academicJournals

Vol. 12(1), pp. 7-16, January 2018
DOI: 10.5897/AJPS2017.1547
Article Number: C14A2BD66937
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Phytochemical analyses and comparative *in vitro* antioxidant studies of aqueous, methanol and ethanol stem bark extracts of *Simarouba glauca* DC. (Paradise tree)

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Received 15 March, 2017; Accepted 31 July, 2017

This study was conducted to evaluate selected phytochemicals (antioxidant potentials of aqueous, methanol and ethanol stem bark extracts of *Simarouba glauca*) relative to standard antioxidants. Sample was harvested, air dried, pulverized and extracted with aqueous and absolute methanol and ethanol; freeze dried at the National Energy Commission Centre, University of Benin. Alkaloids, phenols and tannins were identified, and also flavonoid was detected. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reducing power activity, total antioxidant activity, hydroxyl radical activity, trolox equivalent antioxidant activity and nitric oxide ($NO^{\bullet}$) radical scavenging activity of stem bark extracts of *S. glauca* were evaluated, and all the experiment were conducted in a dose dependent manner. Butylated hydroxytuolene (BHT), ascorbate and trolox were introduced as positive controls (antioxidant). DPPH radical scavenging activity of stem bark extracts did not exhibit anti-radical activity at 50% inhibition but demonstrated less anti-radical activity at percentage inhibition lower than 50%. Extracts yielded significant reducing power and total antioxidant activities (FRAP). Hydroxyl radical scavenging activities of extracts was substantial; extracts exhibited significant anti-radical activity when trolox was introduced as positive antioxidant control, while nitric oxide scavenging activities was unprecedented. The presence of phytochemicals and antioxidant principles proffer high medicinal value to *S. glauca*.

Key words: *Simarouba glauca,* stem bark, oxidants, radical scavenging properties.

INTRODUCTION

Simarouba glauca (*medicinalis*) most commonly referred to as the "Paradise Tree", belongs to the family *Simaroubaceae*. Other common names includes: Aceituno, bitterwood, dysentery bark, palo amargo,

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pitomba, robleceillo and simaba (Moron et al., 1971). The parts of the plant commonly reported to be used locally are the leaves, wood and stem back (Technical Data Report for *Simarouba*, 2002). The leaf extracts of *S. glauca* have previously been reported to possess some essential phytochemicals and anti-free radical potentials (Osagie-Eweka et al., 2016; Umesh, 2015). On the bases of the previous research conducted, further studies of thephytochemicals and anti-free radical potential of the stem bark extracts of *S. glauca* was done.

Description

Simarouba is indigenous to the rainforest and other tropical areas in Mexico, Cuba, Haita and Central America. It grows up to 20 m height and has a trunk 50 to 80 cm in diameter. It produces bright green leaves 20 to 50 cm in length, small white flowers, and small yellow-reddish fruits (Polonsky, 1978). The root system is shallow and suitable for mountain soils. Stem is up to 9 m high with 40-50 cm diameter. It has finely cracked and grey colored outer bark, while inner bark is creamy in color (Molina et al., 1996). The seeds are 1.5 to 2 cm, long pinkish or yellowish in color after ripening (Biswas, 2007). There are two varieties; on the basis of fruit color one produces greenish white fruit and other violet to almost black fruits (Reddy et al., 2003). The most potent active group of chemicals in *S. glauca* is quassinoids that belong to the triterpine family. Practically, all parts of *S. glauca* have several herbal applications; the seed, shell, fruit pulp, leaf, unwanted branches, stem and root bark have been implicated in folk medicine.

Anthropogenic activities and in fact, normal cellular and/or metabolic activities can result in the generation of reactive oxygen species (ROS) and free radicals, capable of initiating oxidative damages to cellular organelles and tissues. Alteration in the cellular redox couple results in oxidative stress (Kalow and Grant, 1995).

Imbalance in oxidants/antioxidants status of a system informed the need to study plants' system capable of supplying proton ions that can enhance and maintain cellular homeostasis even though the body is equipped with its antioxidant defense system. Plants provide natural forms of phytochemicals as rich source of antioxidants that help protect man and animals from a variety of diseases (Umesh, 2015). Many of these dietary components, including flavonoids and phenolic acids and others but not limited to the aforementioned compounds contribute to the protective properties against diseases that affect humans (Ebrahimzadeh et al., 2008; Kaur and Mondal, 2014).

