In vitro free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*

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ABSTRACT

Background: Reactive oxygen species (ROS) are implicated in various pathological conditions. Synthetic antioxidants have adverse health effects, while many medicinal plants have antioxidant components that can prevent the harmful effects of ROS. **Objectives:** This study quantitatively determined the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties of ethanol extract of the stem bark of *Terminalia glaucescens* (EESTG). **Materials and Methods:** The objectives were achieved based on *in vitro* assays. Data were analyzed by Sigma Plot (version 11.0). **Results:** Using gallic acid as the standard compound, TPC value obtained was 596.57 μg GAE/mg extract. TFC content of EESTG, determined as quercetin equivalent was 129.58 μg QE/mg extract. Furthermore, EESTG significantly ($P < 0.001$) displayed higher reducing power activity than the standard compounds (ascorbic acid and butylated hydroxytoluene [BHT]). Total antioxidant capacity assay, measured by phosphomolybdate method, was 358.33 ± 5.77 μg butylated hydroxytoluene equivalents [BHTE]/mg extract. β-carotene-linoleate bleaching method affirmed the potency of EESTG because of its significantly ($P < 0.001$) higher anti-oxidant activity when compared with quercetin and BHT. Based on DPPH assay, EESTG displayed significantly ($P < 0.001$) higher activity than BHT, while the hydroxyl radical scavenging activities of BHT and quercetin significantly ($P < 0.001$) exceeded that of the extract, although EESTG still displayed a high level of activity obtained as 83.77% in comparison to 92.80% of the standard compounds. **Conclusion:** Findings from this study indicate the presence of promisingly potent phytoconstituents in EESTG that have the capability to act as antioxidants and free radical scavengers.

Key words: Antioxidants, extract, free-radicals, standard compounds, *Terminalia glaucescens*

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INTRODUCTION

Free radicals/reactive oxygen species (ROS) are generated from both endogenous and exogenous sources.[1] Typical examples of such sources include enzyme activities (e.g. xanthine oxidase, NADPH oxidase etc.), leakage of electrons from the mitochondrial electron transport chain (ETC), exposure to certain chemicals (e.g. doxorubicin, cigarettes etc.), auto-oxidation (e.g. adrenaline, dopamine etc.), catalytic action of free transition metals (e.g. Fe^{2+}, Cu^{+} etc.) and radiation from the environment (e.g. radon, UV etc.).[1] It has been estimated that one free radical is produced for every -25 oxygen molecules reduced by normal respiration.[2]

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Free radicals have been directly implicated in various pathological conditions including diabetes mellitus, multiple sclerosis, heart disease, Parkinson's disease, inflammation, Alzheimer's disease, atherosclerosis, stroke, cancer, etc.[3,4] Most of the body macromolecules, such as lipids, proteins, deoxyribonucleic acid [DNA] and carbohydrates are susceptible to damage by free radicals.[5]

However, antioxidants have evolved with protective roles against such damage.[5] The negative cellular effects of ROS can be countered by enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), etc.; non-enzymatic, metabolic and nutrient antioxidants including glutathione, vitamin C, vitamin E, etc.; metal binding proteins like

ferritin, lactoferrin, albumin, ceruloplasmin, etc., and phytochemicals such as quercetin, resveratrol, capsaicin etc.[6] The mechanisms of protective actions of antioxidants against ROS toxicity include prevention of the formation of ROS, interruption of ROS attack, scavenging of the reactive metabolites or their conversion to stable molecules or molecules of lower reactivity.[7]

Many medicinal plants, vegetables, and fruits have antioxidant components, especially phenolic compounds, which when consumed, have been confirmed to prevent the destructive/degenerative effects caused by oxidative stress.[8] Aside flavonoids and phenolic compounds which are widely distributed in plants; vitamin C, vitamin E, and carotenoids are some of the other antioxidant components of medicinal plants.[9] These phytoconstituents have been reported to exert various biological effects that include anti-inflammatory, free radical scavenging, anti-carcinogenic, anti-oxidant activities, etc.[10] Research activities focusing on medicinal plants have been encouraging because of their high content of potent antioxidants, accessibility, economic viability and next-to-no side effects.[11] Using four different methodologies, the *in vitro* antioxidant/radical scavenging activities of ethanolic extract of *Cassia occidentalis* leaves have been demonstrated.[12]

