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NUTRITIONAL PROFILE, BIOACTIVE COMPOUND CONTENT AND ANTIOXIDANT ACTIVITY OF ETHANOL LEAF EXTRACT OF EUCALYPTUS TERETICORNIS

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ABSTRACT
This study evaluated the proximate, vitamins, minerals, anti-nutrients composition and bioactive compounds present in the ethanol leaf extract of Eucalyptus tereticornis as well as its antioxidant potential. Standard biochemical methods were used for the various evaluations. The antioxidant potentials were measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, iron reducing power, inhibition of lipid peroxidation and Nitric oxide scavenging antioxidant systems. The proximate analysis revealed that the leaf contains carbohydrate (41.07%), crude protein (13.8%), crude fiber (34.50%), crude fat (2.62%), ash (7.71%) and moisture (0.25%) while the anti-nutrient screening confirmed the presence of tannins, oxalate, phytate and saponin. The study revealed that the leaf extract contains significant amounts of vitamin B1 with appreciable amounts of vitamins B2, B9 and C. The studied mineral composition showed that Iron, Zinc and Sodium were the most abundant elements in the leaves while calcium and cobalt were recorded in trace amounts. The leaf extract also contains rich amount of Phenol, Flavonoids, Beta-carotene and Lycopene. The antioxidant activities of the plant extract increased with increase in concentration. The extract showed potent DPPH and nitric oxide radical scavenging activity comparably with the standard (Vitamin C) used. From the reducing power assay, the extract displayed a high H^+ donating potential. The inhibition of lipid peroxidation of plant extract was also high. The plant has potent antioxidant activity and could serve in protecting the cells from deleterious effects of free radicals. The general results show that the plant contains high amounts of nutrients and bioactive compounds which are believed to have contributed to high antioxidant activities observed.

KEYWORDS: Proximate, Vitamins, Minerals, Bioactive compounds, Antioxidant activity, Eucalyptus tereticornis.

INTRODUCTION
Mankind has continually exploited plants, herbs and fruit yielding trees for their medicinal values.[1] Plants are used for their numerous beneficial effects that embody nutritional and therapeutic (protective and medicinal). Beneficial effects results from the mix of secondary metabolites that are capable of generating physiological actions within the body. The active components in most plants that contribute to its protective effects are the phytochemicals, vitamins and minerals.[2] Studies continually pertains to their pharmacological/therapeutic effects. The particular physiological activity of a specific medicinal plant is shown through the extraction of its bioactive compound. These additionally helps to perform a pharmacological study to synthesize drug from medicinal plants with a reduced toxicity and side effect.[3] Besides several organic compounds, many trace elements also play a vital role in general well-being as well as in the cure of diseases.[4] Vitamins have different biochemical functions like some that act like hormones, for instance, vitamin D which functions as regulators of mineral metabolism, or regulators of cell and tissue growth and differentiation (some forms of vitamin A). Others function as antioxidants (vitamins E and C). The largest number of vitamins (B complex vitamins) function as precursors for enzyme cofactors. A number of minerals essential to human nutrition accumulate in different parts of plants. Plants accumulate minerals

essential for growth from the environment, including metals like Cadmium (Cd), Cobalt (Co) and Silver (Ag) which are of unknown direct benefit to the plant.[5] The major minerals (Ca, P, Mg, S, K, Cl, Na) serve as structural components of tissues and function in cells and basal metabolism, water and acid-base balance.[6] Trace metals in high amounts such as Zn, Fe, Si, Mn, Cu, F, I and Cr constitute significant health hazards for man and have become an area of particular concern and highest priority in environmental research. Trace elements can be directly taken up by the leaves of plants or they accumulate in the soil and reach the plant through their roots. Trace elements also have curative and preventive roles in combating diseases.[7]

Bioactive compounds in medicinal plants provide health benefits, including those that have proven effective in treating and managing debilitating diseases. Vitamin C; a water soluble vitamin is useful for the prevention of scurvy and has an anti-carcinogenic effect.[8] It's considered as a major, naturally occurring nutrient and antioxidant in our daily diet. Beta-carotene, a member of the carotenoid family, found in yellow, orange and red colored fruits and vegetables,[9] due to its nature 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.[10] This is often said to be so because it is two times more effective than β-carotene and up to ten times more effective than α-tocopherol.[11] 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 phytochemical that account for several of the antioxidants activity in plants or plant material. 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 process associated with aging, including Parkinson's and Alzheimer's diseases.[12, 13] 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 α-tocopherol, ascorbic acid, carotenoids, polyphenolic compounds and glutathione.[14] Naturally, there's an equilibrium between the amount of free radicals made within the system and antioxidants to scavenge or quench them, to guard the body against their harmful effects.[15] However, it is possible that the quantity of antioxidants present under normal physiological conditions is also insufficient to neutralize free radicals generated under pathological conditions. Studies have suggested that the consumption of natural antioxidant like polyphenol-rich food, fresh fruits, vegetables or teas have protecting effects against these diseases and its protection has partially been ascribed to the presence of bioactive compounds as vitamins, flavonoids, anthocyanins and different phenolic compounds.[16, 17] These compounds that scavenge free radicals, might scale back the extent of oxidative stress and forestall the oxidation of biomolecules that may break the reaction chains of pathogenesis in the deterioration of physiological functions that may occur leading to coronary heart diseases and cancer.[18]