Studies of these plants (such as *S. glauca*) kingdom by scientist in many countries of the world with respect to their phytochemical constituents (Demiray et al., 2009) have opened up a whole new window of therapeutics of naturally occurring bioactive chemicals against diseases; this has led to identification, isolation and characterization of several plants bioactive compounds with valuable properties beneficial to human health. In this study, selected phytochemicals were determined (Rang et al., 2003); *in-vitro* antioxidant activities of stem bark extracts were evaluated against a number of synthetic antioxidants and radicals; using DPPH free radical scavenging assay, reducing power activity, total antioxidant capacity (FRAP), hydroxyl free radical scavenging capacity, Trolox equivalent antioxidant assay and nitric oxide scavenging assay to ascertain the plants' ability to scavenge free radicals. It is striking to note that extraction solvent and mixtures are known to have significant impact on antioxidant activity (Zhao et al., 2006; Boeing et al., 2014). The stem bark extracts of *S. glauca* displayed high antioxidant potential, capable of scavenging free radicals (Picture 1).

MATERIALS AND METHODS

Plant materials

The stem bark of *S. glauca* was harvested from a private farm at Esan South-East Local Government Area of Edo State, transported to the Department of Plant Biology and Biotechnology for identification and deposited at the herbarium; taken to the Department of Biochemistry Laboratory and air dried at room temperature for twenty eight days all in University of Benin.

Preparation of plant extract

Extract was pulverized and sieved off using a mesh size of 1 mm at the Department of Pharmacognosy, University of Benin. Approximately, 500 g of pulverized stem bark powder was extracted twice in 5 L of distilled water with random shaking to obtain 99.7% extraction, after two days, the extract was filtered through Whatman filter paper No.1, and the filtrate was freeze dried at -50°C at the National Energy Commission Center situated in University of Benin, which yielded approximately 20 g stem bark aqueous extract (SGAE). Similar extraction was done with methanol and ethanol solvents to obtain methanol [SGME] and ethanol (SGEE) stem bark extracts, respectively. All the extracts were stored at 4°C for further phytochemical and *in vitro* antioxidant studies.

Chemicals

Phytochemistry

For phytochemistry, distilled water, methanol, ethanol, hydrochloric acid, saturated picric acid, ferric chloride and H_2SO_4 were used.

Antioxidant studies

2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, butylated hydroxytuolene (BHT), phosphate buffer (pH 6.6), disodium

Picture 1. Harvesting the stem bark of *S. glauca.*

hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate (NaH_2PO_4), potassium ferricyanide, ferric chloride ($FeCl_3$. $6H_2O$), trichloroacetic acid (TCA), acetate buffer (pH 3.6), sodium acetate, glacial acetic acid, ascobic acid, HCl, ferrous sulphate ($FeSO_4$. $7H_2O$), 2,4,6-tripyridyl-triazine (TPTZ), FRAP reagent, 1,10-phenanthroline, phosphate buffer (pH 7.4), hydrogen peroxide (H_2O_2), 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox), sodium nitroprusside, phosphate buffer saline (pH 7.4), sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), sulphanilic acid, naphthylethylenediamine dihydrochloride, quercetin and methanol (analytical grade), were used. All reagents and chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA) and analytical solvents were of analytical grade with 99% purity purchased from Rovelt, Nigeria

Identification of alkaloids

Alkaloids in aqueous, methanol and ethanol stem bark extracts of *S. glauca* were determined by the method described by Sani et al. (2014). Approximately, 2 ml of 10% hydrochloric acid (HCl) was added to 2 ml stem bark extracts with stock concentration of 1 mg/mL (dissolved in appropriate quantity of methanol) in a test tube and vortexed. The presence of alkaloids was confirmed by formation of yellow coloured precipitate.

Identification of total phenol

Phenols in aqueous, methanol and ethanol stem bark extracts of *S. glauca* were qualitatively determined by the method described by Trease and Evans (2002). A Few drops of 10% ferric chloride was mixed with 2 ml stem bark extracts with stock concentration of 1 mg/mL (dissolved in appropriate quantity of methanol) in a test tube and vortexed. Blue-black colour observed confirmed the presence of phenolic compounds.

Identification of tannins

Tannins present in aqueous, methanol and ethanol stem bark extracts of *S. glauca* were determined by the method described by Sofowora (1993). Approximately 5 drops of 0.1% ferric chloride was added to 2 ml stem bark extracts with stock concentration of 1 mg/mL (dissolved in appropriate quantity of methanol) in a test tube and vortexed. Brownish-green colour was observed which indicated presence of tannins.

