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Bioactive Compounds and Antioxidant Activity of Ethanol Leaf Extract of Eucalyptus Tereticornis

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ABSTRACT

This study evaluated the bioactive compounds present in the ethanol leaf extract of Eucalyptus tereticornis as well as its antioxidant potential using standard biochemical methods. The leaf extract contains rich number of bioactive compounds (Phenol 109.41± 0.10GAE/g, Flavonoids 33.10± 0.01mgCE/g, Beta-carotene 3.16± 0.00mg/g and Lycopene 0.32± 0.00mg/g). 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 an 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. The general results indicated that the plant is rich in bioactive compounds which are believed to have contributed to the high antioxidant activities observed and its medicinal value.

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1. INTRODUCTION

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

Bioactive compounds in medicinal plants such as *Eucalyptus tereticornis* provide health benefits, including those that have proven effective in treating and managing debilitating diseases. Phytochemicals, vitamins, and minerals are the bioactive components in most plants that contribute to their protective (pharmacological/therapeutic) effects (Okwu and Ekeke, 2003).

Flavonoids, like other antioxidants, function within the body by mopping up cell-damaging free radicals and metallic ions. 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 such as cancer, rheumatoid arthritis, as well as in the degenerative processes associated with aging, including Parkinson's and Alzheimer's diseases (Ali *et al*., 2008). However, cells are equipped with several defense systems against free radical damage; including oxidative enzymes such as superoxide dismutase and catalase, or compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenolic compounds, and glutathione (Niki *et al*., 1994). Naturally, there's equilibrium between the number of free radicals made within the system and antioxidants to scavenge or quench them, to guard the body against their harmful effects (Udedi *et al*., 2012). These compounds that scavenge free radicals, might scale back the extent of oxidative stress and forestall the oxidation of biomolecules. However, the number of antioxidants present under normal physiological conditions may be insufficient to neutralize free radicals generated under pathological conditions.

2. MATERIALS AND METHODS

2.1. 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.

2.3. 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 (200 g) of powdered leaves were measured using an electronic weighing balance (Model: Adam AFP800L) and soaked with 80% of Ethanol (1000 mL) for 72 hours 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.

2.4. Bioactive Compounds

2.4.1. Total flavonoids assay

The flavonoid content was determined by a modified colorimetry method of (Barros *et al*., 2007). A diluted sample solution of 0.5 mL was mixed with 2 mL of distilled water and 0.15 mL of $NaNO_2$ solution (5%). After 6 min, 0.15 mL of $AlCl_3$ solution (10%) was added and

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allowed to stand for 6 min, then 2 mL of NaOH solution (4%) was added to the mixture. The mixture was made up to 5 mL with water and allowed to stand for 15 min and absorbance 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 grams of sample (mg CE/100g).

2.4.2. Total phenol assay

The method of (Barros *et al*., 2007) was used to determine the total phenol content of the sample. The extract, (1ml; 1mg) solution was mixed with Folin and Ciocalteu‟s phenol reagent (1 mL) and after 3 min, 1 mL of a saturated sodium carbonate solution was added and the mixture was made up to 10 mL with distilled water. The reaction mixture was kept in the dark for 90 min and thereafter the absorbance was read at 725 nm. Gallic acid was used as the standard and the results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

2.4.3. Beta carotene and lycopene

These were determined by the method of (Barros *et al*., 2007). 100 mg of dried ethanol leaf extract was vigorously shaken with 6 mL of the acetone-hexane mixture in the ratio of (4:6) for one minute and filtered with 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) = - 0.458A663 +0.372A505+0.0806A453

β-carotene (mg/100g) = 0.216A663 + - 0.304A505+0.452A453.

2.5. Antioxidant Assay

2.5.1. DPPH scavenging activity

This was assayed with the method of 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 2.5 mL of methanolic solution of DPPH (100µM) in the 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 of Radical Scavenging Activity (RSA): %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 the sample at 50% of RSA was calculated from the curve of %RSA against the sample concentration.

