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Phytochemical Screening and Antioxidant Potentials of Methanol Extract of *Duranta erecta* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author CAA designed the work and managed the literature searches. Author CCU performed the laboratory experiments, statistical analysis, wrote the protocol and the first draft of the manuscript. Both authors read and approved the manuscript.

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ABSTRACT

Plant extracts that have antioxidant activities show promising effect in the management of many diseases initiated by oxidative stress. The present study evaluates the phytochemical composition and *in vitro* antioxidant potentials of methanol extract of *Duranta erecta* leaves. The extract was subjected to phytochemical screening, DPPH inhibition, ferric reducing antioxidant power (FRAP), nitric oxide determination, antioxidant vitamins and minerals composition analysis. All analyses were carried out using standard biochemical methods. The phytochemical evaluation of the plant extract showed the presence of flavonoids (24.20 ± 0.14 mg QE/g), Alkaloids (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannins (9.24 ± 0.03 mg TAE/g), terpenoids (8.90 ± 0.96 mg/g), steroids (2.65 ± 0.55 mg/g) and saponins (5.55 ± 0.76 mg/g). The *in vitro* antioxidant determination showed that the extract had antioxidant properties in a concentration dependent manner. The antioxidant property of the sample was compared to that of ascorbic acid (for DPPH and nitric oxide determination) and gallic acid (for FRAP) as standards. The antioxidant mineral composition of the extract revealed the presence of zinc (1.82 ± 0.03 mg/100g) and selenium (0.59 ± 0.04 mg/100g). The antioxidant vitamins composition of the extract showed moderate concentrations of vitamin C (0.35 ± 0.01 mg/100 g) and vitamin E (0.68 ± 0.07 mg/100g). The result of this study revealed that methanol extract of *Duranta erecta* leaves could be a good source of antioxidants.

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1. INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80 % of the rural population depends on them as primary health care [1]. Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25 % of prescribed medicines in industrialized countries are derived directly or indirectly from plants [2]. However, plants used in traditional medicine are still understudied [3]. In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80 % of the population uses medicinal plants as remedies [3,4].

Phytochemicals refer to group of compounds that occur in medicinal plants naturally. They help in protection against diseases [5]. Phytochemicals are regarded as anti-nutrient that is, they do not have any nutritional value [6]. Phytochemicals have so many effects in the human body such as acting as antioxidants [7], imitating some important hormones and protection against diseases [6]. There are two classifications of phytochemicals based on their functions in plant's metabolic processes. They include primary and secondary metabolites. Primary metabolites consist of amino acids, simple sugar, chlorophyll and proteins while secondary metabolites include terpenes, steroids, saponins, phenols and alkaloids [5].

Duranta erecta is a light green coloured plant which bears orange fruits. It is used as ornamental plant [8]. *Duranta erecta* is a member of the family of *Verbenaceae* and it is made up of 35 species. It is abundant in Asia, Africa, and South and Central America [9]. Previous studies have shown that it has been used for the management of headache, toothache, wound healing, liver protection, [8] and as diuretics [10]. Its anti-malarial activities have been reported [3]. The antioxidant and antimicrobial activities of *Duranta erecta* leaves extracts have been studied by some researchers [8,11].

Reactive oxygen species are produced by processes originating from within or outside of an organism and are harmonised by the activities of antioxidants defences [12]. When there is disharmony in reactive oxygen species and antioxidant defences, it leads to oxidative stress. Antioxidants are a group of substances that hinder oxidative effects and also mitigate the deleterious effect of oxidation in tissues of organisms. Antioxidants when present in food play a pivotal role in cell maintenance. Researchers have shown that medicinal plants contain bioactive compounds that exhibit antioxidant properties. Such compounds include flavonoids, tannins, phenolics, etc [13]. This study is designed to identify the bioactive constituents of the leaves of *Duranta erecta* and its antioxidant potentials.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Duranta erecta* were collected From Amagu-uwani village in Nguru Nsukka and were identified by Mr. Felix Nwafor, a botanist in the University of Nigeria Nsukka.

2.1.1 Extraction of plant material

A known quantity (837.11g) of pulverized leaves of *Duranta erecta* was soaked in methanol (2.5 litres) for 48 hrs. It was then filtered and the filtrate concentrated using rotary evaporator to give a semi-solid residue.

