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Polyphenolic Contents and Free Radical Scavenging Potential of Extracts from Leaves of *Ocimum americanum* L.

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Abstract: This study assessed the polyphenolic contents and antioxidant activity of ethanol, butanol and ethyl acetate extracts of *Ocimum americanum* leaves using *in vitro* models. The ability of the extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$), hydrogen peroxide (H_2O_2), Nitric Oxide (NO) and hydroxyl radical ($OH^{\bullet}$) was investigated. The inhibition of lipid oxidation, reducing power, total flavonoids, phenols and flavonols contents of the extracts were also determined using spectrophotometric methods. The result revealed highest concentration of polyphenolic compounds in the ethanol extract followed by *n*-butanol while ethyl acetate extract contained the least concentration. Free radical scavenging potentials of the extracts were found to be proportional to their respective polyphenolic contents. This study provides evidence that *O. americanum* leaves contain biologically active components with effective antioxidant activity and thus could be used for the management of radical related diseases.

Key words: *Ocimum americanum,* free radicals, antioxidants, polyphenolics, scavenging activity

INTRODUCTION

Oxidative stress occurs as a result of imbalance between the productions of Reactive Oxygen Species (ROS) and the ability of the biological system to neutralize the free radicals (Hadi *et al*., 2007). Free radicals such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) nitric oxide (NO) and organic hydroperoxide (ROOH) are chemically unstable atoms due to the presence of lone pair electrons in their outer shells. They have been implicated as underlying cause of several degenerative diseases in humans by damaging vital macromolecules in the biological system such as lipids, DNA and protein (Farber, 1994). Some of these diseases include atherosclerosis, sickle cell anaemia, myocardial infarction, heart failure and Parkinson disease (Lee *et al*., 2001). However, the presence of enzymatic and non-enzymatic antioxidant defence in the body system has been reported to prevent the pathological action of these radicals by quenching or neutralizing the chain of reactions before vital damages to the cells (Oboh, 2006). Examples of these antioxidants are catalase, superoxide dismutase, glutathione reductase and vitamin C and E. In the last three decades, attention has been shifted to the use of natural antioxidants from plant origin due to the harmful effect of commercially available synthetic antioxidants such as tertbutylhydroxyl toluene (TBHQ), Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) which has been implicated in liver damage and development of cancer cells (Oyetayo, 2009). Medicinal plants are well known to contain secondary metabolites such as phenolics, flavonoids, flavonols and proanthocyanidins which have been reported to possess potent antioxidant properties (Oboh and Rocha, 2007). However, majority of these plants have not been investigated for their possible antioxidant potential in the quest to retard or alleviate the extent of oxidative deterioration.

Ocimum americanum L. (Lamiaceae) is a wild herb that grows in tropical Africa (Steel, 2006). It is known as African basil but popularly called "Efinrin elewe dudu" in south-western Nigeria. The leaf is used in traditional folk medicine in Ghana for the treatment of diabetes (Hogarh, 1996). In Nigeria, it is used by traditional healers for the treatment of constipation, diarrhoea, piles, dysentery and as insect repellent. The leaf is rich in essential oils of therapeutic importance and mostly used for the preparation of delicious local soups as well as flavouring agent in yam and cocoyam porridges in the Yoruba tribe of Nigeria (Ekundayo *et al*., 1989). It is also used as a local condiment because of its aromatic properties (Bassole *et al*., 2005). Previous work conducted on this

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plant reported the inhibitory activity of its acetone extract on some neurotoxins induced brain damage in rats (Oboh, 2008).

There is dearth of scientific information on the antioxidant potentials of *O. americanum* leaves to justify its continuous use in traditional folk medicine. Therefore, the present study was explored to evaluate the polyphenolic contents and the free radical scavenging potentials of its leaves with a view to ascertain its potential health benefits.

