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Phytochemical composition and free radicals scavenging activities of methanolic root extract of *napoleona imperialis*

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Abstract: *Napoleona imperialis* is a wild plant found in south east of Nigeria and is commonly known as *nkpodu* among the Igbo's. It has antihypertensive effect and is also used mostly for the treatment of wounds. In this study, methanol root extract of *Napoleona imperialis* was analysed for the presence of bioactive secondary metabolites and its ability to scavenge 2,2-diphenyl -1-picrylhydrazine (DPPH.) radical, superoxide anion radical (O^{2-}), and nitric oxide radical ($NO^{\cdot}$) Quantitative phytochemical analysis was done spectrophotometrically using standard methods. The result showed that the total phenol concentration was 0.059 ± 0.020 mg QUE and flavonoid was 0.615± 0.008 mg GAE. The extract showed a potent minimal DPPH radical scavenging activity by changing the extract spots from purple to yellow on the thin layer chromatographic (TLC) plate. At the concentration of 2500 g/ml the extract had its maximum inhibition of 88.36 ± 4.72 % compared to 250µg/ml of ascorbate (84.68 ± 10.4 %).The extract also had a low superoxide (O^{2-}) anion radical scavenging ability with IC_{50} of 1472.65 g/mL compared to quecertin (IC_{50} = 17.018 g/mL). In the 5 mM sodium nitroprusside (SNP) only medium, the levels of nitrite and hence nitric oxide ($NO^{\cdot}$) was significantly higher ($p<0.05$) at every time interval compared to the observed levels in the presence of the *Napoleonae root* extract. This therefore suggests that the root extract could be a good source of antioxidants to ameliorate conditions in diseases whose pathogenesis implicates

oxidative stress probably by stimulating the immune system of patients hence mechanism involve for its wound healing capabilitys

Keywords: *Napoleona imperialis.* Phytochemical composition, Oxidative stress, Scavenging activities and Antioxidants.

INTRODUCTION

Today, more than 80% of the population in developing countries in the world depends on plants for their medical needs[1, 2].Traditional medicine has always been part of the cultural and religious life of African people. It is easily accessible and affordable to rural people[3].There have been many validations of traditional remedies through scientific research[4-6].Indeed it has been estimated that 25% of prescribed medicines today are substances derived from plants[7] and a recent example is artemisinin obtained from *Artemisia annu*a for the treatment of malaria.

Napoleona imperialis (N.imperialis) which is a wild plant found in south eastern Nigeria is an evergreen non-timber plant that grows abundantly in bush fallows, secondary bushes and marginal lands in most of the tropical humid zones of West Africa[8]. The plant belongs to the family known as the *lecythidaceae*, along with the cannon ball tree (*corrupita guianensis*), which grows in most regions of Nigeria[9-11]. The tree is about 14.5m high, 10m in girth with a dense rounded crown and dark green foliage. The bark is grey to pale brown and darkens on exposure. The leaves have stout common stalk with broad and elliptic leaflets mostly rounded at the apex. It flowers between January and March, and is usually yellowish white and about 0.7- 1.10mm long, crowded in compact branched stout central stalk. It fruits around April and is broadly globular. The fruits which are fleshy structures are attached directly to the main trunks and limbs. Though *Napoleona imperialis* is one of the lesser known plants, its economic importance has partially been reported by Dalziel[9], and Irvine[12]. These include the use of the roots for medicinal purposes and the twigs as traditional chew sticks. It has been reported that, different parts of the plant are used for different purposes in the region including mulching and fodder (leaves and twigs); and firewood, chewing sticks and ethnomedicine (stem and root)[13, 14]. The juice from the fruits and pods are consumed by man while the seeds are discarded. The chemical composition of the leaf, bark and roots had been partially determined by Ogbonnaya[15]. The leaves have a characteristic bitter taste, foaming properties, and can cause injuries to the digestive mucosa and hemolytic changes in blood[16-18]. *Napoleona imperialis* is commonly found in south eastern Nigeria.

According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrientplant chemical compounds or bioactive components are responsible for protecting the plant against microbial infections or infestations by pests[19-22]. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such asturmeric, beverages such as green tea and red wine, as well as many other sources[22, 23].

The science of application of these indigenous or local medicinal remedies including plants for treatment of diseases is currently called ethnopharmacology but the practice dates back since antiquity. Ethno

pharmacology has been the mainstay of traditional medicines the entire world are currently been integrated into mainstream medicine.