The plant genus *Terminalia* (Combretaceae) has been associated with various biological properties such as antimicrobial, cardiac, hypolipidemic, anti-atherogenic, hepatoprotective effects, etc.[13] It is recognized by various names including idi (Nigeria, Yoruba); wongwong (Ghana, Brong); foni-baji (Sierra Leone, Mende); alotu diésama (Dahomey, Gbe-Fon); en'ga (Ivory Coast, Manding-Dyula); vara sa (Senegal, Manding-Bambara), etc. The genus consists of about 135 species which are predominantly found in the tropical regions of the world.[14] *Terminalia glaucescens* has been used in the treatment of dysentery and microbial infections, and also useful in the last stages of AIDS.[15] Ethanol extract of *T. glaucescens* has been reported to exhibit aldose reductase inhibition,[16] cytotoxic effects[17] and antiplasmodial activity.[18] However nothing has been reported on the free radical scavenging and antioxidant properties of extract of *Terminalia glaucescens*. The objective of this study therefore was to quantitatively determine the total phenolic content, total flavonoid content, antioxidant properties of ethanol extract of the stem-bark of *Terminalia glaucescens* (EESTG) in comparison with standard antioxidant compounds.

MATERIALS AND METHODS

Chemicals

Chemicals used in this study are 2-deoxy-D-ribose, potassium ferricyanide, trichloroacetic acid, Folin-Ciocalteu reagent, gallic acid, L-ascorbic acid, ethylenediamine tetraacetic acid, β-carotene, linoleic acid, Tween 20, trichloroacetic acid, ammonium molybdate, butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium bicarbonate, thiobarbituric acid, ferric chloride ($FeCl_3 \cdot 6H_2O$), ethanol, hydrogen peroxide etc., All reagents used were of analytical grade and products of Sigma Chemical Co. (St. Louis, USA) or BDH Chemical Ltd, Poole, UK.

Plant source and identification

Terminalia glaucescens used in this study was collected from Lokoja, Nigeria in May, 2012. It was identified by a plant taxonomist in the Department of Botany, University of Ibadan, Nigeria and a voucher specimen was deposited in the Herbarium of same Department with the Herbarium number UIH-22404.

Preparation of extracts

The stem bark of the test plant was washed with distilled water to remove any dirt and air-dried under shade until a constant weight was attained after 14 days. The dried sample was grinded to powder, sieved and packed into polythene bags and stored at 4°C. Four hundred grams (400 g) of the powered plant sample was soaked in 70% ethanol (1600 ml) for 72 h with intermittent stirring/shaking.[19] At the end of the extraction, the extract was filtered through Whatman filter paper No. 1 (Whatman Ltd., England). The filtrate was concentrated/evaporated to dryness using a rotary evaporator (RE-52A, Shanghai Ya Rong Biochemistry Instrument Factory, Shanghai) under reduced pressure (in order to speed up the process) at 40°C and stored at 4°C until when needed. The percentage yield of the extraction was 9.39% w/w.

Quantitative phytochemical analyses

Estimation of total phenolic content

The amount of total phenolics in the plant extracts was determined with the Folin-Ciocalteau reagent using the method of Spanos and Wrolstad,[20] as modified by Lister and Wilson.[21] To 0.50 ml of each sample (800 μg/ml), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using UV/VIS spectrometer T70. All tests were performed in triplicate and the graph was plotted with the average of the three determinations. Results are expressed as micrograms of gallic acid equivalents per milligram of dry weight (μg GAE/mg) of extract.

Estimation of total flavonoid content

The determination of the total flavonoid content (TFC) was carried out as described by Nickavar *et al.*[22] Briefly,

2.5 ml of each extract solution (800 μg/ml) was mixed with 2.5 ml $AlCl_3$ reagent in 90% ethanol and allowed to stand for 40 min at room temperature. After that, the absorbance of the mixture was measured at 415 nm using UV/VIS spectrometer T70. The blank was made up of 2.5 ml of 90% ethanol plus sample solution (2.5 ml). Quercetin was used as a reference/standard compound. All tests were performed in triplicate and the TFC for extract expressed as micrograms of quercetin equivalents per milligram (μg QE/mg) of extract was determined on the basis of the linear calibration curve of quercetin (absorbance versus quercetin concentration).