MATERIALS AND METHODS

Collection and identification of plant sample: The leaves of *Ocimum americanum* were collected from a local farmland near Orin Ekiti South-Western Nigeria in the month of May, 2011. The plant was authenticated by Mr. Omotayo (herbarium curator) at the Department of Plant Science, University of Ado Ekiti, Nigeria where the voucher specimen (Aluko 09) was deposited.

Reagents: The reagents used in this study were purchased from Sigma-Aldrich Gmbh, Sternheim, Germany. This includes: tannic acid, quercetin, catechin, Folin-Ciocalteu reagent, aluminium chloride, sodium acetate, vallinin, 2,2 diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, hydrogen peroxide, sodium nitroprusside, sulphanilic acid, naphthylenediamine dichloride, 2-deoxy-D-ribose, ferric chloride, thiobabituric acid, trichloroacetic acid, potassium ferricyanide, ascorbic acid and butylated hydroxyl toluene. All other solvents used were of analytical grade.

Sample extraction: The leaves were air dried for 10 days and then pulverized into fine powder using an electric blender. Fifty gram of the powdered sample was defatted with 250 mL of n-hexane with constant shaking on an orbital shaker (Stuart Scientific Orbital Shaker SO1, Essex, UK) for 12 h. The solvent was removed by filtration using a Buchner funnel with Whatman's No. 1 filter paper and the residue obtained was extracted sequentially with 500 mL of ethyl acetate, *n*-butanol and ethanol, respectively with continuous shaking on an orbital shaker for 24 h. The ethyl acetate and ethanol extracts were concentrated to dryness under vacuum in a rotary evaporator thereafter; the extracts were collected in clean bottles and left opened in a laboratory fume hood for complete evaporation of residual solvent. Meanwhile, butanol extract was allowed to evaporate in a fume hood for 7 days. The percentage yield for ethyl acetate, ethanol and butanol extracts was 0.10, 0.12 and 0.11 w/w, respectively.

Determination of total phenolics: The method of Wolfe *et al.* (2003) was adopted to determine total phenolic contents of *O. americanum* leaves in different solvent systems. A reaction mixture of 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent and 2 mL of 7.5% (w/v) of sodium carbonate was added to 0.5 mL (1 mg mL^{-1}) of the extracts. The mixture was vortexed and incubated at 40°C for 30 min after which the absorbance was measured at 765 nm. The total phenolic content was calculated from the equation obtained from the calibration curve of Tannic acid ($Y = 0.1216x$, $R^2 = 0.936512$) and expressed as mg g^{-1} tannic acid equivalent where x is the absorbance and Y is the tannic acid equivalent.

Determination of total flavonoids: Total flavonoids content of *O. americanum* leaves was determined by the modified method of Ordonez *et al.* (2006). The extract (1 mL in a final concentration of 0.1 mg mL^{-1}) was mixed with 1 mL of 2% (w/v) aluminium chloride prepared in ethanol and left in the dark at room temperature for 1 h. A yellow colour was observed which was measured spectrophotometrically at 420 nm. The total flavonoid content was calculated as mg g^{-1} quercetin equivalent from the equation ($Y = 0.0255x$, $R^2 = 0.9812$) obtained from the calibration curve where x is the absorbance and Y is the quercetin equivalent.

Determination of total flavonols: The determination of the total flavonols content of the extracts was done according to the method of Kumaran and Karunakaran (2007). Two milliliters of the extract was mixed with 2 mL of 2% aluminium chloride in ethanol and 3 mL of 5% sodium acetate solution and allowed to stand for 2.5 h at room temperature and later, measured the absorbance at 440 nm. The flavonols content was expressed as mg g^{-1} quercetin equivalent using the calibration equation ($Y = 0.0255x$, $R^2 = 0.9812$) from standard quercetin where x is the absorbance and Y is the quercetin equivalent.