Identification of flavonoids

Total flavonoid in aqueous, methanol and ethanol stem bark extracts of *S. glauca* were determined by the method described by Santhi and Sengottuvel (2016). Approximately 2 ml stem bark extracts (dissolved in appropriate quantity of methanol) was treated with few drops of concentrated H_2SO_4. Orange colour was observed and indicated the presence of flavonoids.

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

Radical scavenging activities of *S. glauca* stem bark extracts was determined by 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay with some modification. BHT was adopted as the positive reference standard. Its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach DPPH absorption is termed as an antioxidant. The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy et al., 2007).

DPPH is a purple colour dye having absorption maximum of 517 nm and upon reaction with a hydrogen donor, the purple colour disappears forming a stable light gold colour due to conversion of the stable free radical to 2,2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. All determinations were performed in triplicate. A solution of 0.1 mM DPPH in methanol was prepared, 1.0 mL of the solution was mixed with 3.0 mL of extracts in methanol of concentration range (10 to 200 µg). The mixture was thoroughly vortexed and kept in the dark for 40 min at room temperature; and analyzed using the spectrophotometer at 517 nm wavelength. The same procedure was adopted for the reference standard and other extracts. DPPH radical scavenging activity was calculated using the following equation:

Percentage (%) inhibition = [(A0 − A1)/(A0)] × 100.

Where, A0 is of DPPH radical + methanol; A1 is the absorbance of DPPH radical + sample extract/reference standard (Kumar and Kumar, 2009). The IC_{50} value is the concentration of the plant extract required to scavenge 50% of the total DPPH radicals. This assay was previously described by Umesh (2014).

Reducing power assay

The reducing power of the stem bark extracts was determined according to the method described by Ferreira et al. (2007). A measure of 1 ml of different concentrations of extracts ranging from 10 to 100 µg was mixed with 2.5 ml of 0.2 M phosphate buffer, pH 6.6 and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. A measure of 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ were then added and the absorbance measured at 700 nm. Higher absorbance values indicated higher reducing power. Ascorbate served as a positive control.

Radical scavenging activity (%) = [(A0 −A1)/(A0)] × 100.

Total antioxidant assay (FRAP)

The method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant (FRAP) assay, with modification. It is based on the ability of the sample extracts to reduce the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH. Fe(II)-TPTZ has an intensive blue colour which can be read at 593 nm. 1.5 mL of freshly prepared FRAP solution, containing 25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) in 40 Mm HCl and 2.5mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution, was mixed with 1 mL of the extracts (10 to 100 µg), and the absorbance was read at 593 nm. The outcome of the results of stem bark extracts were compared with that of BHT as positive control.

Radical scavenging activity (RSA) (%) = [(A0 −A1)/(A0)] × 100

Hydroxyl free radical scavenging assay

The hydroxyl free radical scavenging activity was conducted according to the method described by Wenli et al. (2004) with a little modification. A reaction mixture containing 1 mL 1,10-phenanthroline (0.75 mM), 1.5 mL of 0.75 mM $FeSO_4$ and 3.8 mL of 0.2 M phosphate buffer solution (pH 7.4) was mixed with 1 mL of sample extracts (10 to 100 µg) and 1.0 mL of 0.01% (v/v) H_2O_2 and the volume was made up to 10 mL with distilled water. The mixture was incubated at 37°C for 60 min, and the absorbance was measured at 536 nm. The scavenging effect was calculated using the following equation: RSA (%) = $[(A_2 - A_1)/(A_0 - A_1)] \times 100$. Where A_2 and A_1 are the absorbance with or without sample, and A_0 is the absorbance without sample and H_2O_2. The effective concentration which scavenges 50% radical (EC_{50}) was concluded from the graph of scavenging effect percentage against the samples concentration.

Trolox equivalent antioxidant capacity (TEAC) assay

This assay was conducted with an improved 2,2'azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) based on the principle of decolourization (Re et al., 1999) as described by Neergheen et al. (2010). The $ABTS^+$ radical was generated by a reaction between ABTS (0.5 mM) and 1 mM potassium persulfate in 0.1 M phosphate buffer. To 3 mL of the $ABTS^+$ solution, A 1.5 mL of the extract with concentration range of 40 to 140 μg was added and the decay in absorbance was followed for 6 min at 734 nm. Trolox was used as a reference standard and TEAC values were expressed as trolox equivalent/µg.

