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Research Article

Phytochemical evaluation and *in vitro* antioxidant status of *Clerodendrum volubile* (an indigenous medicinal plant)

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ABSTRACT

Clerodenrum volubile is an important locally used medicinal plant. It is one of the essential herbs, nature has provided for mankind to be consumed as spices, vegetable and is also used in the treatment of diseases. Qualitative phytoconstituent screening of the plant revealed the presence of steroids, flavonoids, tannins, saponins and phenolic compound while chalcones, alkaloid and anthraquinone were absent. Quantitative phytochemical evaluation showed values of 8.29±1.26mg/g rutin equivalent for flavonoids, 3.53±0.05mg/g gallic acid equivalent for phenol, 3.97±0.03mg/g tannic acid equivalent for tannins and 13.67±1.27% for saponin per dry weight. As it is imperative to extend research work on therapeutic effects of the arsenal of plants, nature has given to us in Africa, so as to obtain a cure for various diseases attacking human's health, antioxidant properties of the plant was evaluated. Antioxidant models used include iron chelating, DPPH(2,2-diphenyl-1-picrylhydrazyl) radical, superoxide ion, hydrogen peroxide, ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical, hydroxyl radical scavenging activities and ferric ion reducing properties. The obtained IC_{50} (concentration of an inhibitor where the response (or binding) is reduced by half) values against DPPH radical were 141.342 and 120.349µg/mL for Clerodendrum volubile and trolox respectively. The chelating effect of the plant extract at 50% inhibition was close to that of ascorbic acid (standard) with 134.34 and 131.19µg/mL concentration respectively. Overall, the aqueous extract of the plant showed antioxidant potential which was close to the effects exerted by known standard antioxidants (ascorbic acid, trolox and EDTA). The plant could hence provide natural antioxidants which are needed to combat numerous free radical-mediated diseases and complications such as aging, cancer and atherosclerosis which are linked with oxidative stress.

Keywords: Ascorbic acid, Natural antioxidants, *Clerodendrum volubile,* Oxidative stress, Phytoconstituent screening.

INTRODUCTION:

The existence of ethnomedicinal claims for *Clerodendrum* species in folk medicines and diverse traditional systems of medicines, across Asian and African continents, for the management of several life-threatening ailments like jaundice, syphilis, typhoid, asthma, cataract, malaria, pyreticosis, hypertension as well as diseases of skin, blood and lung cancer has drawn attention of researchers to these plants (Neeta and Tejas, 2007). *Clerodendrum volubile* is one of the species of the *Clerodendrum* genus. This plant is known as "Eweta", "Dagba" or "Marugbo" among the Ikale, Apoi and Ilaje people in southern-senatorial zone of Ondo State, South West Nigeria. Meanwhile, the plant is known as "Obnettete" among the Itsekiri and Urhobo populace in Niger-Delta. It is an indigenous, locally used medicinal plant. It is also a tropical non-conventional leafy vegetable grown in Nigeria (Erukainure *et al*., 2010). This

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plant is an important medicinal plant especially to people living in the south-south Nigeria. A wide variety of claims have been reportedly made for the medicinal properties of *Clerodendrum volubile* as a treatment for many ailments and clinical conditions such as oedema, rheumatism, dropsy, gout and arthritis (Neeta and Tejas, 2007; Fred-Jaiyesimi and Adekoya, 2012). Hence, the leaf of *Clerodendrum volubile* has often been termed as magic leaf due to its high efficacy when used for management and treatment of numerous ailments such as diabetes, ulcer and other common diseases (Burkill, 1985). Its medicinal value may be the most probable reason for much of the recent attention and increased utilization of the plant as well as its spread into new areas. We have reported the nutritional value of this plant in our previous article (Ogunwa *et al.*, 2015). There have been reports on the contributions of free radicals to several human diseases such as cancer, development of AIDS as well as heart diseases (Kumpulainen and Salonen, 1999; Elekofehinti *et al*., 2012; Ejelonu *et al.*, 2013). In fact, oxidative stress plays a major role in the growth of chronic and degenerative disorders (Willcox *et al*., 2004; Pham-Huy *et al*., 2008). Fortunately, antioxidants can combat such disorders either by inhibiting the formation of free radicals (scavenging them) or increasing the rate of decomposition. The discovery that synthetic antioxidants are carcinogenic with harmful effect on liver and lungs has given birth to a rising interest toward natural antioxidants of herbal sources (Gokhan *et al*., 2011). The fact that plants constituents with antioxidant capacity are capable of providing protective actions against stress induced oxidative damage in biological systems, has been strongly supported by *in vitro* and epidemiological studies on medicinal vegetables and plants (Ness and Powles, 1997; Umesh and Veeru, 2012).