In vitro antioxidant/free-radical scavenging activity assays

Reducing power ability

The reducing power of the extract was investigated by the Fe^{3+}-Fe^{2+} transformation in the presence of the fractions as described by Fejes *et al.*[23] The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[24] One ml of the fraction (50-800 μg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 *g*. About 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water and shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Butylated hydroxytoluene (BHT) and ascorbic acid were used as the standards. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Total antioxidant assay by phosphomolybdate method

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using butylated hydroxytoluene (BHT) as the standard.[25] An aliquot of 0.1 ml of the fractions (50-800 μg/ml) was combined with 1.0 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against the blank using a UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. The total antioxidant capacity was expressed as μg equivalents of BHT by using the standard BHT graph.

β-Carotene-linoleate bleaching assay

The antioxidant activity of the extract was assayed based on the β-carotene bleaching (BCB) method developed by Velioglu *et al.*[9] β-carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were transferred into a round-bottomed flask. The mixture was then added to 0.2 ml of extract or standard (BHT and quercetin) or ethanol (as control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator (RE-52A, Shanghai Ya Rong Biochemistry Instrument Factory, Shanghai). Following evaporation, 50 ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. Two milliliter (2 ml) aliquots of the emulsion were pipetted into test tubes and immediately placed in a water bath at 50°C. The absorbance was read at 20 min intervals for 2 h at 470 nm, using UV/VIS spectrometer T70. Degradation rate (DR) was calculated according to first order kinetics, using the following equation based on Al-Saikhan, Howard, and Miller: [26]

$$\ln (a/b) \times 1/t = DR_{sample} \text{ or } DR_{standard}$$

where ln is natural log, a is the initial absorbance (at 470 nm) at time 0, b is the absorbance (at 470 nm) at 20, 40, 60, 80, 100 or 120 min and t is the initial absorbance (470 nm) at time 0. Antioxidant activity (AA) is expressed as percent of inhibition relative to the control, using the following formula:

$$AA = \left(\frac{DR_{control} - DR_{sample \text{ or } standard}}{DR_{control}} \right) \times 100$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antiradical activity of the extract was estimated according to the procedure described by Nickavar *et al.*[22] Briefly, a 0.1 mM solution of DPPH radical solution in 90% ethanol was prepared and 1 ml of this solution was mixed vigorously with 50 μl of different concentrations (50-800 μg/ml in ethanol) of each extract. After 30 min incubation in the dark and at room temperature, absorbance (A) was measured at 518 nm using a UV/VIS spectrometer -T70. The percentage of the radical scavenging activity (RSA) was calculated based on the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{cont.} - A_{sample})/A_{cont.} \times 100$$

A_{cont} and A_{sample} are the absorbance values (at 518 nm) for the control and sample, respectively.

90% ethanol (1 ml) plus each sample solution (50 μl) was used as blank. DPPH solution (1 ml) plus 90% ethanol (50 μl) was used as negative control. L-ascorbic acid solution (50 μl, at the concentrations of 50-800 μg/ml in ethanol) was used as positive control [i.e. standard/reference]. The EC50 value, defined as the concentration

of the sample leading to 50% reduction of the initial DPPH concentration, was obtained from the linear regression of plots of mean percentage of the antioxidant activity against the concentration of the test extracts (μg/ml) obtained from three replicate assays. The results were also expressed as AEAC (Ascorbic acid equivalent antioxidant capacity) i.e. mg Vitamin C equivalents/mg dry wt, which was calculated as follows: [27]

$$\frac{EC_{50}\ Vit-C(mg/ml)}{EC_{50}\,sample(mg/ml)} = \frac{X.mg\ Vit-C\ equivalents}{mg\,dry\,wt}$$

where EC50 Vit C and EC50 sample are the effective concentrations of vitamin C and sample respectively.

Hydroxyl radical scavenging assay

Non-site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation:

Hydroxyl radical scavenging activity was measured by the ability of the extract to scavenge the hydroxyl radicals generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction).[28] The reaction mixture in a final volume of 1.0 ml contained 100 μl of 2-deoxy-D-ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500 μl of the extract at various concentrations (50–800 μg/ml) in buffer, 200 μl of [1.04 mM EDTA and 200 μM $FeCl_3$] (1:1v/v), 100 μl of 1.0 mM hydrogen peroxide (H_2O_2) and 100 μl of 1.0 mM ascorbic acid. Test samples were kept at 37°C for 1 h. The free radical damage imposed on the substrate, deoxyribose, was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Quercetin and BHT (50–800 μg/ml) were used as positive controls. The scavenging activity on hydroxyl radicals was expressed as

$$\text{Inhibition } \% = (1 - A/A_0) \times 100$$

where A_0 is the absorbance of the negative control (without sample) at 532 nm, and A is the absorbance at 532 nm of the reaction mixture containing sample.