Radical scavenging activity (%) = $[(A_0 - A_1)/(A_0)] \times 100$

Nitric oxide radical scavenging assay

The nitric oxide ($NO^{\bullet}$) radical scavenging activity of *S. glauca* stem bark extracts were estimated according to the method described by Garratt (1964), with some modification. In the present study, naphthylethylenediamine dihydrochloride (0.1% w/v) was used. The reaction mixture containing sodium nitroprusside (10 mM, 2mL), phosphate buffer saline (0.5 mL), and extract or standard solution (20 to 120 μg, 0.5 mL), was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted into new sets of test tubes and mixed with 1 mL sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. Then 1 mL naphthylethylenediamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. BHT was used as positive standard. Results were expressed as percentage radical scavenging activity (% RSA):

$$\% \text{ RSA} = 1 - \frac{\Delta Abs \text{ of sample}}{\Delta Abs \text{ of control}} \times 100$$

RESULTS AND DISCUSSION

Alkaloid

Alkaloids detected in aqueous, methanol and ethanol stem bark extracts of *S. glauca* are shown in Table 1. The strength of the yellow precipitate observed in the aqueous, methanol and ethanol extracts revealed the strong presence of alkaloid. Alkaloid is a phytomedicinal principle classed as a pharmacological agent with a muscarinic receptor agonist property capable of regulating arterial blood pressure in that context. The results of this study concur with the reports of Deepa and Nalini (2013). Although, Deepa and Nalini did not investigate the alkaloids contents of *S. glauca,* their study however revealed that alkaloid is present in stem bark of *Schefflera* species with respect to the same analytical procedure (Trease and Evans, 2002). On the contrary, the results of the present study conflicts the report of Santhosh et al. (2016), apparently due to the difference in plant parts and extraction solvents.

Total phenol

Phenol strength detected in aqueous, methanol and

Table 1. Qualitatively determined phytochemicals present in SB extracts of *S. glauca*.

Phytochemicals	Aqueous extract	Methanol extract	Ethanol extract
Alkaloids	+++	+++	+++
Total Phenols	+++	++	++
Tannins	+	++	+++
Flavonoids	+	++	+++

+,Strong; ++, stronger; +++, strongest.

ethanol stem bark extracts of *S. glauca* are presented in Table 1. The study shows that phenol was stronger in aqueous extract vis-à-vis methanol or ethanol extracts. Phenols are a class of intermediate metabolite compounds synthesized by a number of plants; thus, they constitute precursors for the synthesis of other essential compounds such as flavonoids. The findings of the study agree with the recent reports of Umesh (2015) and Deepa and Nalini (2013). Although, while the present study qualitatively evaluated total phenols in the stem bark extracts, the aforementioned scientists reported quantitatively estimated total phenol content of *S. glauca* leaf extracts and *Schefflera* spp., respectively.

Tannin

Tannin detected in aqueous, methanol and ethanol stem bark extracts of *S. glauca* are presented in Table 1. The study showed that tannin was stronger in ethanol extract as compared to methanol or aqueous extracts. The outcome of the study is in line with the reports of Umesh (2015) and Parul et al. (2013). While Umesh reported an estimated quantitative tannin content of *S. glauca* leaf extracts, Parul and collaborators (2013) reported qualitative tannin contents of *Triumfetta rhomboidae* and *Casuarina littorea* methanol bark extracts. The results of the study completely contradicts the study reported by Santhosh et al. (2016); although, Santhosh and collaborators (2016) utilized leaf extracted with ethyl acetate and petroleum ether while the present study applied stem bark extracted with water, methanol or ethanol. Due to the health benefits of tannins (Chung et al., 1998), the outcome of the study indicate that the stem bark of *S. glauca* could be readily made available in treatment of diarrhea and prevention of cancer as earlier reported by Ruch et al. (1989). Gulcin et al. (2010) reported that the anti-oxidative property of tannins could be related to their anti-carcinogenic potentials, which is important in protecting against cellular oxidative damages, including lipid peroxidation.

Flavonoid

Flavonoids detected in aqueous, methanol and ethanol stem bark extracts of *S. glauca* are shown in Table 1. The study revealed that flavonoid was strongest in stem bark extract of ethanol AS compared to the methanol or aqueous extracts. Flavonoids are generally effective against radicals and have long been reported to reduce inflammation and carcinogenicity. The study agrees with the reports of Lakshmi et al. (2014). Although, the present study evaluated the flavonoid contents of aqueous, methanol and ethanol stem barks extracts of *S. glauca*, Lakshmi and collaborators (2014) reported the flavonoid contents of chloroform, methanol and ethyl acetate leaf extracts of *S. glauca*. The studies of Umesh (2015) which reported the evaluation of flavonoids in aqueous, methanol and ethanol leaf extracts of *S. glauca,* also agrees with the findings of the present study. However, it was quantitative. The findings of the present study contradict the report of Santhosh et al. (2016). The contradictory finding is perhaps due to the different solvent applied in the extraction process.