Despite the fact that many plants have been investigated for phytochemical components and anti-oxidant effects, research is encouraged in this area because discoveries are always acceptable for new plants with these potentials especially those with high therapeutic potential and low toxicity risk. The search for natural antioxidant phytochemicals has been intensified because of their capacity to reduce the impact of free radical reactions, thereby giving protection from diseases (Terao and Piskula, 1997). Hence, the act of discovering new anti-oxidant bioactive compounds from natural sources is still attractive since they are used in folk medicine because they are relatively cheap, easily available with high efficacy and have reduced occurrence of side effects.

The choice to investigate the anti-oxidant properties of aqueous extract of *Clerodendrum volubile* leaves was made based on the revelation that local traditional healers in Ikale land, located in Southern-Senatorial district of Ondo State, Nigeria, employed this part of the plant in the treatment of many oxidative stress mediated diseases and their complications. Therefore, this research work was done to evaluate the phytochemical components and antioxidant potential of *Clerodendrum volubile* leaves to corroborate the traditional claims for its pharmacological properties.

MATERIALS AND METHODS:

Sample collection and preparation

The plant leaves were purchased locally from the people of Ikale land located in Okitipupa Local Government, Ondo State, Nigeria. The sample was identified at the Microbiology and Botany Department, University of Ibadan in Nigeria with herbarium specimen voucher number UIH22481

which was deposited at the Herbarium of the University.

The leaves were detached from their stalks and carefully washed with tap water. They were subsequently rinsed with distilled water so as to remove sand and other impurities. The leaves were then air-dried for twenty (20) days in the laboratory at room temperature. Using a commercial blender, the dried leaves were ground into fine powder. These were kept in polythene bag until used. All analysis was carried out in triplicates.

Aqueous extraction

Plant extract was prepared by soaking 20g of the dry powdered plant leaves in 200 mL of distilled water for 48hrs. The solution was filtered and the filtrate was later concentrated via rotary evaporator and freeze drier.

Qualitative phytochemical analysis

Qualitative phytochemical screening of the aqueous extracts of *Clerodendrum volubile* was carried out using standard procedures (Ajiboye *et al*., 2013; Viswa and Merina, 2014).

Quantitative phytochemical analysis

Phytochemicals which are available in the aqueous extracts of *Clerodendrum volubile* were determined and quantified by established procedures.

Determination of saponin

Saponin was evaluated via the method of Obadoni and Ochuko, 2001. 20g of powdered samples was added to conical flasks. 100mL of 20% aqueous ethanol was poured into the flask. These were heated at about 55^0C for 4hrs over a hot water bath with continuous stirring. The mixture was then filtered while the residue was re-extracted with another 200ml 20% ethanol. The obtained extracts were concentrated to 40mL over water bath. The concentrate was placed in a 250mL separating funnel. 20mL of diethyl ether was added and the mixture was shaken vigorously. The ether layer of the mixture was discarded while the aqueous layer was recovered. The purification process was done again and 60mL of n-butanol was added to the extracts. This was followed by washing with 10mL of 55% aqueous NaCl twice. The obtained solution was heated in a water bath. The extracts were then dried in the oven, after evaporation, to a constant weight. Finally, percentage of saponin content was determined.

Determination of tannin

Total tannins were determined by the method reported by Boham and Kocipai, 1994. Tannic acid was used as standard. 50mL of distilled water was added to 500mg of the sample in a plastic bottle. The content was shaken in a mechanical shaker for 1h. Then, the content was filtered into a 50mL volumetric flask and made up to the mark. 5mL of the filtrate was taken with a pipette into a test tube and 3mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The colored product observed was measured within 10 min at 120nm in a spectrophotometer.