DPPH scavenging assay: A volume of one milliliter of 0.135 mM of DPPH in methanol was mixed with 1 mL of different concentrations (50-300 μg mL^{-1}) of the leaf extract, vitamin C and BHT. The mixture was vortexed and kept in a dark cupboard for 30 min (Liyana-Pathirana and Shahidi, 2005). The observed decolourisation was measured spectrophotometrically at 517 nm and the scavenging ability of the extract was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where, $Abs_{control}$ is the absorbance of DPPH radicals+methanol, Abs_{sample} is the absorbance of DPPH radical+sample or standard.

ABTS scavenging assay: The ABTS scavenging potential of the extract was assayed using the method of Re *et al.* (1999) with some modifications. The stock solution consisting of 7 mM ABTS solution and 2.4 mM potassium persulfate (1:1) was allowed to react in the dark for twelve hours at room temperature. The radical generated was mixed with methanol to obtain an absorbance of 0.702±0.001 unit at 734 nm. One milliliter of the resulting solution was added to 1 mL of the extract or standard (50-300 μg mL^{-1}) and the absorbance was measured at 734 nm after 7 min. The percentage scavenging ability was calculated from this equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where, $Abs_{control}$ is the absorbance of ABTS radicals+methanol, Abs_{sample} is the absorbance of ABTS radical+sample or standard.

Hydrogen peroxide scavenging assay: The modified method of Oyedemi *et al.* (2010) was employed for the hydrogen peroxide scavenging assay of the extracts of *O. americanum* leaves. The stock solution was 4 mM hydrogen peroxide prepared in 0.1 M phosphate buffer (pH 7.4). The solution (0.6 mL) was added to 2 mL of the extract and standard (50-300 μg mL^{-1}) and incubated for 15 min at room temperature. The absorbance was read at 230 nm and the percentage inhibition of hydrogen peroxide was calculated as:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where, $Abs_{control}$ is the absorbance of H_2O_2 radicals, Abs_{sample} is the absorbance of H_2O_2 radical+sample or standard.

Nitric oxide scavenging assay: The nitric oxide scavenging activity of the extracts was evaluated by the method of Igbinosa *et al.* (2011). Two milliliters of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was added to 0.5 mL of plant extract or standard (50-300 μg mL^{-1}) and vortexed. The mixture was incubated for 2.5 h at 25°C and thereafter, 1 mL of the mixture was taken and mixed with 1 mL of Griess reagent (equal volumes of 0.33% sulphanilic acid prepared in 20% glacial acetic acid and 0.1% (w/v) naphthylenediamine dichloride) and incubated at room temperature for 30 min. The absorbance was read at 540 nm and the percentage nitric oxide inhibition by the extracts was calculated using the following equation:

$$\text{NO scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where, $Abs_{control}$ is the absorbance of NO radicals, Abs_{sample} is the absorbance of NO radical+sample or standard.

Hydroxyl radical scavenging assay: The method of Zhang *et al.* (2011) was adopted for the evaluation of the hydroxyl radical scavenging activities of the extracts of *O. americanum* leaves. Briefly, 100 μL of the extract or standard (50-300 μg mL^{-1}) was added to a reaction mixture of 500 μL 5.6 mM 2-deoxy-D-ribose in KH_2PO_4/NaOH (0.05 M, pH 7.4), 100 μL ferric chloride (104 μM), 100 μL EDTA (104 μM), 100 μL H_2O_2 (1 mM) and 100 μL ascorbic acid (1 mM). The mixture was vortexed for 15 sec and incubated in a water bath for 30 min at 50°C. After the incubation, 1 mL of 1% TBA and 1 mL of 2.8% TCA were added to the mixture and further incubated for 30 min. The absorbance of the mixture was read at 532 nm and the percentage inhibition was calculated as:

$$OH^{\bullet} \text{ scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where, $Abs_{control}$ is the absorbance of $OH^{\bullet}$, Abs_{sample} is the absorbance of $OH^{\bullet}$+sample or standard.