There is little or no scientific data on "In *vitro* free radical and nitric oxide scavenging activity of methanol root extract of *Napoleona imperialis* despite its traditional use in medicine for wound healing which may possibly depicts its mechanism for this wound healing and antihypertensive capabilities.. This research study is therefore aimed at determining the phytochemical composition, antioxidants and nitric oxide scavenging potentials of *Napoleona imperialis.root extract.*

MATERIALS AND METHODS

Materials / chemicals: All the chemicals used in this study were of analytical grade. The solvents ethanol, ethyl acetate and hexane were purchased from EMD Biosciences (Gibbstown, NJ). L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, sulphuric acid, sodium nitroprusside (SNP), sodium nitrite, sulpanilamide, phosphoric acid, naphthylethylenediamine dihydrochloride, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), acetic acid, gallic acid, rutin, ferric chloride ($FeCl^{3+}$), ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline (PBS), sodium carbonate (Na_2CO_3), perchloric acid ($HClO_4$), butyrated hydroxyltoluene (BHT), polyvinyl-polypyrrolidone, riboflavin, ferrous sulphate ($FeSO_4.7H_2O$), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), Folin-Ciocalteu's reagent (FCR) and trichloroacetic acid (TCA) were all purchased from Sigma Chemical Co. (St. Louis, MO). Materials used include spectrophotometer, centrifuge, water bath and refrigerator etc.

METHODS

Preparation of extracts: Roots of *N.imperialis*gotten from Mgbirichi community in Imo State were identified at the herbarium, University of Nigeria, Nsukka. Roots were air-dried at room temperature and reduced to fine powder by milling. The powdered plant materials were subjected to extraction with 80% methanol for 48 hours. The hydromethanolic extracts were concentrated using a rotary evaporator (Büchi, Rotavapor R-200) and allowed to paste using a water bath set at 40^{o}C and stored at 4^{o}C until used. Extracts were dissolved in the hydromethanol solvent for the anti-oxidant assays

Rapid DPPH radical scavenging assay using dot-blot: Qualitative screening of the plant extracts for anti-oxidant activity (reflective of the phenolic content) was done using DPPH radical according to the method of Awah *et al.*[24] with slight modifications. Briefly an aliquot (5 µl) of each dilution of plant extract and standard anti-oxidant was carefully loaded on a piece of thin layer chromatography (TLC) plates (silica gel 60 F_{254}, Merck) and allowed to air dry. The sheets were then sprayed with DPPH (0.2 % (w/v) in methanol to reveal the anti-oxidant activity of the extract. The intensity of the yellow colour and the rate at which the colour of the extract spots changed from purple to yellow indicated the scavenging potential of the extract[25] and its phenolic content.

Preparation of solutions

Phytochemical analysis reagents:

Wagner's reagent: Iodine crystals (2 g) and potassium iodide (3 g) were dissolved in 100 ml of distilled water.

Mayer's reagent: Mercuric chloride (1.35 g) was dissolved in 60 ml of distilled water. Also, 5 g of potassium iodide were dissolved in 20 ml of distilled water. The solutions were mixed and the volume made up to 100 ml.

Dragendorff's reagent: Bismuth carbonate (0.85 g) was dissolved in 100 ml of Glacial acetic acid and 40 ml of distilled water, to give solution A. Another solution called solution B was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water. Both solutions were then mixed to give a stock solution- Dragendorff's reagent.

Ferric chloride solution (5%): Ferric chloride (2.5 g) was dissolved in 30 ml of distilled water and the solution made up to 50 ml.

Ammonium solution: Stock concentrated ammonium solution (375 ml) was diluted in 62.5 ml of distilled water and made up to 1000 ml.

Aluminium chloride solution: Aluminium chloride (0.5 g) was dissolved in 50 ml of distilled water and the solution made up to 100 ml.

Dilute sulphuric acid: Concentrated sulphuric acid (10.4 ml) was diluted with 5 ml of distilled water and made up to 100 ml.

Lead sub acetate solution: To 45 ml of 15 % lead acetate solution was added to 20 ml of absolute ethanol and then diluted with 35 ml of distilled water.

Molisch reagent: α-naphthol (0.1 g) was dissolved in 100 ml of absolute ethanol.