Statistical analysis

For statistical analysis, data were analyzed using Sigma Plot (version 11.0). The results were expressed as mean ± SD (standard deviation) and the EC50 values were obtained from the linear regression plots. Pearson's correlation test was used to assess correlations between means. One-way ANOVA was used to assess differences between means; if significant differences were found ($P < 0.001$), the means were pairwise compared using Holm-Sidak Test.

RESULTS

Total phenolic content (TPC) of EESTG

In this present study, we used gallic acid as the standard phenolic compound and presented our results in microgram gallic acid equivalents per milligram (μg GAE/mg) of dry plant extract. We found the value of the TPC of the extract to be 596.57μg GAE/mg extract.

Total flavonoid content (TFC) of EESTG

Our result confirms the presence of flavonoids in the extract based on quercetin as the reference compound and the TFC expressed in microgram quercetin equivalents per milligram (μg QE/mg) of dry extract. The TFC was found to be 129.58 μg QE/mg.

Reducing power activity

Figure 1 shows our result on the reducing power activity of EESTG. At the minimum concentration of extract/standards used in this study (i.e. 50 μg/ml), EESTG, ascorbic acid and butylated hydroxytoluene (BHT) had activity values corresponding to 0.0830 ± 0.0101, 0.0247 ± 0.0055, and 0.1750 ± 0.0113, respectively. Whereas at the highest concentration (i.e. 800 μg/ml), the activity values of EESTG, ascorbic acid and BHT were 0.4897 ± 0.0190,1.1447 ± 0.0580, 1.2560 ± 0.0362, respectively.

Total antioxidant assay by phosphomolybdate method

The total antioxidant capacity is quantitatively expressed as microgram BHT equivalents per mg (μg BHTE/mg) of dry extract. EESTG was found to have 358.33 ± 5.77 μg BHTE/mg dry extract.

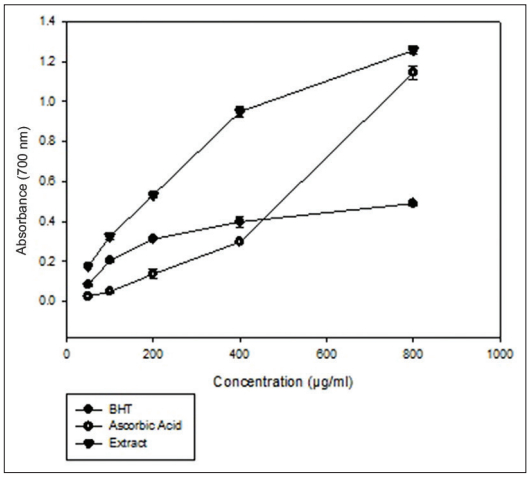

Figure 1: Reducing power of EESTG and the positive controls [BHT and ascorbic acid]. All values are reported as means ± SD ($n = 3$)

Β-carotene-linoleate bleaching assay

Figure 2 shows the degradation rates of EESTG in comparison with the negative and positive controls while a comparison of anti-oxidant activities is displayed in Figure 3.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Figure 4 shows the DPPH radical scavenging activity of ascorbic acid, BHT and EESTG, while Figure 5 is a representation of the corresponding anti-oxidant activities based on DPPH assay.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of EESTG and the positive controls (BHT and quercetin) at different concentrations (50, 100, 200, 400, 800 μg/ml) are shown in Figure 6.

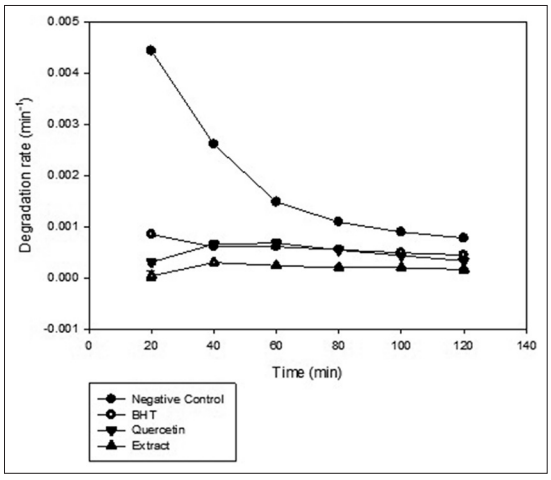

Figure 2: Degradation rate of the ethanol extract of the stem bark of *Terminalia glaucescens* [EESTG] assayed by β-carotene bleaching method ($n = 3$)