Eucalyptus is one among the medicinal plants which belongs to order myrtles and myrtaceae and a large genus of aromatic trees indigenous to Australia, Tasmania, and also the neighbouring island, and now extensively cultivated in many other countries including Nigeria.[19] Eucalyptus species are known for their essential oils that are extracted through steam or hydro distillation. These are widely employed in the perfumery and fragrance industries. Eucalyptus genus is well known in the ayurveda and other local medicine system as treatment and preventive medicine to many human ailments.[20] Eucalyptus tereticornis is one of the species of Eucalyptus genus. The plant is commonly called Mysore gum', 'Mysore hybrid' or 'Eucalyptus hybrid and is mainly used as fiber and timber source. The oil extracted from the plant is utilized for several ailments including muscle pain and anti-septic.[20] The leaf of the plant have shown antimicrobial activity in vitro and anti-hyperglycemic action in in-vivo models.[21, 22]

MATERIALS AND METHODS

Collection and Identification of sample

The fresh leaves of Eucalyptus tereticornis were collected from school farm of Enugu state University of Science and Technology, Enugu, Nigeria. It was authenticated by a taxonomist in the department of Botany, Nnamdi Azikiwe University, Awka. The sample was deposited at the herbarium of University with Voucher number; NAUH 169A.

Sample Preparation and Extraction

The leaves were washed and shade-dried under room temperature for two weeks. The dried leaves were then pulverized to powder using an electric grinding machine. Three hundred grams (300g) of powdered leaves were measured using electronic weighing balance (Model: Adam AFP800L) and soaked with 80% Ethanol (1000ml) for 72hrs with intermittent stirring with the aid of a spatula. The mixture was then filtered into a conical flask using Whatman no 1 filter paper and the filtrate was evaporated to dryness in a water bath at 50^0C. 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.[23] The carbohydrate was determined by difference method as reported by Onyeike et al.[24] 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 Atwater 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.[25]

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

ANTI-NUTRIENT ANALYSIS

Qualitative determination of anti-nutrients

Qualitative analysis of the anti-nutrients were done according to the method described by Harborne[26] and Trease and Evans.[27]

Quantitative determination of anti-nutrients

Tannin and Saponin were determined according to the methods described by Harborne,[26] Oxalate was determined according to the method of Osagie,[28] Phytate was determined using the method of Young and Greaves[29] modified by Lucas and Markakes.[30]

Vitamin analysis

Vitamin C was determined using the method of AOAC.[31] Vitamin B1 was determined using the method described by Okwu and Ndu,[32] while Vitamin B2 and B9 were determined using the methods described by Okwu and Josiah.[33] HPLC was used for the analysis.

Mineral analysis.

Mineral contents were determined using atomic absorption spectrometry and flame photometry according to the methods of AOAC.[34]

CHEMICAL ASSAY

Total flavonoids Assay

The flavonoid content was determined by a slightly modified colorimetry method described previously by Barros et al.[35] A 0.5 ml aliquot of appropriately (1mg/ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 min, 0.15 ml of 10% AlCl3 solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared with catechin concentrations. The results were expressed as mg Catechin equivalents per 100 gram of sample (mg CE/100g).

Total phenol Assay

The total phenol content of the samples was determined using the method of Barros et al.[35] The extract solution (1 ml; 1mg) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction will be kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used as the standard and the results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

Beta Carotene and Lycopene

These were determined by the method of Barros et al.[35] The quantity of 100 mg of dried ethanol extract was vigorously shaken with 6 ml of acetone-hexane mixture in the ratio of (4:6) for one minute and filtered using Whatman No.4 filter paper. The absorbance of the filtrate was read at 453, 505 and 663 nm respectively. The content of Lycopene and β-carotene was calculated according to the following equations:

Lycopene (mg/100g) = $-0.458A_{663} +0.372A_{505}+0.0806A_{453}$

β-carotene (mg/100g) = $0.216A_{663} +-0.304A_{505}+0.452A_{453}$.