Lipid peroxidation assay: The lipid peroxidation inhibitory activity of the extract was evaluated by the method of Duh *et al.* (2001). Egg lecithin (a creamy powder) was prepared by repeated washing of egg yolk with acetone until the yellow colour was removed. A concentration of 3 mg mL^{-1} of egg lecithin in phosphate buffer was added to 10 μL ferric chloride (400 mM) and 10 μL L-ascorbic acid (400 mM). Then, 100 μL of different concentrations of the extracts or standard drug was added and incubated at 37°C for 1 h. The reaction was stopped by the addition of 2 mL (1:1:1) TBA-TCA-HCL reagent (0.37% TBA, 15% TCA and 0.25N HCL). The mixture was boiled for 15 min, cooled, centrifuged at 2000 rpm for 10 min and absorbance of the supernatant was measured at 532 nm. The percentage inhibition was determined as follows:

$$\text{Lipid oxidation inhibition (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of LPO, Abs_{sample} is the absorbance of LPO+sample or standard.

Determination of reducing power: The reducing power of the extracts was determined by the modified method of Yen and Chen (1995). Various concentrations (50-300 μg mL^{-1}) of the extracts or standard drugs in a volume of 0.5 mL was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated for 20 min at 50°C. Thereafter; 1 mL of 10% (w/v) TCA was added and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and 1.25 mL of the solution was allowed to react with 1.25 mL distilled water and 0.25 mL of 0.1% (w/v) of ferrous chloride for 5 min and the absorbance was read at 700 nm. The observed increase in absorbance with increasing concentration indicated the ferric reducing potential of the extracts.

Statistical analysis: All results were expressed as Mean±standard Deviation (SD) of three replicates and were subjected to Analysis of Variance (ANOVA) using the student Minitab release version 12, Windows 95. Significant levels were tested at $p<0.05$.

RESULTS

The various extracts of *O. americanum* were evaluated for their polyphenolic contents. The ethanol extract showed the highest polyphenolic content with total phenol (94.00 mg TE g^{-1}), flavonoids (38.69 mg QE g^{-1}) and flavonols (15.64 mgQE g^{-1}). This was followed by butanol extract which contained total phenol (79.00 mg TE g^{-1}), flavonoids (34.35 mg QE g^{-1}) and flavonols (5.37 mg QE g^{-1}). Generally, ethyl acetate extracts had the lowest concentration of the polyphenolic compounds. The findings of our study revealed high concentrations of total phenolics and flavonoids but low concentrations of flavonols in the three extracts of *O. americanum* leaves (Table 1). The high phenolics content in this plant might be responsible for the strong antioxidant activity observed in this study.

Figure 1 showed the DPPH radical scavenging activity of the extracts and standard antioxidants (BHT and Vitamin C). The extracts and drugs exhibited a concentration-dependent inhibition of DPPH radical. The observed percentage inhibition was in the ascending order of ethyl acetate<butanol<ethanol<BHT and vitamin C.

ABTS radical scavenging potential of the extracts at the concentrations investigated in this study was determined together with standard antioxidants (Fig. 2).

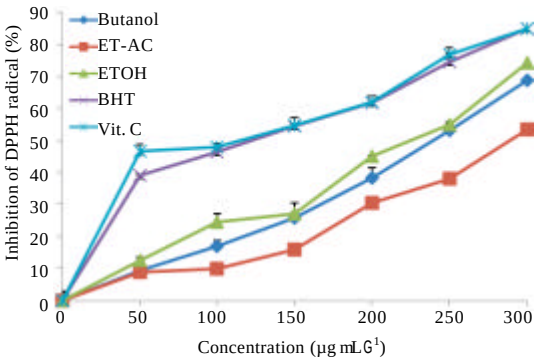

Fig. 1: Scavenging effects of extracts of *O. americanum* leaves on DPPH radical. The results are Mean±SD (n = 3)