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

The triplicate results of free radical scavenging activity of aqueous, methanol and ethanol stem bark extracts of *S. glauca* are presented in Figure 1, coupled with the IC_{50} values shown in Table 2. The stable radical DPPH has been widely used to test the radical-scavenging activities of various dietary antioxidants (Brand-Williams et al., 1995). It was observed that the aqueous, methanol or ethanol extracts did not display significant radical scavenging activity as compared to BHT; however, there was geometric increase of the extracts' activity as concentration increased whereas, BHT showed maximum activity of 94.0% at 200 µg concentration. Comparatively, at 50% inhibition, it was observed that the control BHT had a concentration of 21.0 µg; effective concentration at which 50% of radicals are scavenged; whereas, no IC_{50} values were recorded for aqueous, methanol or ethanol extracts. The results indicate that ethanol extract has the least free radical scavenging activity, while the aqueous extract seems to be the most potent of the stem bark extracts. Although, the results of the present study did not show significant DPPH radical scavenging activity at 50% IC, however, it agrees

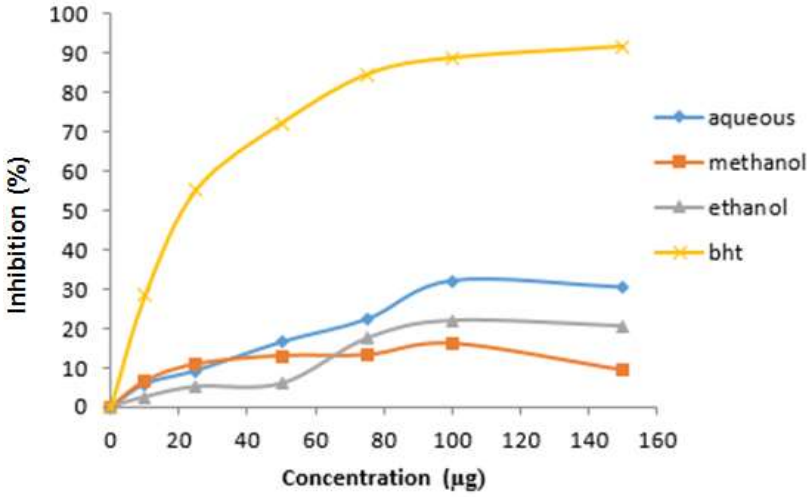

Figure 1. Comparative DDPH radical scavenging activity of *S. glauca* stem bark extracts and BHT.

Table 2. Percentage (%) inhibition concentrations (IC_{50}) of *S. glauca* stem bark extracts and standards against radicals.

Antioxidant radical	Inhibition concentration (IC_{50}) (µg/ml)				
	Aqueous extract	Methanol extract	Ethanol extract	BHT	Ascobate
DPPH radical	--	-	-	21.00	-
Reducing power (EC_{50})	3.70	3.50	4.00	-	4.80
FRAP	4.80	4.70	4.90	5.00	-
Hydroxyl radical	4.75	4.78	5.10	4.80	-
$ABTS^{+}$ radicals	22.50	27.60	27.50	-	Trolox (18.00)
Nitric oxide radical	10.00	11.90	19.00	18.00	-

with the reports of Umesh (2015). The findings of this study also agrees with the studies of Deepa and Nalini (2013) who reported the significant DPPH radical scavenging activities of leaf, bark and flower extracts of *Schefflera* spp.

Reducing power activity

The reducing activity of stem bark extracts was determined according to the method described by Ferreira et al. (2007); the mean results presented in Figure 2, coupled with the EC_{50} values are shown in Table 2. Reducing power of aqueous, methanol and ethanol stem bark extracts of *S. glauca* were compared with ascorbate; the methanol extract exhibited the highest reducing power which was more than the aqueous, followed by ethanol and the control ascorbate as depicted from their individual effective concentration at 50%. The reducing capacity of each extract was concentration dependent; its percentage effective concentration also followed same pattern, comparable to that of the control ascorbate but all the extracts displayed better reducing activities than the ascorbate. The results of the reducing activities of extracts further validate the findings as presented in Table 1, especially with the identification of phenolic compounds which is ascribed a major reducing power. The findings in this study is in line with the studies of Deepa and Nalini (2013) who reported that reducing potency of bark extracts was concentration dependent; although, Deepa and Nalini evaluated the reducing power activity of ethanol, methanol and aqueous bark extracts of *Schefflera* spp. Umesh (2015) who also reported high reducing activities of methanol, aqueous and ethanol leaf extracts of *S. glauca* also supports the claim of the present study.