ANTIOXIDANT ASSAY

DPPH Scavenging Activity

The stable 2, 2-diphenyl-1-picryl hydrazylradical (DPPH) was used for the determination of free radical scavenging activity measuring the decrease in DPPH radical absorption after exposure to radical scavengers. This was assayed using the method of Ebrahimzadem et al.[36] Different Concentrations of the extract (0-1000µmol; 0.3ml) were mixed with 2.7ml of methanolic solution of DPPH (100µM) in test tube. The mixture was shaken and kept in dark for 60mins. The absorbance was taken at a wavelength of 517nM using spectrophotometer. Ascorbic acid was used as standard. The percentage scavenging activity was calculated using the formula:

$$\%RSA = (ADPPH- As)/ADPPH) \times 100.$$

Where As is the absorbance of the test solution with the sample; ADPPH is the absorbance of DPPH solution.

The IC_{50} (Concentration 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.[35] Determination of the extent of inhibition of lipid peroxidation was carried out using homogenate of brain of a goat of approximately 90kg as the source of polyunsaturated fatty acids. The brain was dissected and homogenized with pestle and mortar in an ice cold Tris-HCL buffer (pH7.4, 20mM) to produce 10%w/v brain homogenate which was centrifuged at 3000g for 10mins. An aliquot (0.1) of the supernatant was incubated with 0.2ml of the sample extract at various concentrations (0-100µglml).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 followed by the addition of 0.38ml of

2% TBA. The mixture was heated at 80°c for 20mins. After centrifugation at 3000g for 10mins to remove the precipitated protein, the colour intensity of the Malondialdehyde (MDA) - TBA complex in the supernatant was measured by its absorbance at 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 (IC50) was calculated from the graph of antioxidant activity percentage against the extract concentrations. Ascorbic acid was used as standard.

Reducing Power Assay

The reducing power was determined according to the method of Barros et al.[35] This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Various concentrations of the plant ethanol leaf extract (0-1000µg/ml) were mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 min. and 2.5ml of 10% Trichloroacetic acid was added. The mixture was centrifuged at 1000rpm for 8mins. The upper layer (5 ml) was mixed with 5 ml of deionised water followed by the addition of 1 ml of 0.1% of ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer. The extract concentration providing 0.5 of absorbance (IC50) was calculated from the graph of absorbance at 700 nm against extract concentration.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was estimated by method of Rozina et al.[37] The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing nitric oxide (NO). Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric ions. For the experiment, 2.7ml of sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations (0-1000µg/ml; 0.3ml) of ethanol extract of the plant incubated at 30°c for 2hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5ml of Griess reagent was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylenediamine dilydrochloride was immediately read at 550nm. Inhibition of nitrite formation by the plant extract and standard antioxidant ascorbic acid were calculated relative to control. Inhibition data (percentage inhibition) were linearized against the concentration of each extract and standard antioxidant. IC50 which is an inhibitory concentration of each extract required to reduce 50% of nitric oxide formation was determined.

Statistical Analysis

The experimental data was analyzed using ANOVA and values were expressed as means ± SD.

RESULTS

Proximate Composition

Table 1 shows the proximate composition of the ethanol leaf extract of E. tereticornis. The carbohydrate has the highest content (41.07 ± 0.02%) while the least was moisture (0.25 ± 0.01%). The leaf also contains appreciable amount of energy; 243.26 Kcal/100g.

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

Parameter	Composition (%)
Carbohydrate	41.07 ± 0.02
Crude Protein	13.85 ± 0.01
Crude fiber	34.50 ± 0.10
Fat	2.62 ± 0.30
Total ash	7.71 ± 0.21
Moisture	0.25 ± 0.01
Total energy (Kcal/100g)	243.26

Anti-nutrient Composition

The result of the qualitative and quantitative analysis of anti-nutrient determined in the ethanol leaf extract of E. tereticornis is presented in table 2. The leaf has a very high content of Oxalate and a low content of Phytate.

Table 2: Qualitative and quantitative anti-nutrient composition of ethanol leaf extract of E. tereticornis.

Anti-nutrient	Qualitative composition	Quantitative composition (mg/g)
Tannin	++	2.06 ± 0.01
Oxalate	+++	254.62 ± 0.21
Saponin	+	0.65 ± 0.30
Phytate	+	0.095 ± 0.25

Key+++=highly present, ++=moderately present, += present in low concentration

Vitamin content

The composition of analyzed vitamins of Eucalyptus tereticornis leaves are shown in Table 3. The leaf extract contains considerable amount of Vitamins B1 and B2. Vitamins B9 and C are in moderate amounts.