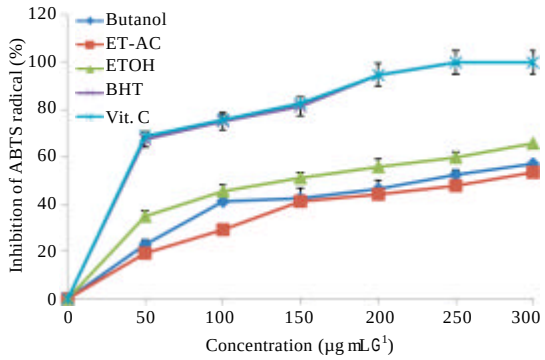

Fig. 2: Scavenging effects of extracts of *O. americanum* leaves on ABTS radical. The results are Mean±SD (n = 3)

Table 1: Polyphenolic contents of ethanol, butanol and ethyl acetate extracts of *O. americanum* leaves

Extract	Total phenolics (mg TE g^{-1})	Total flavonoids (mg QE g^{-1})	Total flavonols (mg QE g^{-1})
Ethanol	94.00±2.24	38.69±1.20	15.64±1.55
Butanol	79.00±0.69	34.35±0.18	5.37±0.09
Ethyl acetate	57.76±4.30	22.28±0.52	2.57±0.10

Data expressed as Mean±SD (n = 3). TE: Tannic acid, QE: Quercetin, CE: Catechin equivalent

The inhibitory activities of both the extracts and reference drugs occurred at all the concentrations tested in an increasing order.

O. americanum extracts demonstrated hydrogen peroxide decomposition activity in a concentration-dependent manner (Fig. 3). The scavenging activities of the extracts were found to be lower than those of the standard drugs. At a concentration of 300 μg mL^{-1}, the hydrogen peroxide radical inhibition exhibited by the extracts and known antioxidants followed the order: ethyl acetate<butanol<ethanol<BHT<vitamin C.

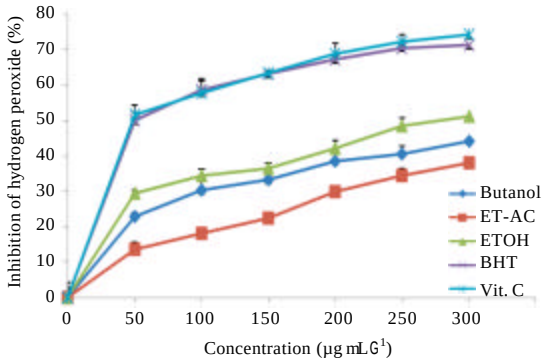

Fig. 3: Scavenging effects of extracts of *O. americanum* leaves on hydrogen peroxide radical. The results are Mean±SD (n = 3)

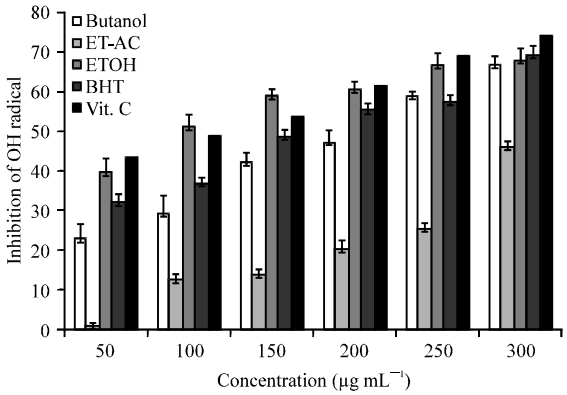

Fig. 5: Scavenging effects of extracts of *O. americanum* leaves on hydroxyl radical. The results are Mean±SD (n = 3)

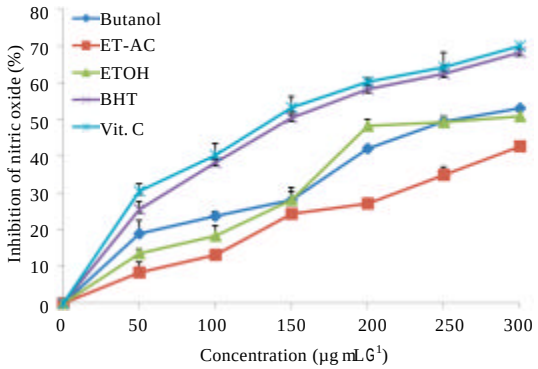

Fig. 4: Scavenging effects of extracts of *O. americanum* leaves on nitric oxide radical. The results are Mean±SD (n = 3)