Determination of total flavonoid content

The method of Meda *et al*., 2005 was used to measure the flavonoids content using rutin as standard. In brief, samples of aqueous extract were mixed with 50µL of $AlCl_3$ and KCH_3COOH. Standard rutin solutions were prepared from 0.01g rutin dissolved in 20mL of ethanol. The amount of flavonoids in both extracts was expressed as rutin equivalent (RE).

Determination of total phenolic content

Phenolic constituents were determined by the method reported by Kamdem *et al*., 2012. Samples of the extract were placed in test tubes. Distilled water was added to make the volume 1.4 mL. This was followed by addition of 0.2 mL of 10 % Folin-Ciocalteu reagent (diluted 1:1 with water) and 0.4

mL of Na_2CO_3 solution (7.5 %) which was added to the test tube in sequential order. The test tubes were incubated at 45°C for 40mins. The absorbance was read at 725 nm in a spectrophotometer. Various solutions (0, 1, 2, 2.5, 5, 10 and 15 mg/mL) of gallic acid (0.1mg/mL) was used to prepare the standard curve. The value for total phenolic content was then calculated and expressed as the microgram gallic acid equivalent of dry extract.

***In vitro* antioxidant assay**

The *in vitro* antioxidant assays were done in line with approved procedures.

DPPH antiradical assay

Antiradical assay on DPPH was measured according to Gyamfi *et al*., 1999 method. Trolox was the positive control. Briefly, 2 ml of DPPH (0.1mM in methanol) was added to various concentrations (50, 100, 200 and 500µg/mL) of the plant extract as well as the standard (trolox) and mixed vigorously. 30 min incubation at room temperature was done for the obtained mixture and absorbance was measured at 517 nm.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the plant extract was determined using method of Puntel *et al*., 2005. Standard antioxidant, EDTA was used as a positive control. The reaction was carried out in Tris-HCL buffer (0.1 mol/L, pH 7.4). The plant extracts (50, 100, 200 and 500µg/mL) were added to 100µmol/L $FeSO_4$ solution. The mixture was subsequently incubated at 50ºC for 5min, followed by addition of 13ml 1,10-phenanthroline (0.25% w/v). The absorbance was then read at 510nm.

Reducing antioxidant power

The method of Pulido *et al*., 2000, using ascorbic acid as reference, was adopted for determining the reducing antioxidant property of the plant extract. 0.25mL of the extract was mixed with 0.25ml of 200mM of sodium phosphate buffer pH 6.6 and 0.25mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20min, followed by addition of 0.25ml of 10% TCA (Trichloroacetic acid). The mixture was centrifuged at 2000rpm for 10min and 1ml of the supernatant was mixed with equal volume of distilled water as well as 0.1% of $FeCl_3$. The absorbance was measured at 700nm.

ABTS antiradical assay

For ABTS (2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) antiradical properties, the methods described by Re *et al*., 1999 and Raghavendra *et al*., 2013 were used while trolox was the reference. The $ABTS^{\cdot+}$ was generated by reacting an 7mM ABTS aqueous solution with $K_2S_2O_8$ (2.45mM, final conc.) in the dark for 16hrs. The different concentrations of plant extract were to 0.3mL of ABTS solution. The mixture volume made up to 2mL by addition of water. The absorbance was measured after 15mins at 732nm.

Superoxide anion antiradical assay

The method of Selvakumar *et al*., 2001 was adopted for the superoxide anion antiradical assays using concentrations of 50, 100, 200 and 500µg/ml for both the sample and standard (ascorbic acid). The superoxide anion radicals were produced in 2mL of phosphate buffer (100mM, pH 7.4) with 78µM β-nicotinamide adenine dinucleotide (NADH), 50µM nitro blue tetrazoliumchloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature for 5min. It was then added with 10µM 5-methylphenazinium methosulphate to initiate the reaction and was incubated for 5min at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560 nm. The reaction mixture without test sample was

used as control while mixture without 5-methylphenazinium methosulphate was used as blank.