2.2 Qualitative and Quantitative Phytochemical Screening of Methanol Extract of *Duranta erecta* Leaves

2.2.1 Qualitative phytochemical screening

The qualitative phytochemical screening of the plant extract was evaluated following a previously reported method [14,15].

2.2.1.1 Test for alkaloids

A quantity of the sample (0.2 g) was boiled with 5 ml of 2 % HCl on a water bath and then filtered. To 1 ml each of the filtrate was treated with the following reagents:

Two drops of Dragendorff's reagent; An orange precipitate indicated the presence of alkaloids.

Two drops of Mayer's reagent; A creamy-white precipitate indicated the presence of alkaloids.

2.2.1.2 Test for flavonoids

A quantity of the sample (0.2 g) was heated with 10 ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered. 4 ml of the filtrate was shaken with 1 ml of 1 % aluminium chloride solution and observed for light yellow colouration that indicated the presence of flavonoids.

2.2.1.3 Test for phenolic compounds

About 2.0 ml of the extract was measured in a test tube and 0.01 mol/dm^3 Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated the presence of phenolic compounds.

2.2.1.4 Test for glycosides

A quantity of the sample (2.0 g) was mixed with 30 ml of distilled water and 15 ml of dilute H_2SO_4 respectively and heated in a water bath for 5 minutes and then filtered. 5 ml of the filtrate was added 0.3 ml of Fehling's solutions A and B until it turned alkaline plus heat on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycosides.

2.2.1.5 Test for saponins

A quantity of the sample (0.1 g) was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. To a 1 ml of the filtrate was added two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

2.2.1.6 Test for tannins

A quantity of the sample (2 g) was boiled with 5 ml of 45 % ethanol for 5 minutes. The mixture was cooled and then filtered. To 1 ml of the filtrate was added 0.5 ml of bromine water and then observed for a pale brown precipitate.

2.2.1.7 Test for terpenoids and steroids

Ethanol (9 ml) was added to 1 g of the sample and refluxed for 10 minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1hour, and the waxy matter filtered off. The filtrate was extracted with chloroform (2.5 ml) using a separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids.

Another 0.5 ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicated the presence of terpenoids.

2.2.2 Concentration of alkaloids

The alkaloid present in the plant extract was determined following the method that has been previously reported [16]. To a 5 g of dried sample was added 200 ml of acetic acid in ethanol. Mixture was allowed for 4 hours, then filtered and concentrated till it was a quarter of the original volume. Drops of conc. NH_4OH was added till formation of precipitate was complete, the solution was allowed to stand and precipitate collected and washed with dilute NH_4OH and then filtered, dried and weighed.

2.2.3 Concentration of saponins

An already reported method was used for the concentration of saponin determination [17]. To a 20 g of the sample was added 100 ml of 20 % aqueous ethanol, and then heated for 4 hours in a water bath at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. Both extracts were combined, and heated on a water bath 90 °C till the volume was reduced within 40 ml, then it was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. To the aqueous layer was added 60 ml of n-butanol, then washed twice with 10 ml of 5 % aqueous NaCl. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated.

2.2.4 Estimation of flavonoid content

A method that has been previously reported was used to estimate the flavonoid content of the extract [18]. Sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30ml of $NaNO_2$ solution (10%). After 5 min, 0.30ml $AlCl_3$ solution (10%) was added followed by 2.0ml of NaOH solution (1%) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus blank. Standard curve of quercetin was prepared (0-12 mg/ml) and the results were expressed as quercetin equivalents (mg quercetin/ g dried extract).

2.2.5 Concentration of terpenoids

The method earlier reported was used in determining the quantitative terpenoids content of the plant extract [19]. Extract (2g) was weighed and soaked in 50 ml of 95 % ethanol for 24 hrs. The mixture was filtered and the filtrate extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as total terpenoids.

2.2.6 Estimation of total phenolic content

Folin-Ciocaltaeu assay was used to determine the total phenolic content of the plant extract [20]. The sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent and allowed to stand for 5 min. Then 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23^0C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution.