Total antioxidant activity (FRAP)

The mean results of total antioxidant activity of stem bark extracts of *S. glauca* are reported in Figure 3 and the IC_{50} (concentration at which 50% of Fe (III) is

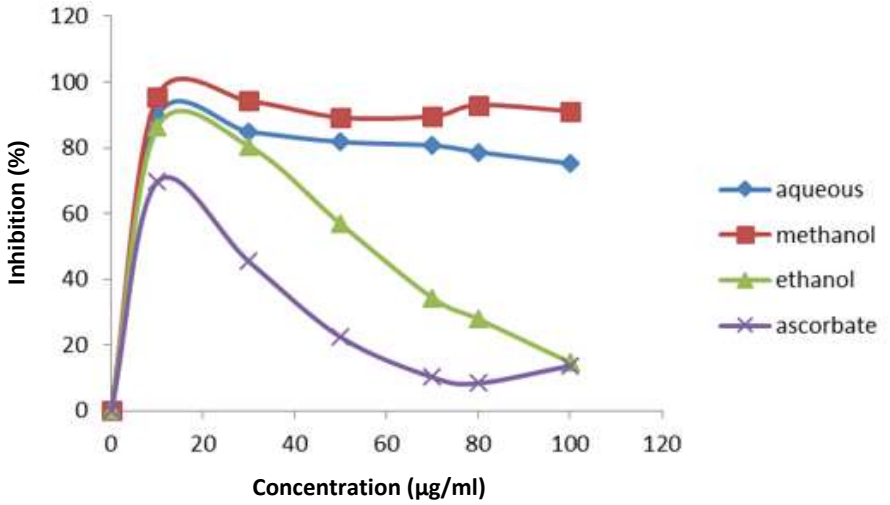

Figure 2. Comparative reducing power activities of *S. glauca* stem bark extracts with ascorbate.

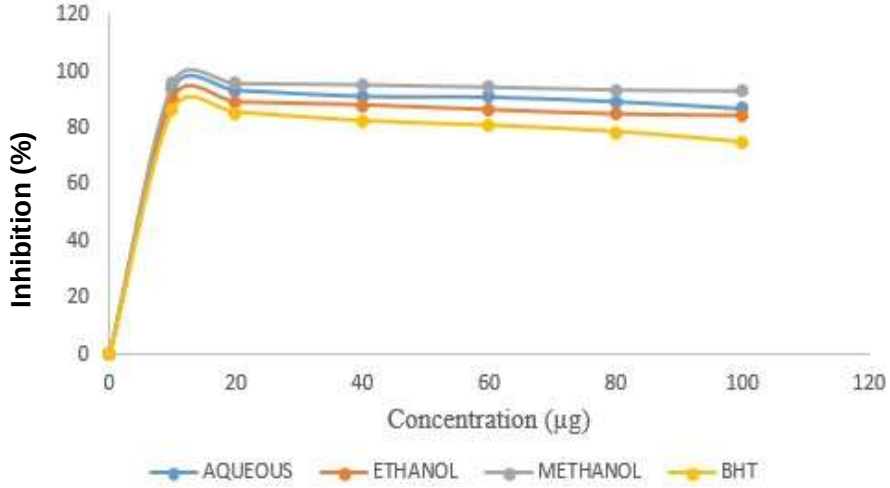

Figure 3. Comparative total antioxidant activities (FRAP) of *S. glauca* stem bark extracts and BHT.

reduced to Fe (II) in test sample) is shown in Table 2. The antioxidant effect of the stem bark extracts of the plant was measured by assessing its reducing capacity using the FRAP assay. *S. glauca* stem bark extracts reducing potential was estimated by its capacity to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex, based on a blue-colored product. It was observed that the TPTZ-Fe (III) to TPTZ-Fe (II) complex reducing activity of the extracts including BHT was concentration dependent, as reducing activity increased with increased concentration. It was observed that the methanol extract remarkably demonstrated a high reducing potential, tracked by aqueous and ethanol extracts, and lastly by the control BHT with IC_{50} 4.70, 4.80, 4.90 and 5.00 µg, respectively. The results of the study is in line with the report of Osagie-Eweka et al. (2016); although, they neither reported studies on stem bark nor methanol extracts, but of aqueous and ethanol leaf extracts of *S. glauca.* The results of the present study, however disagrees with the report of Parul et al. (2013), obviously due to the difference in plants applied and methodology. The outcome of the study, however, indicates that the bioactive compounds inherent in the plant stem bark could be beneficial in treatment of oxidative stress-related diseases such as cancer as earlier reported by Umesh (2015) who drew some suggestive conclusion from outcomes of their cytotoxicity studies with leaf extracts of *S. glauca.*