Hydrogen peroxide scavenging assay

Hydrogen peroxide (H_2O_2) scavenging assay was carried out according to a previously established method (Ruch *et al*., 1989) with ascorbic acid as the positive control agent. Briefly, 40mM H_2O_2 solution was prepared in phosphate buffer (0.1M, pH 7.4). Various concentrations of aqueous plant extract as well as ascorbic acid were added to 0.6mL of 40mM H_2O_2 solution. The absorbance value of the reaction mixture was measured at 230nm 10min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated for the extracts and standard (ascorbic acid).

OH^- radical scavenging ability

Determination of the extract power to prevent Fe^{2+}/H_2O_2-induced deoxyribose decomposition was done using the method reported by Ejelonu *et al*., 2013. The reaction mixture used contained 120.0µl, 20mM deoxyribose; 400.0µl, 0.1M phosphate buffer pH 7.4; 40.0µl, 20.0mM hydrogen peroxide and 40.0µl, 500.0mM FeSO4. The extracts were added to the mixture. Distilled water was then added to make the mixture volume 800.0µl. This was incubated at 37ºC for 30min. The reaction was stopped by the addition of 0.5mL of 2.8% TCA. 0.4mL of 0.6% TBA (tertiary butyl alcohol**)** solution was subsequently added. The reaction tubes were then incubated in boiling water for 20min. The absorbance was taken at 532nm in spectrophotometer.

Statistical analysis

Data generated in triplicates were expressed as means of 3 determinations ± S.D. The MS Excel version 7 software was used for the analysis as well as for plotting of graphs. The IC_{50} values were obtained from the inhibition curves. The percentage inhibition of various radicals was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{absorbance (control)} - \text{absorbance}}{\text{absorbance (control)}} \times 100$$

RESULTS AND DISCUSSION:

According to this study, the weight of the aqueous extract was 3.81g giving a percentage yield of 19.05%. It is known that the extraction yield is usually employed to measure the efficiency of a solvent to extract, specific and desired components from a given sample. Qualitative phytochemical evaluation of the plant extract identifies presence of steroids, saponins, flavonoids, tannins, and phenolic compounds (Table 1) which had been reported to have potent biological and therapeutic properties (Jamuna *et al.,* 2014) while alkaloid, anthraquinone and chalcones were absent. This indicates that the plant will have many medicinal uses. Quantitative phytochemical evaluation (Table 2) showed values of 8.29±1.26mg/g rutin equivalent for flavonoids, 3.53±0.05mg/g gallic acid equivalent for phenol, 3.97±0.03mg/g tannic acid equivalent for tannins and 13.67±1.27% for saponin per dry weight.

According to these results, saponin has the highest concentration, followed by flavonoids (Table 2). The total phenolic and flavonoid content, in this study, are reported as mg GAE (Gallic acid equivalent)/g and mg RE (Rutin equivalent)/g of dried weight. Saponins, as the most prominent phytochemicals in this plant, are usually steroid or triterpenoid glycosides and are commonly found in a several plants and plant products. Ample biological effects including antioxidant, antiperoxidative, hypoglycaemic, anti-carcinogenic and hypolipidemic properties have

Table 1: Qualitative Phytochemical Compositionof *Clerodendrum volubile*

Phytoconstituents	*Clerodendrum volubile*
Alkaloids	-
Cardiac glycosides	+
Steroids	+
Anthraquinones	-
Phenolic compounds	+
Saponins	+
Flavonoids	+
Tannins	+
Chalcones	-
Carbohydrate	+
Proteins	+

+ Presence, – Absence of phytoconstituents

Table 2: Quatitative Phytochemical Composition of *Clerodendrum volubile*

Phytoconstituents	*Clerodendrum volubile*
Saponins	13.67 ± 1.27
Flavonoids	8.29 ± 1.26
Phenol	3.53 ± 0.05
Tannins	3.97 ± 0.03

Each value is a mean of three determination ±SD. Total phenol content is expressed as mg gallic acid equivalent/g, flavonoid content is reported as mg rutin equivalent/g and tannin is shown as mg tannic acid equivalent/g while saponin is presented as percentage (%) of dry weight.

been ascribed to saponins (Hung *et al*., 2009; Elekofehinti*et al*., 2012).