2.2.7 Estimation of total tannin content

Folin-Ciocalteu assay was used to determine the total tannin content [21]. To a volumetric flask containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate was added 0.1 ml of the sample extract and then made up to 10 ml with distilled water. Mixture was shaken and maintained at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared following the same process. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer.

2.2.8 Concentration of steroids

The amount of steroids present in the plant extract was determined following an earlier reported method [16]. The extract (1 g) was marcarated with 20 ml of ethanol and filtered. To the filtrate (2 ml), was added 2 ml of chromagen solution and allowed to stand for 30 min. The absorbance was read at 550 nm.

2.3 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) Radical-Scavenging Activity

Different concentrations of the extract was prepared (20-100 µg/ml) using methanol. From each of the concentrations, 1.0ml was drawn and mixed with 1.0ml of 0.3mM DPPH in methanol. It was thoroughly mixed and kept in the dark for 25 mins at room temperature. The control was prepared in a similar way without the extract. Ascorbic acid was used as standard. The absorbance was read at 518 nm with UV/Visible spectrophotometer [22].

$$\frac{\mathrm{Ab0} - \mathrm{Ab1}}{\mathrm{Ab0}} \times 100$$

Ab_0 = absorbance of the control, Ab_1 = absorbance of the test samples. IC_{50} values were determined.

2.4 Nitric Oxide Scavenging Activity

The plant extract at different concentrations (20-100 µg/ml) mixed with phosphate buffer was incubated with sodium nitroprusside (5 mM) 25^0C for 5 hours. Identical solution was prepared without the plant extract and served as a control. Griess reagent (0.5ml) was used to dilute 0.5ml each of the incubated solution. Absorbance (Ab) was read at 546 nm [23].

Calculation,

$$\text{NO Scavenging activity (\%)} = \frac{\text{Ab of Control} - \text{Ab of Test}}{\text{Ab of Control}} \times 100$$

The antioxidant activity of the plant extract was expressed as IC_{50}.

2.5 Ferric Reducing Antioxidant Power Assay

A volume (1 ml) of varying concentrations (20-100 µg/ml) of the plant extract mixed with 2.5 ml each of phosphate buffer and potassium ferricyanide was incubated for 20 min at a temperature of 50°C. The mixture was centrifuged at 3000 rpm for 10min upon addition of 2.5 ml aliquots of trichloroacetic acid. Freshly prepared 1 % ferric chloride solution (0.5ml) was added to the mixture containing 2.5 ml each of the supernatant and distilled water and the absorbance read at 700 nm. The standard used for comparison of the activity was gallic acid [23].

Calculation,

$$\text{(\%) increase in reducing power} = \frac{\text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

The antioxidant activity of the plant extract was expressed as IC_{50}.

2.6 Determination of Antioxidant Vitamins Concentrations

The vitamin C and E estimation was performed using the method of Pearson [24].

2.7 Determination of Antioxidant Mineral Contents

The minerals were determined using Atomic Absorption Spectrophotometer after the digestion of the sample [25].

2.8 Statistical Analysis

The Statistical Product and Service Solutions (SPSS) IBM version 20 was used to analyse all data from this research. The results were expressed as means ± SD of triplicate determinations.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative phytochemical constituent of *Duranta erecta* leaves

The result in Table 1 showed the presence of steroids in low concentration; tannins, saponins and terpenoids in moderate concentration while alkaloids, flavonoids and phenolics were in high concentration.

3.1.2 Quantitative phytochemical composition of methanol extract of *Duranta erecta* leaves

Table 2 shows the quantitative phytochemical composition of the extract with values as follows: Flavonoids has the highest concentration (24.20 ± 0.14 mg QE/g) followed by alkaloids (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannin (9.24 ± 0.03 mg Tanic acid/g), terpenoid (8.90 ± 0.96 mg/g), steroid (2.65 ± 0.55 mg/g) and saponin (5.55 ± 0.76 mg/g).

3.1.3 DPPH radical scavenging activity of methanol extracts of *Duranta erecta*

Duranta erecta leaves extract showed a concentration dependent DPPH radical scavenging activity which was comparable to that of ascorbic acid used as a standard. The percentage inhibitory activity of methanol extract of *Duranta erecta* leaves was concentration dependent with a half maximal inhibitory concentration (IC_{50}) of 40.91 ± 0.19 µg/ml compared to that of standard with IC_{50} of 26.88 ± 0.57 µg/ml.