Table.3: Vitamin composition of ethanol leaf extract of Eucalyptus tereticornis.

Vitamin Composition	Concentration (mg/100g)
Vitamin B1	1.237 ± 0.31
Vitamin B2	0.789 ± 0.24
Vitamin B9	0.259 ± 2.10
Vitamin C	0.336 ± 0.10

Mineral Content

The result of the analysis of the some mineral content of leaves of Eucalyptus tereticornis is presented in Table 4. From the result it was observed that Sodium is the most abundant element followed by Iron while Calcium and Cobalt were recorded in trace amounts. Manganese was not detected.

Table.4: Mineral composition of ethanol leaf extract of Eucalyptus tereticornis.

Mineral Composition	Value (mg/L)
Iron (Fe)	0.76 ± 0.01
Zinc (Zn)	0.30 ± 0.21
Manganese (Mn)	Nil
Sodium (Na)	0.83 ± 0.30
Calcium (Ca)	0.06 ± 0.10
Cobalt (Co)	0.02 ± 0.25

Bioactive compounds

Table 5 shows the result obtained for the total phenol, flavonoid, beta-carotene and lycopene content of the plant extract. The phenolic content of the extract was expressed in milligrams of gallic acid per gram of the dry matter. The result shows that the extract has a very high content of phenol per gram of dry extract. The flavonoid content was expressed in milligram of Catechin per gram of dry extract. From the result the flavonoid content was relatively high. The lycopene content was low while the beta-carotene was fairly high.

Table 5: Bioactive compound compositions of the ethanol leaf extract of Eucalyptus Tereticornis.

Parameter	Concentration
Total Phenol (mgGAE/g)	110.79 ± 0.10
Flavonoid (mgCE/g)	35.13 ± 0.45
Beta-Carotene (mg/g)	2.89 ± 0.00
Lycopene (mg/g)	0.28 ± 0.00

ANTIOXIDANT ACTIVITY

DPPH Scavenging Activity

The Radical Scavenging Activity (RSA) of E. tereticornis ethanol extracts and the standard; Vitamin C are presented in fig 1. The scavenging activity of the plant extract was found to be concentration-dependent and comparable with that of Vitamin C. At the highest concentration tested, the RSA for the plant extract was 93.57% while that of Vitamin C was 96.31%. The DPPH scavenging ability interpolated from the graph (Fig 1) as effective concentration; i.e. concentration of sample at 50% RSA (IC50) shows that the E. tereticornis has a lower DPPH scavenging ability when compared with the standard; Vitamin C (Table 6).

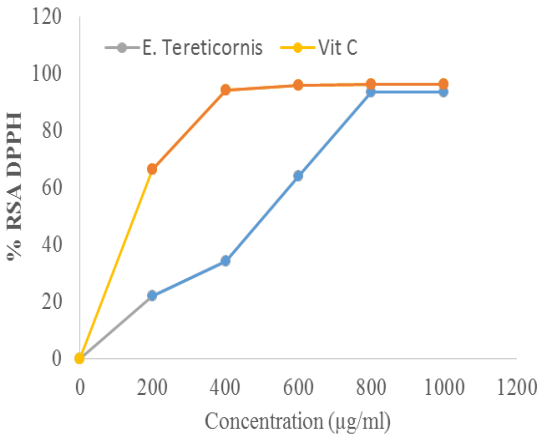

Fig. 1: DPPH Radical Scavenging Activity of ethanol leaf extract of E. tereticornis and the standard; Vitamin C.

Table 6: IC_{50} values of E. tereticornis and Vitamin C DPPH radical scavenging ability.

Extract	IC_{50} (µg/ml)
E. tereticornis	520
Vitamin C	150

Inhibition of lipid peroxidation assay

Fig 2 illustrates the percentage inhibition of lipid peroxidation by the plant extract and vit. C. The percentage inhibition increased with increasing concentration with both samples producing more than 50% inhibition at the highest concentration (1000µg/ml) tested. The inhibitory ability of the plant extract was nevertheless lower than that of the standard as shown by the IC_{50} values in table 7 interpolated from the graph below (fig 2).

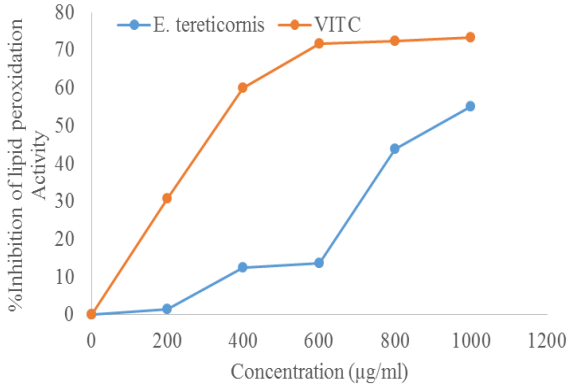

Fig 2: Inhibition of Lipid Peroxidation by ethanol extract of E. tereticornis and Vitamin C.