Figure 1-7 showed the results for various antioxidant models used in this study. It became pertinent to use several models because the total antioxidant capacity of an antioxidant cannot be measured by using just a single method. Several assays have evaluated the overall antioxidant potential or reducing capacity, as a measure of an organism total ability to withstand free radical stress (Gulcin *et al*., 2010). The most commonly used methods for *in vitro* antioxidant screening include metal ion chelating, ferric ion reducing, scavenging assays against superoxide anion radical, hydroxyl radical, hydrogen peroxide, ABTS radical and DPPH free radical (Zhao *et al*., 2006). It has been advised that, to accurately evaluate the total antioxidant activity of a product, more than one method should be employed.

DPPH radical scavenging activity

DPPH model is a simple, precise, relatively quick and acceptable method which is widely used to measure free radical scavenging activity. It is a stable radical even at room temperature and becomes a stable diamagnetic molecule upon receiving an electron or hydrogen radical. It has been employed in the determination of antioxidant ability of numerous natural products. The interaction of DPPH with antioxidants is visually noticeable because the purple color changes to yellow, demonstrating the reduction of the stable DPPH radical to its diphenylpicryl hydrazine. This reaction is used to reveal the capacity of neutral products to scavenge free radicals. It is believed that antioxidants act on DPPH via hydrogen donation or electron transfer mechanism (Knezevic *et al*., 2011) and this is often measured as a decrease in absorbance taken spectrophotometrically at 517nm. *Clerodendrum volubile* leaf extract and the standard antioxidant (Trolox) exhibited an inhibition of DPPH radical in a concentration-dependent pattern (Figure 1). However, the extract showed a lower radical

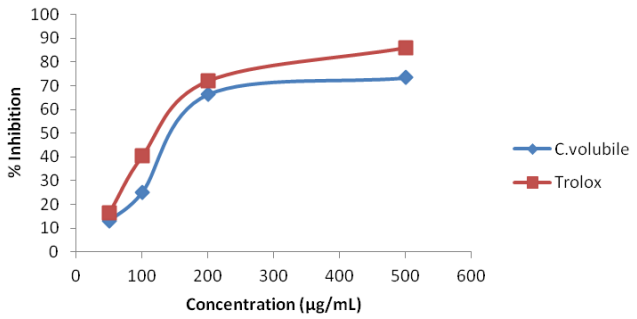

Figure 1: DPPH radical scavenging properties of *Clerodendrum volubile* (Marugbo) and Standard (Trolox). Data represents means of triplicates of different concentrations analyzed.

scavenging ability for DPPH radical when compared to Trolox. The IC_{50} values of *Clerodendrum volubile* and Trolox were 141.342 and 120.349µg/mL respectively (Figure 1). This result revealed the electron and/or hydrogen donating potential of the plant extract.

Superoxide chelating activity

Superoxide anion (O_2^-) is generally considered as a weak oxidant. However, it has been said to cause reduction or oxidation of solutes which ultimately can lead to production of dangerous and powerful hydroxyl radicals and also singlet oxygen, both of which play a role in oxidative stress (Meyer and Isaksen, 1995). Reports have shown that superoxide anions cause direct or indirect damage to biomolecules because it forms H_2O_2, singlet oxygen, hydroxyl radical or peroxynitrite. This occurrence is associated with aging and leads to pathological events like ischemic reperfusion injury. According to this study, both the *Clerodendrum volubile* leaf extract and standard reagent (ascorbic acid) displayed superoxide scavenging activity indicating that our plant may be a good scavenger against the super oxide radical. The mechanism of action of the plant extract may possibly be via inhibition of the generation of superoxide in the *in vitro* reaction system.

Hydroxyl radical scavenging ability

The OH^- scavenging capacity of the extract was compared to a known antioxidant (Ascorbic acid). The result indicated that *Clerodendrum volubile* possess hydroxyl radical scavenging capacity. The percentage of inhibition was 48.22±0.21% and 80.11±0.15% at 500µg/mL concentration for the sample and standard (Trolox) respectively. Hydroxyl radical is one of the potent reactive oxygen species generated in the human body under physiological conditions, where it interacts with polyunsaturated fatty acid components, especially phospholipids of cell membrane leading to cell damage. They have the capacity to abstract hydrogen atoms from membrane lipids (Yen and Duh, 1994), attack proteins as well as effect breaks in DNA via oxidation. Rollet-Labelle *et al*., 1998 claimed that hydroxyl radicals are the most reactive of all the dioxygen (in reduced forms) and that they can easily be generated from the reaction of different hydroperoxides with transition metal ions. In the present study, hydroxyl radical scavenging ability of the standard (ascorbic acid) is however higher than the plant extract.

