315.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 1978; **95**: 351–358.

Okwu DE, Ekeke O. Phytochemical screening and mineral composition of chewing sticks in south Eastern Nigeria. *Global journal of Pure and Applied Science*, 2003; **9**: 235-238.

Oladiji AT, Mih FO. Proximate composition mineral and phytochemical constituents of Eleusine coracana (finger millet). *African Journal of Biotechnology*, 2005; **4**(12): 1440-1441.

Onwuka GI. Food analysis and instrumentation: theory and practice. Naphtali prints, Nigeria, 2005; 95-96.

Onyeike EN, Ehirim FC. Chemical and Sensory Evaluation of Melon fungus (Pleurotustuberregium) and Melon fungus Cake. *Journal of Biochemistry & Molecular Biology*, 2001; **16**(1): 77-81.

Onyeike EN, Olungwe T, Uwakwe AA. Effect of heat treatment and defatting on the proximate composition of Some Nigerian local soup thickeners. *Food Chemistry*, 1995; 173-175.

Pamela CC, Richard AH, Denise RF. Lippincott"s Illustrated Reviews Biochemistry 3rd Ed, Lippincott Williams and Wilkins, Philadelphia, 2005; 335-388.

Prasad MNV. Heavy Metal Stress in Plants, 2nd Edition, *Springer*, United Kingdom, 2004; 484-487.

Rozina P, Sukalayan KK, Pijush S. In Vitro Nitric Oxide Scavenging Activity Of Methanol Extracts Of Three Bangladeshi Medicinal Plants. The Pharma Innovation – *Journal*, 2013; **1**(12): 83-88.

Scarfiotti C1, Fabris F, Cestaro B, Giuliani A. Free radicals, atherosclerosis, ageing, and related dysmetabolic pathologies: pathological and clinical aspects. *Eur J Cancer Prev*., 1997; **6**(1): S31-6.

Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, 1992; **32**: 67–103.

Sharma S, Vig AP. Evaluation of In Vitro Antioxidant Properties of Methanol and Aqueous Extracts of Parkinsonia aculeata L. Leaves, *The Scientific World Journal*, 2013; 1-7.

Singh HP, Kaur S, Negi K, Kumari S, Saini V, Batish DR. Assessment of in-vitro antioxidant actuivity of essential oil of Eucalyptus citriodora (lemon-scented Eucalypt; Myrtaceae) and its major constituents. *LWT Food Sci. Technol*, 2012; **48**(2): 237–241.

Udedi SC, Ani ON, Anajekwu BN, Ononamadu CJ, Igwilo IO, Ibeabuchi CG, Ifemeje JC, Lukong CB, Ogbuozobe GO. Nutritional composition and antioxidant activity of African Walnut, Tetracarpidium conophorum, *The Journal of Applied Biochemistry*, 2012; **107**(2014): 170-180.

Ullah Z., Baloch MK., Baloch IB, Bibi F. Proximate and nutrient analysis of selected medicinal plants of Tank and South Waziristan Area of Pakistan. *Middle East J. Sci. Res*., 2013; **13**(10): 1345–1350.