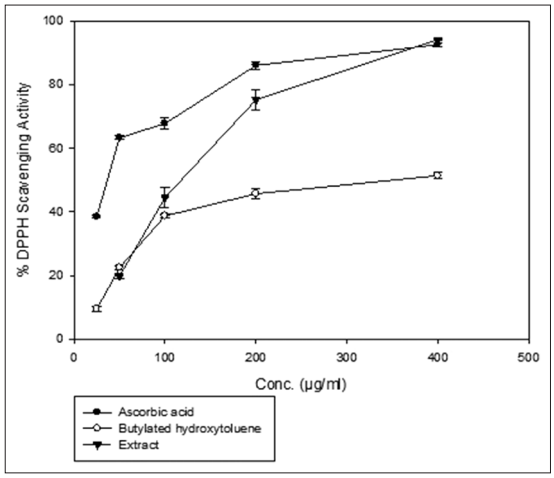

Figure 4: DPPH radical scavenging activity of ascorbic acid, BHT and ethanol extract of the stem bark of *Terminalia glaucescens* [EESTG]. Values are the average of duplicate experiments and represented as mean ± standard deviation

DISCUSSION

In order to ascertain whether there is any link between the ethnomedicinal applications of *Terminalia glaucescens* and its antioxidant activities, different methods were employed to evaluate the free radical scavenging and antioxidant activities of ethanol extract of stem bark of *Terminalia glaucescens* (EESTG). We evaluated the total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging, reducing power, total antioxidant based on phosphomolybdate method, hydroxyl radical scavenging and β-carotene-linoleate bleaching activities of the extract.

The total phenolic content was assayed using Folin-Ciocalteau reagent. This method which is routinely employed to study phenolic antioxidants is fast, convenient, simple and most importantly reproducible.[29] The value

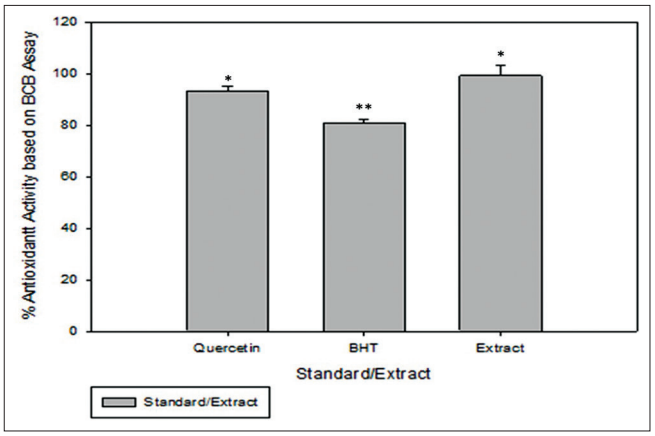

Figure 3: Antioxidant activity (%) of ethanol extract of the stem bark of *Terminalia glaucescens* [EESTG] assayed by β-carotene–linoleate bleaching. Values are mean ± SD for triplicate assay. *$P < 0.001$; not significantly different.**$P < 0.001$; significantly different from quercetin and extract

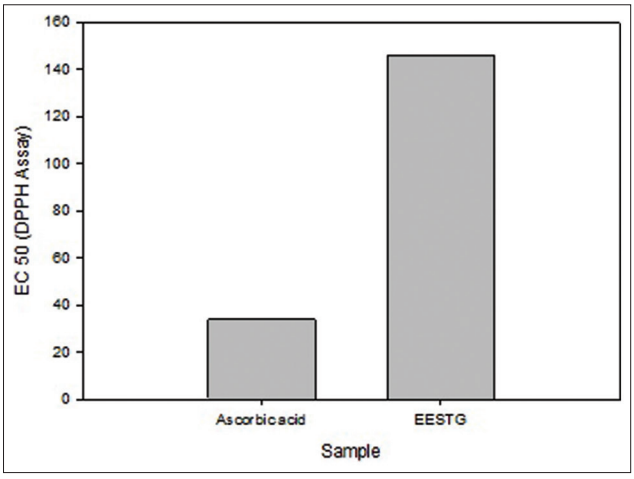

Figure 5: EC 50 values of -ethanol extract of the stem bark of *Terminalia glaucescens* [EESTG] and the standard, vit. C (ascorbic acid)