Table 7: IC_{50} values of E. tereticornis and Vitamin C inhibition of lipid peroxidation ability.

Extract	IC_{50} (µg/ml)
E. tereticornis	700
Vitamin C	240

Reducing Power Assay

The reducing power activity of the ethanol extract of E. tereticornis and Vitamin C is illustrated in figure 3 and found to be concentration-dependent. It was observed that at lower concentrations, E. tereticornis exhibited higher reducing power ability. In the assay, the yellow colour of the test solution changed to various shades of green and blue depending on the reducing power of each sample. The plant extract also exhibited higher reducing power ability with IC50 of 520µg/ml than the standard with IC50 of 580µg/ml as shown in table 8.

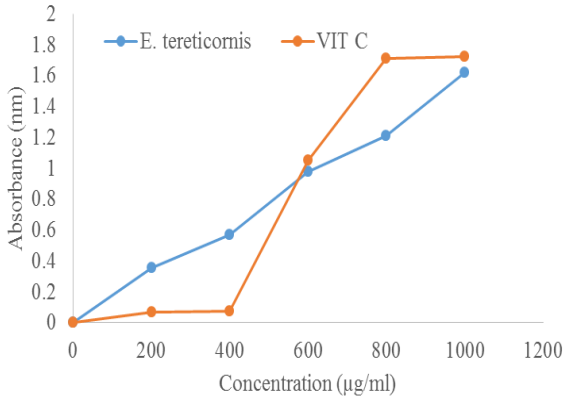

Fig. 3: Reducing power of ethanol extract of E. tereticornis and Vitamin C.

Table 8: IC_{50} values of ethanol extract of E. tereticornis and Vitamin C reducing power capacity.

Extract	IC_{50} (µg/ml)
E. tereticornis	520
Vitamin C	580

Nitric Oxide Scavenging Activity

Fig 4 shows the nitric oxide (NO) radical scavenging activity of the leaf extract of E. tereticornis and Vitamin C. NO scavenging ability is determined by the decrease in the absorbance at 550 nm induced by antioxidants. Inhibition of nitrite formation by the E. tereticornis extracts increased with increase in concentration. Nitric oxide scavenging ability measures the ability of antioxidant in scavenging radicals present and inhibit the formation of nitrite. The scavenging potency was interpolated from a graph of percentage inhibition against concentration of the extract (fig 4) and expressed as the concentration that inhibits 50% radical (IC50) as shown in table 9.

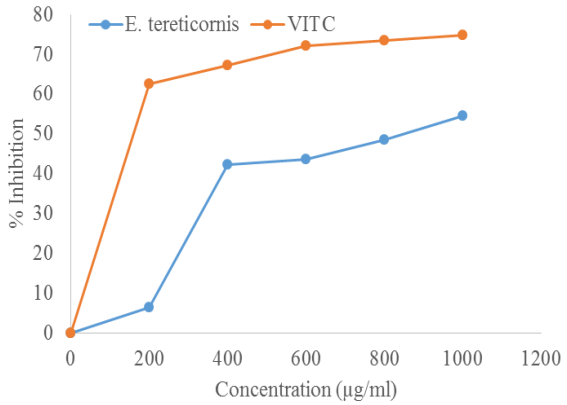

Fig. 4: Nitric oxide scavenging activity of ethanol extract of E. tereticornis compared to the standard (Vitamin c).

Table 9: IC_{50} values of E. tereticornis and Vitamin C.

Extract	IC_{50} (µg/ml)
E. tereticornis	320
Vitamin C	100

DISCUSSION

Proximate Analysis

From the results of proximate composition of Eucalyptus tereticornis given in Table1, it was shown that the leaf has carbohydrate content of 41.07 ± 0.02%. This value is high enough being a leaf. Carbohydrates generate energy required by the body as they are essential nutrient required for adequate diet.[38] They supply energy to various cells such as brain, muscle and blood.[39] From the result, the leaf of E. tereticornis could serve as a source of energy. The crude protein content of Eucalyptus tereticornis was 13.85 ± 0.01%. The plant is a moderate source of protein but small when compared to the recommended dietary allowance (RDA) for protein of 56g for individual weighing 70kg and 46g for adult weighing 50kg.[40] Being a plant, proteins from plant sources are said to have lower quality but when combined with other sources of protein like animal protein may produce adequate nutritional value.[41] Furthermore, any plant food that provides more than 12% protein is considered a good source of protein.[42] Crude fiber in food or plant is an indication of the level of non-digestible carbohydrate and lignin.[43] The crude fiber obtained was 34.50 ± 0.10%. This is high enough and considered to be appropriate. Studies have shown that increased fiber consumption may contribute to a decrease in the occurrence of digestive disorders and some diseases such as cardiovascular diseases, colon cancer, diabetes, hypertension, obesity, and several digestive disorders.[44,45] This is because it aids digestion and absorption of glucose and fat. Although crude fiber enhances digestibility, its presence in high level can cause gastro-intestinal disturbances and decreased nutrient usage[46] because of high content of cellulose and a little lignin which is indigestible in human.[47] The