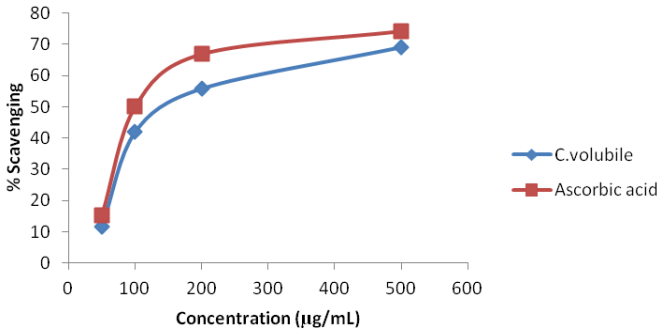

Figure 2: Superoxide radical scavenging activities of *Clerodendrum volubile* (Marugbo) versus ascorbic acid. Data represents means of triplicates of different concentrations analyzed.

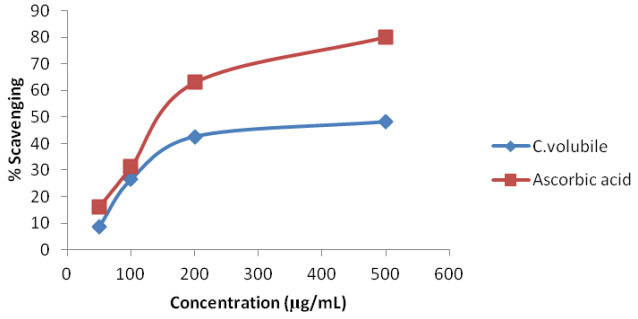

Figure 3: Hydroxyl radical scavenging properties of *Clerodendrum volubile* and ascorbic acid. Data represents means of triplicates of different concentrations analyzed.

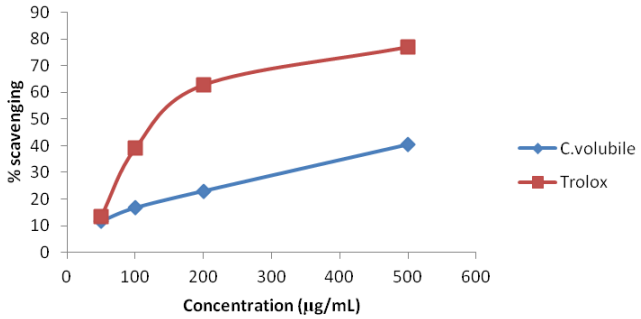

Figure 4: ABTS radical Scavenging effects of *Clerodendrum volubile* and Ascorbic acid. Data represents means of triplicates of different concentrations analyzed.

The ABTS radical scavenging activity

This is a more sensitive model for estimating the antioxidant potential of products which can be natural or synthetic (Ejelonu *et al*., 2013). Addition of potassium persulfate converts ABTS to its radical cation which is blue in color and said to absorb light at 734 nm. ABTS assay is based on the measurement of the absorbance produced by the inhibition of the $ABTS^{+}$ radical, which has a characteristic long wavelength absorption spectrum (Sanchez-Moreno *et al.*, 2002). The potency of antioxidants to deactivate free radical species, via donation of electrons or hydrogen atoms, is observed through decoloration of ABTS radical cation as the reduced ABTS radical is usually colourless in a colour-quenching reaction (Elekofehinti *et al.*, 2013).