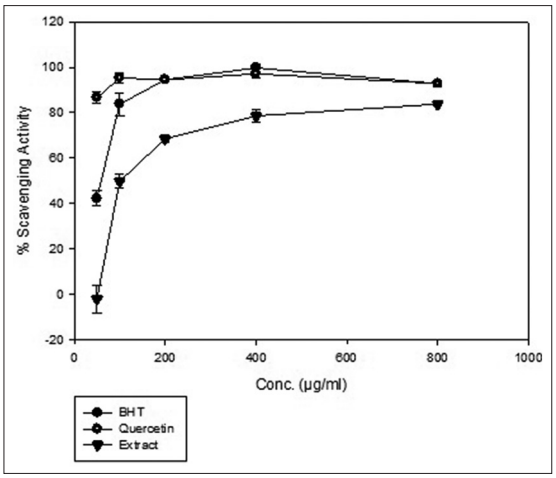

Figure 6: Hydroxyl radical scavenging activity of the ethanol extract of the stem bark of *Terminalia glaucescens* [EESTG] and the positive controls [BHT and quercetin]. All values are reported as means ± SD (*n* = 3)

of the TPC of the extract obtained confirms that the extract is very rich in phenolic contents. Phenolics have received much scientific attention because they are the most widely-spread secondary metabolites in the plant kingdom and aside this, they are also known as sources of potential natural antioxidants because of their abilities to act both as efficient radical scavengers and metal chelators.[30]

Our result on total flavonoid content shows that the flavonoid content of EESTG is lower in quantity as compared to phenolic content just in consonance with the results earlier obtained by some other researchers.[31,32] The antioxidant activities of phenolic compounds and flavonoids in biological systems have already been established based on their abilities to act as scavengers of singlet oxygen and free radicals;[33] thus validating the presence of antioxidants and free radical scavengers in the extract.

We determined the reductive ability of EESTG by measuring the transformation of Fe^{3+} to Fe^{2+} which has been known to take place in the presence of extracts/samples that possess reducing property.[34] It is evident from our findings that the extract possesses antioxidant activity in a concentration-dependent manner, which may imply its relevance in attenuating oxidative damage to cellular components and thereby prevent oxidative stress. At both minimum and maximum concentrations, EESTG significantly ($P < 0.001$) displayed higher reducing power activity than the reference compounds. These results seem to validate the basis for the therapeutic use of the extract in traditional medicine because correlations are known to exist between the reductive ability of a compound and its antioxidant activity, although it should be of note that a reductant is not necessarily an antioxidant, but an antioxidant is commonly a reductant.[35]

Apart from the advantage of being employed for the spectrophotometric quantitation of total antioxidant capacity, the determination of the total antioxidant capacity by phosphomolybdate method also employs cost-effective reagents.[36] The principle of this assay involves the activity of an antioxidant compound which leads to reduction of the hexavalent form of molybdenum [Mo (VI)] to the pentavalent form [Mo (V)], and the formation of a green phosphate/Mo (V) complex at acidic pH and at higher temperature. This is spectrophotometrically measured at 695 nm. The result we obtained based on this assay confirms the antioxidant potency of EESTG in comparison to BHT as a standard antioxidant.

The β-carotene-linoleate bleaching (BCB) method employs an emulsified lipid and therefore applicable especially to investigate lipophilic antioxidants such as the antioxidant activity of essential oils. If polar compounds such as ascorbic acid, rosmarinic acid, etc., are tested by the BCB method, they would be considered as weak antioxidants;[37] as a result of this factor, the reference compounds that we used in this study were quercetin and BHT. In this assay, β-carotene, a biologically oxidizable substrate, gives direct information on the ability of an extract to prevent oxidation.[38] When linoleic acid is oxidized, it produces hydroperoxide-derived free radicals which bleach the yellow color of β-carotene. Hence, this assay quantifies the ability of the extract to prevent, impede or reduce the formation of free radicals and thus the anti-oxidant activity of the extract is directly measured by the extent to which the bleaching of β-carotene can be prevented. This ability of an extract is a product of the presence of different antioxidants which can neutralize the linoleate-free radical and other free radicals formed in the system.[25] A correlation between degradation rate and bleaching of β-carotene displays that EESTG with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. This affirms the potency of the extract because of its higher anti-oxidant activity as compared with the controls. Specifically, EESTG displayed a significantly ($P < 0.001$) higher anti-oxidant activity when compared to BHT, and the activity of EESTG was higher than that of quercetin although not significant ($P = 0.139$).