crude fat content of E. tereticornis leaf extract was 2.62 ± 0.30%. This is low and expected for a leafy plant as studies have shown that leafy vegetables are poor sources of lipids.[48] A diet that provides 1–2 % of its caloric energy as lipid is said to be sufficient to human beings in that excess lipid consumption results in cardiovascular disorders[49] and obesity. More so, lipid is needed in diet because it serves as a good source of energy, aids in transport of fat-soluble vitamins, contributes to important cell processes, insulates and protects internal tissues.[41, 50] Ash content of 7.71 ± 0.21% was obtained from the analysis. Ash content is a measure of the mineral content of the food. It is the residue remaining after all the moisture has been removed and the organic material (fat, protein, carbohydrates, vitamins, organic acid) have been incinerated at a temperature of about 500^{o}C. The moisture content obtained was 0.25 ± 0.01%. Moisture content is the amount of water in a material. Water is an essential compound of many foods,[51] 20% of the total water consumption is through food moisture.[52] Moisture content is an indication of the food's water activity.[53, 54] It is used to measure how stable and susceptible a food material is to contamination by microbes.[55] The moisture content of E. tereticornis is low and within the range of values required as safe limit for storage for plant food materials.[56] This low content indicates that the leaves can be stored for a long time without the development microbial growth.

The energy (calorific) value was 243.26Kcal/100g. This is moderate in that it has been observed that vegetables have low energy values.[57] But it is comparable with reported values of some medicinal plants which ranged from 261.33 to 485.70Kcal/100g.[58, 59, 60] However, this leaf has higher calorific value than most of the common leafy vegetables like cabbage (27 kcal/100 g), spinach (26 kcal/100 g) and lettuce (21 kcal/100 g).[61] Plants with a high calorific value can be considered as a good diet and indicates that they can be used as food or may be included as a part of dietary supplements.[51]

Anti-nutrient composition

Anti-nutrients are said to be essential chemical compounds found at different amounts in virtually all parts of plants.[62] They are said to limit the use of many plants because they occur ubiquitously as natural compounds capable of eliciting deleterious effect in man and animals.[63] The result of the anti-nutrient analysis of ethanol leaf extract of Eucalyptus tereticornis in table 2 revealed the presence of oxalate (258.12mg/g), Tannin (2.06mg/g), Saponin (0.65mg/g) and phytate (0.12%). The high amount of these anti-nutritional factors shows the pharmacological activities of the leaves of this medicinal plant. These compounds confer health benefits when consumed in the right proportion as some of these nutrients could hinder bio-availability of some important minerals and other nutrients in the body. Oxalate binds and removes extra calcium. It complexes with calcium and forms calcium crystals which deposits as renal stones leading to blockage of renal tubules.[64] The high level of oxalate in Eucalyptus tereticornis plant in this study shows that the plant may not be good for consumption as it could hinder the availability of essential minerals in the body. However, studies have shown that most of these toxicants are removed during processing and cooking.[65] Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and can precipitate proteins from aqueous solution. They occur in all vascular plants. Tannin binds to proteins making them bio-unavailable. Tannins inhibits microbial activities through hydrogen bounding, iron deprivation or specific interactions with certain important proteins such as enzymes in microbial cells.[66] They have shown notable activity in cancer prevention.[67] Tannins act as an astringent, hastening wound healing and inflamed mucus membrane.[68] Phytate is the major storage form of phosphorus in leafy vegetables[69] and in many plant tissues, especially brain and seeds. It can form complexes with multivalent metals like zinc, iron and calcium or proteins and therefore reduce their bio-availability in the gastrointestinal tract.[64] However, it is an antioxidant by inhibiting iron-mediated free radical generation.[64] From this study the phytate content was the lowest and so may not cause bio-unavailability of essential minerals in the body. Saponins are glycosides such as steroid saponins and triterpenoid saponins.[70] Saponin is associated with formation of foams in aqueous solution, hemolytic activity against Red Blood Cell and cholesterol binding properties.[71] Excess level of saponins causes hypocholestrolaemia because it binds cholesterol making it unavailable for absorption.[72] When Saponin forms complexes with protein, it can hinder the digestion of protein.[73, 74] It was reported that saponins in high amount in feed affect intake of feed and reduces the rate of growth in poultry.[70, 73, 75] The presence of these bioactive compounds supports the reported pharmacological activities of E. tereticornis plant.[76, 77, 78]