Raghavendra and colleagues had earlier reported that the ABTS radical cation is reactive towards most antioxidants and that the blue ABTS radical cation is converted back to its colorless neutral form during an interaction with antioxidants (Raghavendra *et al*., 2013). The plant extract exhibited maximum scavenging activity of 40.35% at 500µg/mL concentration against ABTS radical while Trolox showed 77.17% inhibition at the same concentration (Figure 4). The ability of the *Clerodendrum volubile* extract to decolorize ABTS solution is an indication of its potential to quench organic radicals (ABTS*) (Nenadis *et al*., 2004). The ABTS scavenging model showed that the extract exerts antioxidant activity by hydrogen-donation and chain breaking (Leong and Shui, 2002). Hence, it is suggested that the plant extract can help reduce or prevent oxidative damages which might have been caused by organic radicals.

Hydrogen peroxide scavenging activity

Hydrogen peroxide may penetrate into the human body via various routes such as eye or skin contact and inhalation of vapor or mist. In a concentration dependent manner, *Clerodendrum volubile* leaf extract also promotes hydrogen peroxide decomposition (Figure 5) having an IC_{50} of 142.521µg/mL. Even as a weak oxidizing agent, hydrogen peroxide can directly inactivate a few enzymes, via oxidation of important thiol (-SH) moieties. H_2O_2 can enter into the cell by crossing the cell membranes rapidly and likely react with Fe^{2+}, and/or Cu^{2+} ions to form hydroxyl radical. This process has been suggested as the possible origin of many toxic effects of hydrogen peroxide (Halliwell and Gutteridge, 1981). Scavenging of H_2O_2 by *Clerodendrum volubile* leaf extract could be because of its phenolic compounds which, through donation of electron, reduce H_2O_2 to water (Banerjee and Bonde, 2011). This activity of the plant extract is biologically advantageous since cells must prevent accumulation of H_2O_2.

Fe^{2+} - chelating activity

The plant showed a close chelating effect compared to the standard (ascorbic acid) at 50% inhibition with 134.34 and 131.19µg/mL concentration respectively (Figure 6). The percentage Fe^{2+} chelated was 66.29 for *Clerodendrum volubile* and 86.09 for ascorbic acid at 500µg/mL concentration respectively.

As a major strategy in preventing generation of reactive oxygen species, chelation of metal ion is often done to access active metal catalysis. According to the results, the reaction leading to 1,10-phenanthroline-Fe^{2+} complex formation may

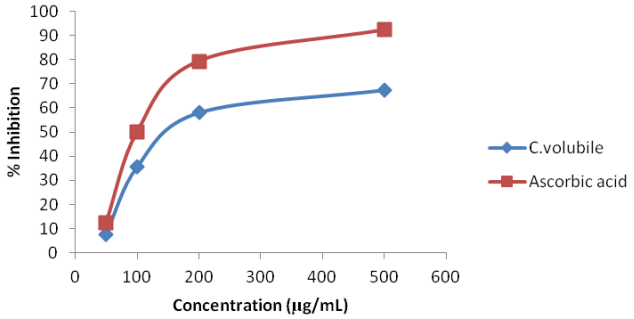

Figure 5: Hydrogen peroxide scavenging activities of *Clerodendrum volubile* (Marugbo) versus Ascorbic acid. Data represents means of triplicates of different concentrations analyzed.

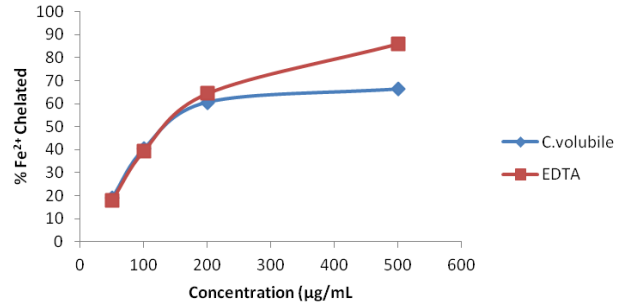

Figure 6: Fe^{2+}- chelating activities of *Clerodendrum volubile* versus Ascorbic acid (standard).Data represents means of triplicates of different concentrations analyzed.