3.1.4 Nitric oxide radical scavenging activity of methanol extracts of *Duranta erecta*

Table 4 shows a concentration dependent effect of the extract in scavenging nitric oxide radical with a half maximal inhibitory concentration (IC_{50}) of 56.89 ± 3.39 µg/ml while the standard (ascorbic acid) has an IC_{50} of 32.16 ± 3.79 µg/ml. The highest percentage inhibition of nitric oxide activity (68.51 ± 1.44 %) was observed at the highest dose of the extract (100 µg/ml) while the lowest inhibition (35.40 ± 2.11 %) was at the concentration of 20 µg/ml.

3.1.5 Ferric reducing antioxidant power of the extract

Table 5 shows a concentration dependent reducing power of the extract with a half maximal inhibitory concentration (IC_{50}) of 97.23 ± 0.68 µg/ml while the standard (gallic acid) has an IC_{50} of 60.64 ± 2.81 µg/ml. The highest percentage inhibition (52.58 ± 0.53 %) was observed at the highest dose of of the extract (100 µg/ml) while the lowest inhibition (30.86 ± 1.28 %) was at the concentration of 20 µg/ml.

Table 1. Qualitative phytochemical constituent of methanol extract of *Duranta erecta* leaves

Phytochemicals	Inference
Alkaloids	+++
Flavonoids	+++
Phenolics	+++
Glycosides	ND
Saponins	++
Tannins	++
Terpenoids	++
Steroids	+

Key: + Present but low in concentration, ++ present in moderate concentration, +++ present in high concentration, ND = Not Detected

Table 2. Quantitative phytochemical composition of methanol extract of *Duranta erecta* leaves

Phytochemical constituent	Concentration
Total phenol (mg GAE/g)	12.73 ± 0.61
Flavonoid (mg QE/g)	24.20 ± 0.14
Alkaloid (mg/g)	15.87 ± 1.71
Tannin (mg Tanic acid/g)	9.24 ± 0.03
Terpenoid (mg/g)	8.90 ± 0.96
Steroid (mg/g)	2.65 ± 0.55
Saponin (mg/g)	5.55 ± 0.76

Values are presented as mean ± SD (n = 3)

Table 3. DPPH radical scavenging activity of methanol extract of *Duranta erecta* leaves

Concentration (µg/ml)	% Inhibition by extract	% Inhibition by standard
20	41.67 ± 0.76	48.11 ± 1.14
40	47.97 ± 1.33	58.64 ± 1.58
60	57.20 ± 1.14	61.74 ± 2.69
80	70.83 ± 1.14	86.74 ± 0.58
100	77.90 ± 2.31	89.37 ± 2.53
IC_{50} (µg/ml)	40.91 ± 0.19	26.88 ± 0.57

Values are presented as mean ± SD (n = 3)

Table 4. Effect of methanol extract of *Duranta erecta* leaves on nitric oxide radical

Concentration (µg/ml)	% Inhibition by extract	% Inhibition by standard
20	35.40 ± 2.11	35.43 ± 2.81
40	41.60 ± 2.61	57.93 ± 1.24
60	49.20 ± 0.80	71.72 ± 2.12
80	61.38 ± 3.45	76.55 ± 1.42
100	68.51 ± 1.44	84.21 ± 0.14
IC_{50} (µg/ml)	56.89 ± 3.39	32.16 ± 3.79

Values are presented as mean ± SD (n = 3)

Table 5. Effect of methanol extract of *Duranta erecta* leaves on ferric reducing antioxidant power

Concentration (µg/ml)	% Inhibition by extract	% Inhibition by standard
20	30.86 ± 1.28	39.28 ± 1.21
40	33.83 ± 0.32	43.01 ± 2.12
60	38.85 ± 3.85	47.98 ± 1.01
80	44.59 ± 1.06	56.90 ± 0.23
100	52.58 ± 0.53	62.02 ± 1.21
IC_{50} (µg/ml)	97.23 ± 0.68	60.64 ± 2.81

Values are presented as mean ± SD (n = 3)

Table 6. Antioxidant minerals composition of methanol extract of *Dutanta erecta* leaves