Vitamin Content

The result in table 3 shows the content of the analyzed vitamins present in E. tereticornis leaf extract. Vitamin B1 (thiamin) and B2 (riboflavin) are involved in macronutrient metabolism. Vitamin B2 specifically is necessary for oxidative phosphorylation and for coenzyme formation.[79] Vitamin B9 (Folate) is needed for purines and pyrimidines synthesis that are required for production of DNA and erythropoiesis.[80] Vitamin C (ascorbic acid) is generally involved in protein metabolism and collagen synthesis;[81] possesses an antioxidant property and required for maintenance of normal connective tissues, healing of wound and also hasten the intestinal uptake of dietary iron.[82] Vitamin B1 was present in high amount (1.237mg/100g) while vitamins B2 (0.789mg/100g), B9 (0.259mg/100g) and C (0.336mg/100g) are in low amounts compared with Recommended Daily Allowance of 1.2, 1.4, 0.4 and 60mg/100g respectively.[80, 81, 83]

Mineral content

The result in table 4 shows the compositions of the analyzed minerals present in the leaf extract of E. tereticornis. It shows the presence of Iron (0.76 ± 0.01), Zinc (0.30 ± 0.21, Sodium (0.83 ±0.30), Calcium (0.06 ± 0.10) and Cobalt (0.02± 0.25). Minerals are essential for the right functioning of tissues and functions as second messengers in cascade of some biochemical mechanisms.[84] Iron is an essential constituent of proteins and acts as catalyst for certain enzymes such as cytochrome oxidase[85, 86] and needed for formation of hemoglobin.[87] It participates in energy transfer within the plant and also controls obesity by facilitating biomolecules oxidation.[85] The iron content of E. tereticornis, though in small amount and less than the recommended dietary allowance of 8mg/day can be used in diets for reducing anemia. Zinc is important in protein synthesis, replication and cellular differentiation as well as in immunity and sexual functions.[88] Sodium is present in highest amount in the leaves of Eucalyptus tereticornis, and it is a principal cation of extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body.[89] E. tereticornis leaves had low calcium content and less than the recommended dietary allowance of 1000mg/day. Calcium forms component of bones and teeth, important for coagulation of blood and muscle contraction as well as act as co-factor in enzyme reactions.[81, 89, 90] Cobalt although classified as heavy metal is beneficial to human. It is a major part of cobalamin (vitamin B_{12}) which is the primary biological reservoir of cobalt as an ultratrace element.[4] Heavy metals like cobalt are toxic because of their solubility in water.[91] Exposure to high levels results in lung and heart diseases and dermatitis. Cobalt (Co) causes an increase in hemoglobin in anemic patients with diseases such as cancer, nephritis and chronic infections. The average daily intake of cobalt is estimated to be 5 to 40 µg per day (0.04mg).[92] E. tereticornis with concentration of 0.02mg is within safe daily intake limits.

Bioactive compounds

The result of the analysis of the bioactive compounds shown in table 5 revealed that the plant is rich in phenol, flavonoid and beta-carotene with a low amount of lycopene. The total phenolic was 110.79mgGAE/g and was the highest bioactive compound found. Phenolics are said to be the most abundant secondary metabolite in plants and have given rise to more medical interest as antioxidants in terms of their ability to act as both efficient radical oxygen species (ROS) scavengers and metal ion chelator.[93] A direct relationship exists between antioxidant activity of plants and total phenolic content.[94, 95] Thus, this plant can serve as a source of phenolic antioxidant. Flavonoids are also a class of secondary plant phenolics with powerful antioxidant properties.[93] The plant is also rich in flavonoids with flavonoid content of 35.13 mgCE/g. Flavonoids are modifiers known for their antioxidant activity and also potentiate the body's reactions to allergens, viruses and carcinogens.[96] With a relatively high content of flavonoid, this plant may be useful in therapeutic roles. Carotenes are made up of specific groups like lycopene and beta-carotene.[93] The antioxidant property of carotenoids is due to singlet oxygen quenching which results in excited carotenoids that dissipate the newly acquired energy through a series of rational and vibrational interactions with the solvent, thus returning to the lower energy i.e. unexcited state and permitting them to eliminate more radical species.[93] In the present study, the beta-carotene content was 2.89mg/g while the lycopene content was 0.28mg/g. Lycopene hinders lipid peroxidation, programmed cell death and DNA damage and is considered as the most potent oxygen quencher in the carotenoid family (Chauhan et al, 2011).[10] This is because it is two-times more effective than beta-carotene, and up to ten times more effective than α-tocopherol.[11]

Antioxidant Activity

The antioxidant activity of E. tereticornis leaves were determined by measuring the DPPH and nitric oxide radical scavenging activity, inhibition of lipid peroxidation and the reducing power activity.