2.5.2. Inhibition of lipid peroxidation using TBA (Thiobarbituric acid) reactive substance

This was determined by the method of Barros *et al*., (2007). A homogenate of the 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 0.2 mL of the sample at various concentrations (0 – 500 and or 0 – 1200 µg/mL) in the presence of 0.1 mL of 10 µM Ferrosulphate and 0.1 mL of 0.1mM ascorbic acid at 37°C for 1hr. The reaction was stopped by the addition of 0.5 mL of TAC (28%) and 0.38 mL of TBA (2%) and the mixture was heated at 80°C for 20mins, centrifuged at 3000 g for 10mins to remove the precipitated protein. The absorbance of the Malondialdehyde (MDA) - TBA complex in the supernatant was read at a wavelength of 532 nM. The inhibition

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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% of lipid peroxidation inhibition (IC_{50}) was calculated from the curve of antioxidant activity percentage against the extract concentrations using ascorbic acid as the standard.

2.5.3. Reducing power assay

The method of Barros *et al*. (2007) was used to determine the reducing power. It uses the principle that an increase in the absorbance of the reaction mixture is an indication of an increase in antioxidant activity. Different concentrations of the test sample were mixed with 2.5mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%) and incubated at 50° C for 20 min followed by the addition of 2.5 mL of trichloroacetic acid (10%). The mixture was centrifuged at 1000 rpm for 8mins and the supernatant was mixed with 5 mL of deionized 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 curve of absorbance at 700 nm against extract concentration.

2.5.4. Nitric oxide scavenging activity

The method of Rozina *et al*. (2013) was used in the determination. In an 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, 2.7 mL of sodium nitroprusside (10 mM) in phosphate buffer was mixed with various concentrations of the sample and incubated at 30°C for 2 hours. The control had the same reaction mixture without the extract. The absorbance of the chromophore that formed during diazotization was read after incubation at 550 nm. Inhibition of nitrite formation by the plant extract and standard antioxidant ascorbic acid was 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.

3. RESULTS

For bioactive compounds, the result of bioactive compounds is presented in **Table 1**. The phenolic content of the extract expressed in milligrams of gallic acid per gram of the dry matter was high. The flavonoid content was expressed in milligrams of Catechin per gram of dry extract was relatively high while lycopene was low and beta-carotene was fairly high.

For DPPH scavenging activity**, t**he RSA of ethanol leaf extract of *E. tereticornis* using vitamin C as standard is presented in **Figure 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 curve of DPPH radical scavenging ability at 50% of RSA shows that the *E. tereticornis* has a higher DPPH scavenging ability when compared with the standard; Vitamin C in **Table 2**.

For inhibition of lipid peroxidation assay, **Figure 2** presents the percent inhibitory ability of the extract on lipid peroxidation with vitamin C as standard. The inhibition increased with an 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 3** interpolated from the curves in **Figure 2**.

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Table 1. Bioactive compound compositions of the ethanol leaf extract of *Eucalyptus Tereticornis*.

Parameter	Concentration
Total phenol (mgGAE/g)	109.41±0.10
Flavonoid mgCE/g	33.10±0.45
Beta-carotene mg/g	3.16±0.00
Lycopene (mg/g)	0.32±0.00

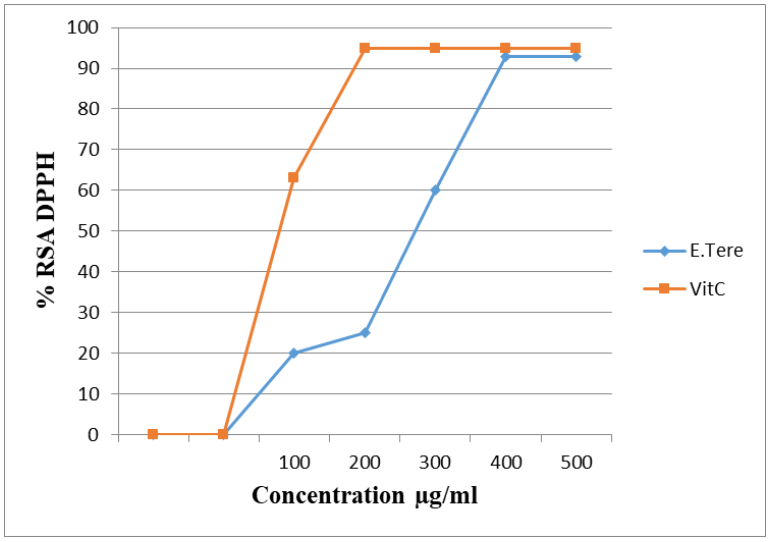

Figure 1. DPPH radical scavenging activity of ethanol leaf extract of *E. tereticornis* and the standard; vitamin C.