have been disrupted, demonstrating the chelating power of *Clerodendrum volubile* aqueous extract. The decrease observed in the colour (orange) is a confirmation that the plant chelates the ferrous ion instead of the phenanthroline (Elekofehinti *et al*., 2013). This effect may not be unassociated with the availability of phenolic compounds (which can complex with iron) in the plant extract. Iron is important to life because it is needed in many biochemical and physiological processes like cellular respiration and oxygen transport. Iron is also involved in enzymatic reactions where it serves as co-factor to some metallic enzymes. However, excessive free iron can give rise to reactive oxygen species via Fenton reaction, inducing toxicity that leads to oxidative damages in proteins, lipids and other cellular constituents (Elekofehinti *et al*., 2013). The net effect is

occurrence of diseases which include neurodegenerative and cardiovascular diseases. Prevention of lipid peroxidation that can lead to oxidative stress involves blocking free iron from participating, through metal-catalyzed reaction, in the initiation and progression of reactions that will generate highly reactive and biologically-damaging lipid and hydroxyl peroxyl radicals.

Ferric reducing ability

Figure 7 revealed the ferric reducing potential of *Clerodendrum volubile*. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain or by donating a hydrogen atom. The presence of reductants was observed in *Clerodendrum volubile* leaf as the plant extract reduced Fe^{3+}/ferricyanide complex to its ferrous form. This result indicated that reductones exist in the plant.

The antioxidant properties of the plant could be due to the presence of its phytochemicals. Kessler *et al*., 2003 and Cook and Samman, 1996 had earlier claimed that flavonoids show antioxidant activity and that their antioxidant mechanisms of action are through scavenging or chelating process. The potency of flavonoids as antioxidant in lipid systems has also been recognized because they reduce the oxidative effect characterized on membrane phospholipids thereby reducing the access of free radicals to the lipid bilayer hydrophobic membrane matrix. The existence of a free 3-OH and hydroxyl groups in flavonoids structure are essential for its free radical scavenging capacity. This phytochemical has been mentioned to reduce capillary fragility as well as exerting cortisone-like effect on tissues thus protecting against cancer and cardiovascular

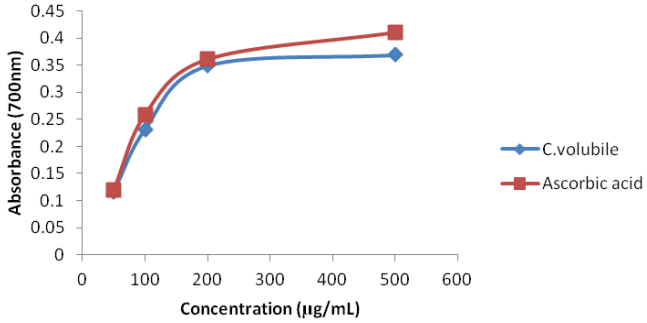

Figure 7: Ferric reducing properties of *Clerodendrum volubile* and Ascorbic acid.Data represents means of triplicates of different concentrations analyzed.

diseases (Ejelonu *et al.*, 2013). Meanwhile, Shahidi and Wanasundara, 1992 reported that phenolic compounds, as class of antioxidant agents, act as oxidants terminators. Xu *et al*., 2007 added that many anti-oxidative phenolic compounds in plants usually occur in a covalently-bound form. The antioxidant effects of phenolic compounds are actually because of their redox capacity and they have been given much attention as potential natural antioxidant because they possess ability to act as efficient radical scavengers and metal chelator. Rajlakshmi *et al*., 2003 had earlier claimed that aqueous extracts of genus *Clerodendrum* showed strongest inhibitory activity over organic (alcohol, petroleum ether and ethyl acetate) extracts. This is in agreement, as observed in this study, that aqueous extract of *Clerodendrum volubile* leaves displayed potent antioxidant properties.

CONCLUSION:

The antioxidant activity of *Clerodendrum volubile* is evident from its effectiveness in scavenging the free radicals in a dose-dependent manner in antioxidant assays. The antioxidant property, as observed in this investigation, could be as a result of the phytochemicals such as phenolic compounds, flavonoids, saponins and tannins in this plant. These have been shown to possess potent antioxidant activities. The findings in this study revealed the potential of *Clerodendrum volubile* for treatment of many free radical-mediated life-threatening diseases and their complications, thus validating its use in folkloric and ethnomedicines. It is therefore suggested that maximum potential of these plant should be explored in pharmaceutical sciences and medicinal field for their appropriate application.

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