The DPPH and nitric oxide radical scavenging activity of plant extract at different concentrations are shown in figures 1 and 4 respectively. These were found to increase with increase in concentration and comparable with that of the standard; vitamin C. DPPH radicals are model system widely used to determine the scavenging activity of several natural bioactive compounds.[97] The result in this study indicates that the plant was potentially active in scavenging free radicals. The plant extract showed percentage RSA of 93.57 with no significant difference ($p>0.05$) when compared to the standard which showed 96.31% at the highest concentration of 1000 µg/ml (Fig 1). However, the leaf extract showed IC_{50} value of 520µg/ml which is significantly ($p<0.05$) less active than the standard with IC_{50} value of 150µg/ml as shown in table 6. IC_{50} is the concentration of the extracts to quench 50% of DPPH in the solution under the experimental conditions. IC_{50} value is inversely related to the activity as it is the measure of inhibitory concentration and a lower value would reflect greater antioxidant activity of the extract. The plant extract also exhibited scavenging activity through competing with oxygen to scavenge for the nitrite radical generated in aqueous environment. The plant extract removed the nitrite radical as there was increase in activity especially at higher concentrations comparable with the standard. The nitric oxide radical scavenging potency (IC_{50}) as shown in table 9 was interpolated from Fig 4. The plant extract was also significantly ($p<0.05$) less potent with IC50 value of 320µg/ml than vitamin C with IC_{50} of 100µg/ml. The observed high free radical scavenging activity of the plant extract could be linked to the high phenol content of the plant as phenols have been shown to possess the ability to scavenge free radicals through hydrogen donation or electron donation.[98] This ability to

scavenge free radicals is a confirmation of their capacity as radical scavengers which could find application as natural antioxidant.

The reduction of fe3+ complex to the ferrous form by the extract was observed using the reducing power assay. The reducing antioxidant power assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. It is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Increasing absorbance indicates an increase in reductive ability. The reducing capacity of compounds may serve as a significant indicator of their potential antioxidant activity.[99] In this study, the reducing power of the extract increased with increasing concentration non-significantly ($p<0.05$) when compared with the standard. It exhibited higher reductive ability at lower concentration than the standard. The reductive potency as shown in table 8 interpolated from fig 3 shows a lower IC_{50} value of 520µg/ml for the extract than that of the standard (580µg/ml). This implies that the reducing power potency of the extract is higher compared to the standard; albeit non-significantly ($p>0.05$). The extract of E. tereticornis contains reductones which exert antioxidant activity by hydrogen atom donation; and thus, has demonstrated its antioxidant potential as the reducing capacity of compounds indicates its potential antioxidant properties.[100, 101]

Degradative products such as malonaldehyde (MDA) are generated from oxidative degradation of polyunsaturated fatty acids in the cell membrane. This process is called lipid peroxidation and found to cause the destruction of cell membrane and cell damage in bio-systems.[102] Several pathological disorders such as atherosclerosis, inflammation and liver injury are associated with lipid peroxidation of cell membranes.[103] 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.[104] The reaction of MDA with thiobarbituric (TBA) has been used widely as a sensitive assay method for lipid peroxidation.[105] The generation of Fe^{2+} ascorbate in the brain homogenate was inhibited by E. tereticornis extract as shown in table 7. 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 240µg/ml as against the extract with an IC_{50} of 700µg/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. From studies, it was suggested that phenolic compounds and other chemical components have the ability to suppress lipid peroxidation either through free radical quenching, electron transfer, radical addition or radical recombination.[106]

CONCLUSION

It is evident from this study that Eucalyptus tereticornis leaf has a high nutritive value. This could serve in meeting the nutritional needs of man and animals but needs further processing to eliminate anti-nutrients present. From the result of the analysis, it was revealed that the plant is rich in bioactive compounds and this justifies its ethno-medicinal uses. Hence, Eucalyptus tereticornis is recommended as an alternative source of potential antioxidants and can provide an effective means to combat the deleterious effects of reactive oxygen species. This plant could also be used in the prevention and management of degenerative diseases such as diabetes, mellitus, and cardiovascular disorders, as well as in the prevention of postpartum distraction arising from oxidative stress.

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