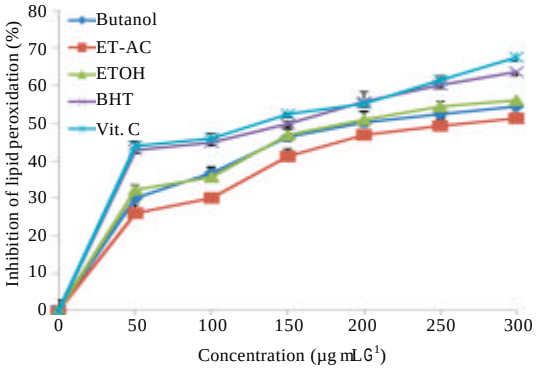

Fig. 6: Inhibitory effects of extracts of *O. americanum* leaves on lipid peroxidation. The results are Mean±SD (n = 3)

Figure 4 illustrates the scavenging activities of the extracts and standard drugs against nitric oxide released by sodium nitroprusside. The inhibitory activities of the extracts and reference drugs were found in the following order: Vitamin C>BHT>butanol>ethanol>ethyl acetate extract.

The effect of *O. americanum* extracts on the inhibition of hydroxyl radical production was assessed by the iron (II)-dependent deoxyribose damage assay. Figure 5 presents the results of the effects of the extracts and standard drugs on OH^* radical production. The extent of inhibition occurred in a dose-dependent manner. At a concentration of 300 µg mL^{-1}, the ethanol and butanol extracts showed maximum inhibitory effect of 67.95 and 66.67%, respectively which was comparable to that of BHT (69.23%).

The anti-lipid peroxidation effect of the extracts and reference drugs is presented in Fig. 6. The addition of Fe^{2+}/ascorbate to the lecithin of egg yolk caused increased lipid peroxidation which was inhibited by the addition of the extracts and standard antioxidants in a concentration-dependent manner. The highest inhibition of lipid peroxidation was exhibited by vitamin C while the lowest inhibitory activity was demonstrated by the ethyl acetate extract of *O. americanum* leaves.

The reductive capabilities of the extracts of *O. americanum* leaves as compared with standard antioxidants are shown in Fig. 7. The transformation of Fe^{3+}-Fe^{2+} was investigated in the presence of the extract and reference drugs in a concentration-related manner. The presence of reductants was confirmed by the changes of yellow colour of the test solution to greenish-blue and the absorbance increased with increasing concentration. The reducing power is shown in the ascending order of ethyl acetate<ethanol<butanol<BHT< vitamin C.

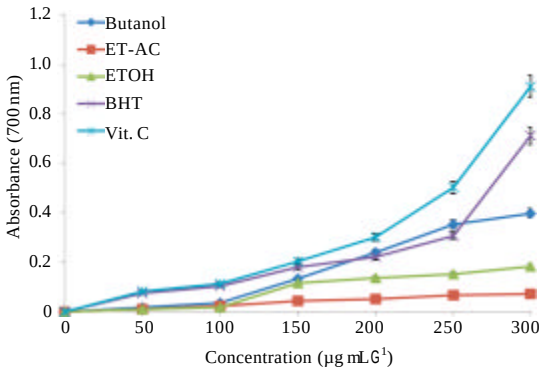

Fig. 7: Ferric reductive abilities of extracts of *O. americanum* leaves. The results are Mean±SD (n = 3)