DPPH is a stable, nitrogen-centered free radical which produces violet/purple color in ethanol solution and fades to shades of yellow color in the presence of antioxidants. One peculiarity of this method is that it allows testing of both lipophilic and hydrophilic compounds[37,38] in comparison to other methods that are restricted in the nature of antioxidants that they can be used to quantify. Based on these facts, DPPH assay is one of the most widely employed methods for screening antioxidant activities of plant extracts.[39] The purple color of DPPH

solution was reduced to a yellow colored product, diphenylpicryl hydrazine on the addition of EESTG in a concentration-dependent manner. At the least concentration of standard/extract (50 μg/ml), the percent DPPH radical scavenging activities of vitamin C, BHT and EESTG were 63.2145 ± 0.2593, 0.8621 ± 0.2155, and 19.7557 ± 0.8973, respectively; while at the highest concentration (800 μg/ml), the corresponding activities were 92.6995 ± 0.1698, 27.0833 ± 1.3856, 93.2471 ± 0.1244, respectively. These results show that EESTG displayed significantly ($P < 0.001$) higher activity than BHT but lower activity than ascorbic acid at 50 μg/ml. Also, the extract was significantly ($P < 0.001$) more active than BHT, but of no significant difference from the activity of vitamin C at 800 μg/ml. Based on DPPH assay, we also expressed the antioxidant activities as the 50% effective concentration (EC50) and ascorbic acid equivalent antioxidant capacity (AEAC). EC50 value, defined as the concentration of the sample leading to 50% reduction in the initial DPPH concentration, was obtained from a calibration curve for the extract. The lower the EC50 value the higher the antioxidant activity of a sample. The EC50 values of the root, leaf, whole plant and stem of *Sida rhombifolia* have been reported by Kamlesh *et al.*[40] to be 546.1, 852.8, 983.8 and 1,222.5 μg/ml respectively, as compared to 145.54 μg/ml and 33.72 μg/ml which we obtained for EESTG and ascorbic acid respectively. The higher the AEAC value, the greater is the antioxidant activity. EESTG showed an AEAC value of 1.43 mg Vitamin C equivalents/g dry wt of extract. Summed up together, the EC 50 and AEAC values obtained for EESTG imply that the extract contains phytochemicals with antioxidant properties.

Hydroxyl radical (HO•) is one of the most powerful free radicals directly implicated in the irreversible damage inflicted by oxidative stress.[41] It is generated mainly through Fenton reaction; and other routes such as the reaction between hypochlorous acid and superoxide anion as well as the decomposition of peroxynitrous acid. The overall effects of hydroxyl radicals have the inclination of causing mutagenesis, carcinogenesis and aging.[41] In this assay, the incubation of ferric-EDTA with H_2O_2 and ascorbic acid at pH 7.4 led to the production of hydroxyl radicals. These radicals were detected by their ability to degrade 2-deoxy-D-ribose into fragments, on heating with thiobarbituric acid (TBA) at low pH forming a pink chromogen.[42] The presence of anti-oxidants in EESTG induced the removal of hydroxyl radicals and thus prevented the degradation of 2-deoxy-D-ribose in a concentration-dependent manner. Our results show that the hydroxyl radical scavenging activities of the standard compounds (BHT and quercetin) significantly ($P < 0.001$) exceeded those of the extract at both the lowest (50 μg/ml) and highest (800 μg/ml) concentrations used in this study. It is crucial to note that the extract displayed a high level of potency although not as much as the standards as can be seen that at 800 μg/ml in which EESTG had a scavenging activity of 83.77% while both standard compounds had 92.80%. This fact still promisingly indicates the presence of potent phytoconstituents in EESTG that have the capability to scavenge hydroxyl radicals although the activities of such components may have been shielded by the presence of other components in the heterogenous extract.

CONCLUSION

A thorough examination of the various *in vitro* antioxidant and free radical scavenging assays carried out on ethanol extract of stem bark of *Terminalia glaucescens* points to the fact that the extract contains some phytocomponents with potent antioxidant activity as evident most emphatically from β-carotene-linoleate bleaching assay and reducing power activity. There is also a very high tendency that such constituents might be phenolic in nature based on the high value of the total phenolic content of the extract in comparison with its total flavonoid content.

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