Table 2. IC_{50} values of *E. tereticornis* and Vitamin C interpolated from the curve of DPPH radical scavenging ability in **Figure 1**.

Extract	IC_{50} (ug/mL)
E. tereticornis	280
Vitamin C	80

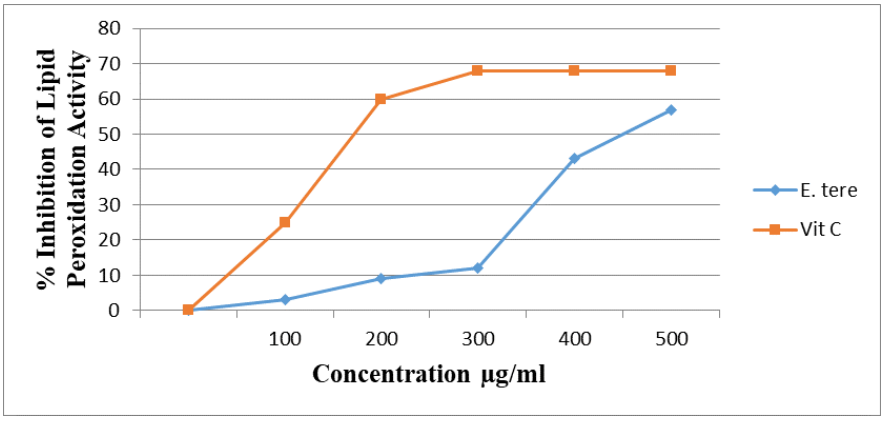

Figure 2. Inhibition of Lipid Peroxidation by ethanol extract of *E. tereticornis* and Vitamin C.

Table 3. 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

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For reducing power assay, the reducing power activity of the ethanol leaf extract of *E. tereticornis* with Vitamin C standard is presented in **Figure 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 concerning concentration as shown in **Table 4** interpolated from **Figure 3**.

For nitric oxide scavenging activity, the nitric oxide (NO) radical scavenging ability of the leaf extract and Vitamin C as standard is presented in **Figure 4**. Inhibition of nitrite formation by the *E. tereticornis* extracts increased with an increase in concentration relative to the standard vitamin C and IC_{50} interpolated from the graph as shown in **Table 5**.

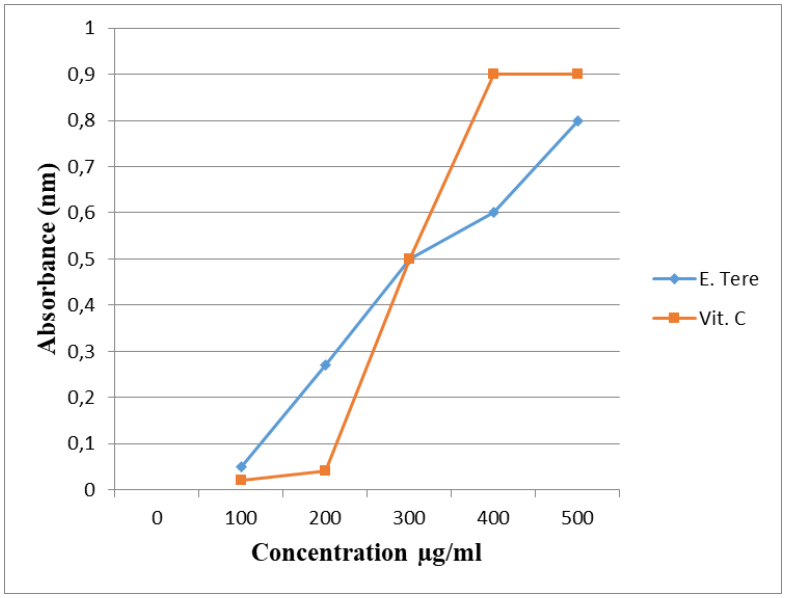

Figure 3. Reducing power of ethanol extract of *E. tereticornis* and Vitamin C.