Mineral/Vitamin Constituents	Concentration (mg/100g)
Selenium	0.59 ± 0.04
Zinc	1.82 ± 0.03
Vitamin C	0.35 ± 0.01
Vitamin E	0.68 ± 0.07

Values are presented as mean ± SD (n = 3)

3.1.6 Antioxidant minerals and vitamin composition of methanol extract of *Dutanta erecta* leaves

Table 6 shows the composition of antioxidant minerals and vitamin of the methanol extract of *Duranta erecta* leaves. Selenium has a lower concentration (0.59 ± 0.04 mg/100g) compared to zinc having a concentration of 1.82 ± 0.03 mg/100g. It also shows that the extract has moderate concentrations of vitamin C (0.35 ± 0.01 mg/100g) and vitamin E (0.68 ± 0.07 mg/100g).

3.2 Discussion

Free radicals are associated with the initiation and progression of many diseases that are most common in ageing people [26]. Antioxidant enzymes like superoxide dismutase, catalase, and glutathione as well as phytochemicals through their activities naturally protect human against oxidative stress [27]. The present research was carried out to investigate the phytochemical composition and antioxidant potentials of methanol extract of *Duranta erecta* leaves in *in vitro* models.

In the phytochemical study, the presence of flavonoids (24.20 ± 0.14 mg QE/g), alkaloids (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannins (9.24 ± 0.03 mg Tanic acid/g), terpenoids (8.90 ± 0.96 mg/g), steroid s(2.65 ± 0.55 mg/g) and saponins (5.55 ± 0.76 mg/g) were revealed. The absence of glycosides was not consistent with an earlier report, which stated that glycosides were present in extract of *Duranta erecta* leaves [11]. The result indicates that the leaves possess some biologically active components which could serve as good antioxidants. The presence of a functional hydroxyl groups in flavonoids confer an antioxidant properties on flavonoids. These hydroxyl groups scavenging free radicals and as well chelate metal ions. This effect helps in the reduction in generation of free radicals that leads to oxidative stress and several disease conditions [28]. Flavonoids suppress reactive oxygen species formation through the inhibition of some key enzymes. It also regulates the antioxidant defense system [29]. Flavonoids also inhibit lipid peroxidation [28] by sugar substitution in the phenolic C ring [30]. Alkaloids exhibit their antioxidant properties through alleviation of H_2O_2-induced oxidative damage [30]. Tannins are very powerful antioxidants. They have effect on Fenton reaction thus retarding oxidation. It also helps in chelating metal ions [11]. It has been reported previously that saponins possess antioxidant ability through scavenging of hydrogen peroxide [31]. Evidence suggests that steroids also possess antioxidant and anti-inflammatory activities [32]. An important bioactive compound; β-sitosterol has been identified and isolated from *Duranta erecta* leaves [33]. It has been reported that β-sitosterol, a phytosterols improves BPH symptoms [34]. It has an anti-inflammatory effect through inhibition of prostaglandins synthesis and stimulation, inhibition of aromatase activity and also inhibition of 5-alpha-reductase activity [35]. There are elevated levels of prostaglandins in BPH patients of which β-sitosterol helps to reduce [35]. Terpenoids also have anti-inflammatory properties. Two important triterpenes (oleanolic and ursolic acids) has been identified in *Duranta erecta* leaves [36]. It has been reported that oleanolic, and ursolic acid have anti-inflammatory activity in the connective tissue of prostate gland [37].

DPPH assay is a vital assay used in determining the antioxidant activity of plant extracts in *in vitro* model [38]. DPPH is a free radical that interacts readily with antioxidant compounds. Compounds with antioxidant properties donate an atom of hydrogen to DPPH and thus a colour change. The strength of the colour change is measured calorimetrically. As the intensity of the colour change increases the inhibition of DPPH increases [23]. In this study, the inhibition of radicals of DPPH increased linearly with increasing extract concentrations. The highest inhibition (77.90 ± 2.31 %) was observed at a concentration of100 µg/ml. In the DPPH assay, lower half maximal inhibition concentration (IC_{50}) value implies higher antioxidant activity. The IC_{50} value is the concentration that caused half maximal inhibition of the radical. Researchers have proposed that extracts with IC_{50} less than 50 µg/ml are regarded as very strong antioxidants, 50-100 µg/ml; strong antioxidants, 101-150 µg/ml; moderate antioxidants, and those above 150 µg/ml weak antioxidants [39,40]. The IC_{50} of the extract was found to be 40.91 ± 0.19 µg/ml indicating that the extract is a very strong antioxidant. This finding is in consistent with an earlier report which stated that *Duranta erecta* leaves extract effectively inhibited DPPH radicals [11].