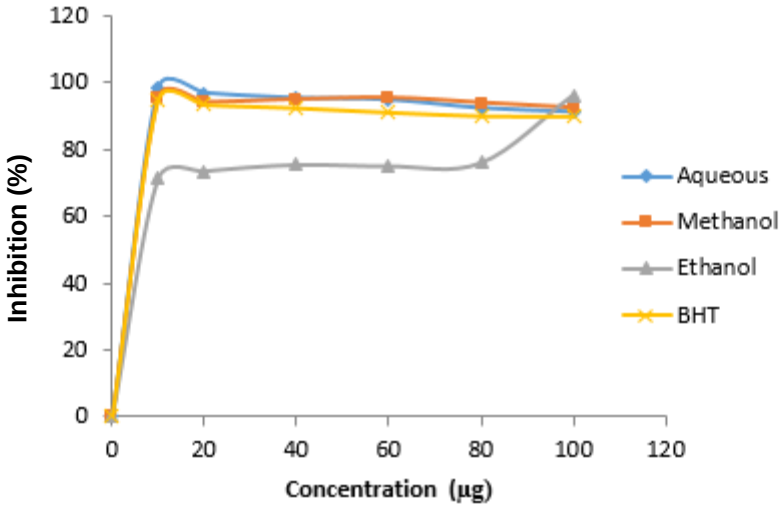

Figure 4. Comparative hydroxyl free radical scavenging activities of *S. glauca* stem bark extracts and BHT.

Hydroxyl free radical scavenging activity

The mean results of hydroxyl ($OH^{\bullet}$) radical scavenging activity of stem bark extracts of *S. glauca* and standard BHT are presented in Figure 4 and the IC_{50}, in Table 2. Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism (Harsh, 2010). A single hydroxyl radical results in a type of chain reaction, with formation of many molecules of lipid hydroxyl peroxides in the cell membrane which may severely disrupt its function and lead to cell death (Harsh, 2010). It was observed that the aqueous stem bark extract remarkably exhibited the highest hydroxyl radical scavenging activity with IC_{50} 4.75 µg. The methanol extract demonstrated the second highest scavenging activity with IC_{50} 4.78 µg, followed by the BHT with IC_{50} 4.80 µg, while the ethanol extracts displayed the least hydroxyl radical scavenging activity with IC_{50} 5.10 µg. The results of the study revealed that the aqueous and methanol extracts demonstrated a higher anti-hydroxyl radical activity than the control, BHT. The findings further validate the presence of phenolic compounds in the aqueous extract as shown in Table 1 which indicate a greater reducing power, having the capacity to donate a proton to create a balance in the redox potential.

Trolox equivalent antioxidant capacity (TEAC)

The mean results of the antioxidant activity of *S. glauca* stem bark extracts on $ABTS^{+}$ radicals are reported in Figure 5 and the IC_{50} values in Table 2. The capacity of aqueous, methanol and ethanol stem bark extracts of *S. glauca* to reduce $ABTS^{+}$ (radical) generated by a reaction between $ABTS^{+}$ and potassium persulfate was assessed vis-à-vis the standard trolox as positive control. It was observed that trolox demonstrated the highest activity, aqueous extract demonstrated second highest activity, ethanol extract displayed the third highest activity and lastly, is the methanol stem bark extract of *S. glauca* as activities increased with gradient increase in concentration of extracts. The results also revealed that the ethanol and methanol extracts demonstrated comparable $ABTS^{+}$ scavenging activity. Trolox, aqueous, ethanol and methanol activities displayed IC_{50} of 18.00, 22.50, 27.50 and 27.60 µg, respectively.