Table 4. IC_{50} values of ethanol extract of E. tereticornis and Vitamin C reducing power capacity interpolated from **Figure 3.**

Extract	IC_{50} (ug/mL)
E. tereticornis	300
Vitamin C	300

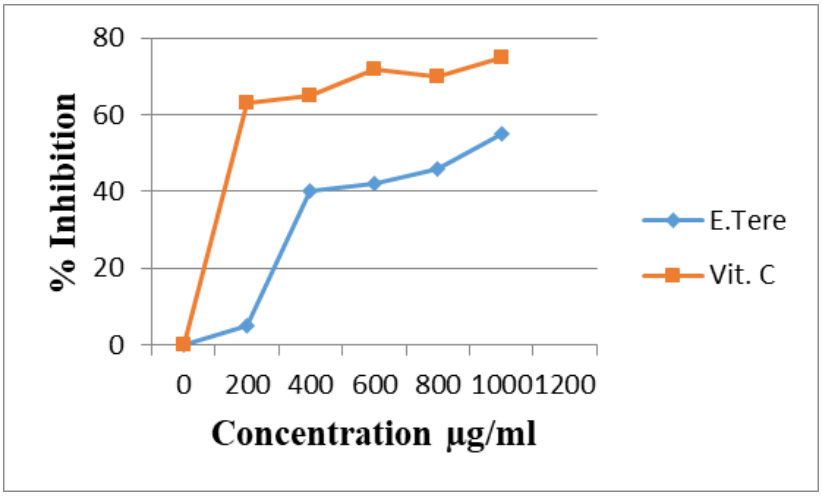

Figure 4. Nitric oxide scavenging activity of ethanol extract of *E. tereticornis* compared to the standard (Vitamin C).

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Table 5. IC_{50} values of *E. tereticornis* and Vitamin C.

Extract	IC_{50} (ug/mL)
E. tereticornis	900
Vitamin C	100

4. DISCUSSION

The result of the analysis of the bioactive compounds shown in **Table 1** revealed that the plant is rich in phenol, flavonoid, and beta-carotene with a low amount of lycopene. The total phenol was 109.41 mgGAE/g and was the highest bioactive compound found. Phenolics are said to be the most abundant secondary metabolite in plants with antioxidant activity and flavonoids are one of them. The plant with a relatively high content of flavonoid, 33.10 mgCE/g may be useful in therapeutic roles. Carotenes are made up of specific groups like lycopene and beta-carotene with antioxidant properties. In the present study, the beta-carotene content was 3.16mg/g while the lycopene content was 0.32 mg/g. Lycopene hinders lipid peroxidation, programmed cell death, and DNA damage, which is considered the most potent oxygen quencher in the carotenoid family (Chauhan *et al*., 2011).

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 an 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 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 an IC_{50} value of 280µg/mL which is less active than the standard vitamin C with an IC_{50} value of 80µg/mL as shown in **Table 2**.

Oxidative degradation of polyunsaturated fatty acids in the cell membrane produces malonaldehyde (MDA) which is degradable. This process is called lipid peroxidation and is found to destroy cell membrane and cell damage in bio-systems. 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 the goat was inhibited by *E. tereticornis* extract as shown in **Table 3**. The percentage inhibition activity increased with an 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 can 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

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biomolecules and the 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 an aqueous environment. The extract removed nitrite radicals as there was an increase in activity at higher concentrations concerning the standard. The nitric oxide radical scavenging potency (IC_{50}) as shown in **Table 4** was interpolated from **Figure 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 concerning vitamin C standard could be attributed to the 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 a ferric–ferrous complex that has an absorption maximum at 700 nm. This is merely a reduction of Fe^{3+} to Fe^{2+} and an increase in absorbance is an indication of an increase in reductive ability which is an indication of its antioxidant potential ability (Shahidi and Wanasundara, 1992). In this study, the increase in reducing the power of the extract paralleled that of the standard with an equimolar concentration of IC_{50} of 300 µg/mL as interpolated from **Figure 3**. The extract contains reductones that 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).

5. CONCLUSION

From the result of the analysis, Eucalyptus tereticornis has antioxidant activity against excited oxygen species. Its high content of bioactive compounds is a justification for its medicinal value. The secondary metabolites, phenols, flavonoids account for the plants' therapeutic roles. 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.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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