In nitric oxide (NO) radical scavenging assay, NO radicals which are generated from sodium nitroprusside interacts with oxygen to form nitrite.

The antioxidant activity of the extract is then measured as its ability to inhibit nitrite formation [41]. Although nitric oxide radical has some potential health benefits, its role in causing oxidative damage cannot be overemphasised [42]. The extract under study exhibited nitric oxide radical scavenging activity that was concentration dependent. The IC_{50} of the standard and the extract were 32.16 ± 3.79 µg/ml and 56.16 ± 3.39 µg/ml respectively. The result indicates that the *Duranta erecta* leaves extract might serve as an excellent nitric oxide radical scavenger due to the high level of flavonoids. Research had shown that flavonoids are potent nitric oxide radical scavengers [42].

FRAP assay relies on the ability of a plant extract to transfer electron. It measures the ability of a given extract to reduce ferric iron to ferrous iron [23]. The result obtained showed that the ferric reducing antioxidant power of the extract was concentration dependent. From the result, it could be concluded that the extract may be effective in minimizing oxidative damages in tissues.

In the present study, the concentration of zinc and selenium in the methanol extract of *Duranta erecta* were 1.82 ± 0.03 mg/100g and 0.59 ± 0.04 mg/100g respectively. Selenium is found in ionic form, bound to amino acids; methionine and cysteine. It bioavailability very high, and selenium helps in the formation of connective tissues by acting as a prosthetic group in enzymes which form cross-links in collagen and elastin [6]. Selenium; mineral nutrient is a key component of the enzyme, Glutathione peroxidase (an antioxidant enzyme), which targets hydrogen peroxide (H_2O_2) in the body and converts it to water. It is an antioxidant and plays crucial roles in various tissues and organs of the human body [43]. Zinc is present at a reasonable quantity in the plant extract. The concentration of zinc in the plant extract falls below the FAO/WHO permissible limit of zinc in both edible and medicinal plants which is 50 mg/kg [44]. Zinc is an essential trace mineral, when present in the body serves as a co-factor for superoxide dismutase (SOD); an endogenous antioxidant enzyme [45]. Other anti oxidative roles played by zinc within the body include; protection of cells from oxidative damage, lipid membrane stabilization, and inhibition of NADPH-Oxidase. [46]. It also plays a crucial role in the synthesis of metallothioneins; a protein that reduces free radicals generated in a stressful situations [47]. Extracts of *Duranta erecta* leaves contains vitamin C and vitamin E in the concentrations of 0.35 ± 0.01 mg/100g and 0.68 ± 0.07 mg/100g respectively. Vitamin C and E have been reported to show antioxidant properties through their ability to scavenge free radicals [48]. Vitamin C stabilizes the human connective tissues, facilitates the healing of wounds and regeneration of vitamin E. Vitamin C reduces Fe^{3+} to Fe^{2+} in the intestine there by aiding its absorption [49]. Vitamin E is lipophilic in nature, and thus found in lipid membrane where they scavenge free radicals thus inhibitng lipid peroxidation [50].

4. CONCLUSION

Findings from the present study showed that extract of *Duranta erecta* leaves contains a reasonable quantity of important phytochemicals like flavonoids, alkaloids, tannins, phenolics, saponins, etc which are essential sources of antioxidants. It also contains both antioxidant minerals; zinc and selenium as well as antioxidant vitamins; vitamin C and E which adds up to its antioxidant strength. The various *in vitro* antioxidant assays (DPPH, FRAP and NO radical scavenging assay) showed that extract of *Duranta erecta* leaves displayed antioxidant properties in a concentration dependent manner. The finding of this study therefore suggests that *Duranta erecta* leaves could serve as a promising source of antioxidants. These antioxidants could have great relevance as therapeutic agents in preventing or slowing the progress of diseases caused by oxidative stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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