Nitric oxide radical scavenging activity

The triplicate results of nitric oxide scavenging activity of *S. glauca* stem bark extracts are reported in Figure 6 and IC_{50} presented in Table 2. $NO^{\bullet}$ is a very unstable species and when it reacts with an oxygen molecule, it can produce stable nitrate and nitrite. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured spectrophotometrically at 540 nm. The aqueous stem bark extract demonstrated a remarkable nitric oxide scavenging activity with IC_{50} of 10.00 µg; methanol extract also demonstrated a significant nitric oxide scavenging activity with IC_{50} 11.90 µg against BHT that served as a synthetic antioxidant. The ethanol stem bark extract displayed the least nitric oxide scavenging activity with IC_{50} of 19.00 µg when compared with the aqueous, methanol stem bark extracts and BHT (IC50 18.00). The results of the study indicate that the activity of

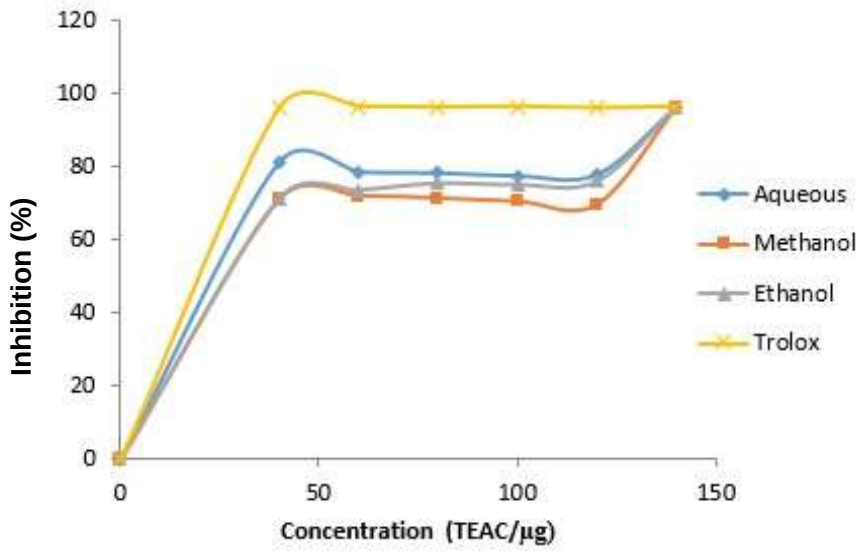

Figure 5. Comparative Trolox equivalent antioxidant capacity (TEAC) of *S. glauca* stem bark extracts and Trolox.

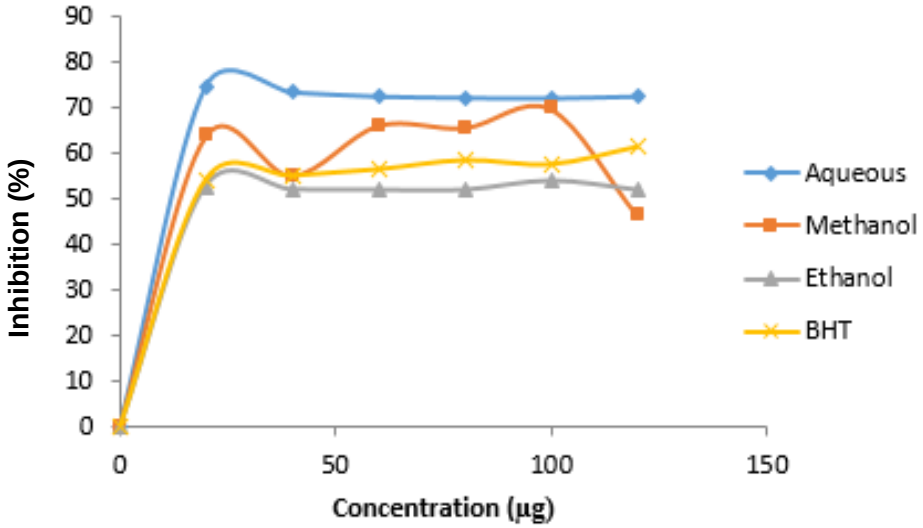

Figure 6. Comparative nitric oxide radical scavenging activities of *S. glauca* stem bark extracts and BHT.

each extract was concentration dependent; while the aqueous and methanol extract demonstrated radical inhibitory effect vis-à-vis the inhibitory strengths of BHT, ethanol extract demonstrated less but significant inhibitory effect. The findings of the present study agrees with the earlier studies of Shahriar et al. (2012) who reported that the activity of test extracts appeared to be slightly better than standard ascorbic acid (27.685) and BHT (27.294) at 50% inhibition although, Shahriar and collaborators (2012) conducted studies on the bark extracts of *Terminalia arjuna*.

Conclusion

The results obtained from the study on the quality of phytochemical constituents, particularly the phenol and alkaloid content and antioxidant potency of aqueous, methanol or ethanol stem bark extracts of *S. glauca,* provided evidence that the plant is a promising source of natural antioxidant. The plant's natural antioxidant potential with presence of beneficial active principles avails pharmaceutical industries a huge benefit towards developing potentially improved drugs to treat or manage

a number of diseases and health related conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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