DISCUSSION

Polyphenols are pharmacologically active components of plants which are capable of neutralizing free radicals, chelating metal catalysts and inhibiting the activity of oxidizing enzymes in biological systems (Miliauskas *et al*., 2004). They are also capable of regenerating endogenous α-tocopherol in the phospholipid bilayer of the membrane to its active antioxidant form. This mechanism of antioxidant action confers health beneficial potentials on polyphenolic compounds. Several workers have attributed therapeutic potential of various medicinal plants to their significant antioxidant potential due to the presence of phenolic compounds (Shahidi *et al*., 1994; Oyedemi *et al*., 2010; Basma *et al*., 2011). These compounds have been reported to reduce the risk of cardiovascular diseases, cancer, urinary tract diseases and metabolic syndrome (Wang *et al*., 1998). The high levels of phenolics in the solvent extracts of *O. americanum* leaves could justify the ethnotherapeutic usage of this plant by the traditional healers. Our study revealed that there was a relationship between the polyphenolic contents and the antioxidant activities of the extracts. These appreciable levels of polyphenols in the extracts of the leaves might be responsible for the use of this plant for the treatment of radical related problems such as diabetes and gastrointestinal lesions (Nyarko *et al*., 2002).

DPPH is a stable radical commonly used to determine the antioxidant activity of various compounds. It is a purple radical that decolorizes to either yellow or colourless due to the antioxidant potential of test samples. The ethanol extract of *O. americanum* leaves exhibited the highest inhibition of DPPH radical which corresponds to its phenolic contents. This was followed by butanol extract whereas, ethyl acetate extract showed the least inhibition. It is worth noting that the inhibition of DPPH radical by vitamin C and BHT used as reference drugs was significantly higher than those of the extracts. This observation is in tune with the previous findings of Ganie *et al*. (2011) on the antioxidant effect of ethyl acetate extract of *Podophyllum hexandrum*. The strong inhibition displayed on DPPH radical could be linked to polyphenolic compounds which are capable of donating electrons or transferring hydrogen atom to neutralize free radicals and thus, could be a promising therapeutic agent to treat stress induced pathological conditions.

ABTS is a blue green chromophore with characteristic absorbance maxima at 734 nm and mostly reactive toward phenolics, thiols and other antioxidants (Walker and Everette, 2009). The reactions of the solvent extracts with the pre-formed radical decolorized the chromophore with increasing concentrations. In this study, ABTS radical scavenging activity of ethanol extract of the leaf of *O. americanum* was higher than other solvent extracts but lower when compared with vitamin C and BHT. At 300 µg mL^{-1}, the percentage inhibition of ABTS radicals was found to be 65.70, 56.98, 53.33 and 100% for ethanol, butanol, ethyl acetate extracts, BHT and vitamin C, respectively. A similar trend for the inhibition of DPPH radical was observed in the inhibitory activities of the extracts on ABTS radical. This is contrary to the findings of Wang *et al*. (1998), who reported that compounds which posses $ABTS^{\bullet+}$ activity may not exhibit $DPPH^{\bullet}$ scavenging potential. Though, the mechanism of action was not investigated in this study, but could be assigned to the hydrogen proton donating ability of the extracts to $ABTS^{\bullet+}$. The donor could be adduced to the polyphenolic contents. The observation from this present study gives scientific credence to the traditional usage of *O. americanum* leaves for the treatment of oxidative stress induced diseases in man.

Hydrogen peroxide is an oxidant with capability of oxidizing biological compounds by penetrating biological membranes. It can be reduced to hydroxyl radical through the action of gluthatione peroxidase and catalase in the presence of iron or copper. The formation of this radical is an underlying cause of tissue damage and if not prevented may result to cell death (Reddy *et al*., 2010). The hydrogen peroxide inhibition increased with increasing concentration of the extracts and the standards. The ethanol extract showed the highest scavenging potential against H_2O_2; followed by butanol while the least inhibition was exhibited by ethyl acetate extract at 300 µg mL^{-1}. Although vitamin C and BHT showed better scavenging activity, the inhibition of H_2O_2 by the extracts can be attributed to the proton donating abilities of their polyphenolic contents.

Nitric Oxide (NO) radical is generated from sodium nitroprusside at physiological pH. It is associated with inflammatory conditions such as atherosclerosis, arthritis, ulcerative colitis and carcinomas (Hazra *et al*., 2009). It is a highly reactive compound that is capable of changing the structural and functional behavior of many cellular components (Ashokkumar *et al*., 2008). The extract of *O. americanum* leaves inhibited nitric oxide radical in a concentration dependent manner. The percentage inhibition of ethanol, butanol and ethyl acetate extracts was lower than that of vitamin C or BHT. The inhibitory potentials of the extracts against NO radical can be attributed to their ability to compete with oxygen and its derivatives (Marcocci *et al*., 1994).

Hydroxyl radicals have been implicated in the oxidative damage of DNA, proteins and lipids (Spencer *et al*., 1994). The formation of hydroxyl radicals in biological systems has been attributed to the interactions of metal ions such as ferrous or copper with hydrogen peroxide (McCord and Day, 1987). The hydroxyl radical generated by Fe^{2+}-ascorbic acid and EDTA-H_2O_2 system (Fenton's reaction) was scavenged by the extracts and standards in a concentration dependent manner. At 300 μg mL^{-1}, the percentage inhibition of ethanol and butanol extract against OH radical was 67.95 and 66.67%, respectively which was comparable to that of BHT (69.23%). Ethyl acetate extract depicted the least inhibition (46.15%). This observation suggests that the extracts of *O.americanum* can be used as an alternative remedy to synthetic antioxidants in combating the oxidative activity of hydroxyl radical. In addition, the weak antioxidant potential of ethyl acetate extract may be due to poor extraction of polyphenolic content.

The effect of lipid peroxidation on biological system has brought about an increasing interest in herbal preparations capable of preventing lipid peroxidation and oxidative damage in biological systems (Yagi, 1987). In this study, lecithin extracted from egg yolk was employed as a lipid rich media. The ethanol extract exhibited the highest inhibition of lipid peroxidation followed by butanol while the least inhibition was shown by ethyl acetate extract. Even though, BHT and vitamin C showed better inhibition against lipid oxidation than the plant extracts, the inhibition of lipid peroxidation by the extracts can be adduced to the presence of phenolic compounds. This supports the earlier reports correlating the presence of polyphenolic compounds to antioxidant activity of natural plant products (Oyedemi and Afolayan, 2011; Abalaka *et al*., 2011; Aiyegoro and Okoh, 2010).

The reducing capacity of a compound is as an indication of its potential antioxidant activity due to the presence of reductants (Duh *et al*., 1999). The ability of *O. americanum* extracts to reduce ferric ions to its ferrous form is evident in the concentration dependent formation of Perl's Prussian blue which was monitored spectrophotometrically at 700 nm. At a concentration of 300 μg mL^{-1}, the reducing ability of butanol, ethanol and ethyl acetate extracts of *O. americanum* leaf was significantly lower than that of BHT and vitamin C. Nevertheless, our study revealed that O. *americanum* contain components capable of electron transfer which can react with free radicals to convert them to stable products and terminate the chain of reactions that leads to oxidative stress.

CONCLUSION

This study revealed that the leaves of *O. americanum* contain appreciable amounts of polyphenolic compounds that are capable of eliciting potent antioxidant activities. The antioxidant profile of this plant can be harnessed to treat radical related pathological conditions. The mechanism of antioxidant action was based on the ability of its extracts to donate electrons, reduce ferric ions, scavenge nitric oxide, hydrogen peroxide and hydroxyl radicals. The antioxidant activity exhibited by the solvent extracts of *O. americanum* leaves could justify the ethnotherapeutic usage of this plant by the traditional healers. The antioxidative potential of the plant was dependent on the solvent of extraction which means that ethyl acetate may not be a good solvent of extraction in the exploitation of the antioxidant property of *O. americanum* leaves.

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