Qualitative phytochemical analysis: Tests for flavonoids, tannins, carbohydrates/ glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard methods [26]; [27] described below:

Test for tannins: Pulverised sample (0.5 g) of each plant was boiled in 20 ml of distilled water in a test tube and then filtered with Whatman No. 1 filter paper. Then 0.1 % $FeCl_3$ was added to the filtrate and observed for brownish green or a blue black colouration, which shows the presence of tannins.

Test for saponins: A quantity (2 g) of pulverized samples of each plant was boiled together with 20 ml of distilled water in a water bath and filtered. Then 10 ml of the filtered sample was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for resins: Pulverized plant material (0.5 g) was extracted with 15 ml of 96 % ethanol. The ethanol extract was then poured into 20 ml of distilled water in a beaker. A formation of resinous precipitate indicates the presence of resins. Furthermore, 0.12 g of the extract was extracted with chloroform and the extract concentrated to dryness. The residue was re-dissolved in 3 ml acetone and 3 ml concentration HCl added. This mixture was heated in a water bath for 30 min. A pink colour that changes to magnets red confirmed the presence of resins.

Test for alkaloids: To 0.5 g of pulverized plant material, 5 ml of 1% HCl were added and boiled for 5 min. in a steam bath. This was filtered and 1 ml of the filtrate treated with a few drops of Draggendorf's reagent, a second 1 ml portion treated similarly with Wagner's reagent and a third portion with Mayer's reagent. The formation of red, reddish-brown and creamy white precipitates respectively was an indication of the presence of alkaloids.

Test for glycosides: To 0.5 g of pulverized plant samples, 10 ml of distilled water was added and boiled for 5 min. This was filtered and 2 ml of the filtrate hydrolyzed with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. A reddish–brown precipitate showed the presence of glycosides.

Test for flavonoids: Pulverized plant samples (0.2 g) were heated with 10 ml of ethylacetate in boiling water for 3 min. The mixture was filtered and the 4 ml of the filtrate was shaken with 1 ml of 1 % aluminium chloride solution and observed. A yellowish coloration in the ethylacetate layer indicated the presence of flavonoids.

Test for steroids and terpenoids: To 10 ml of ethanol, was added 1 g of pulverized plant materials and boiled for a 10 minutes. The filtrate was concentrated to 2.5 ml in a boiling water bath and 5 ml of hot water added. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using separatory funnel. To 0.5 ml of the chloroform extract in a test tube was added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface shows the presence of steroids

Test for proteins: Distilled water (5 ml) was added to 0.1 g of the pulverized samples and left to stand for 3 hours and then filtered. To 2 ml portion of the filtrate was added 0.1 ml of Millon's reagent, shaken and kept for observation. Formation of yellow precipitate shows the presence of proteins.

Carbohydrates: To a 2 ml of the sample in a test tube, 2 drops of α-naphthol solution was added then 1 ml of conc. H_2SO_4 was carefully poured down the sides of the tube to form two layers. A colour change to brown at the junction of the two layers indicates the presence of carbohydrates.

Quantitative phytochemical analysis

Determination of total phenolic contents: Total phenolics were determined using Folin-Ciocalteau reagent (FCR) as described by Velioglu *et al.*[28], with slight modifications[24]. FCR consist of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR forming a blue coloured molybdenum oxide whose colour intensity is directly proportional to the phenolic contents. Briefly, 100 l of the extract dissolved in methanol (1 mg/ml) was mixed with 750 l of Folin-Ciocalteu reagent (diluted 10-fold in dH_2O) and allowed to stand at 22 °C for 5 min; 750 l of Na_2CO_3 (60 g/l) solution was then added to the mixture. After 90 min the absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

Determination of tannin contents: Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by Makkar *et al.*[29]. Briefly, 1 ml of extract dissolved in methanol (1 mg/ml), in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, left for 15 min at 4 °C and then centrifuged for 10 min at 3000 rpm using a Fischer Scientific centrifuge. In the clear supernatant, the non-tannin phenolics were determined the same way as the total phenolics. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Determination of flavonoids and flavonols: The flavonoids content was determined according to the method described by Kumaran and Karunakaran[30] with slight modifications[24]. This method is based on the formation of a flavonoid-aluminum complex, which absorbs maximally at 415 nm. Briefly, 100 l of

plant extracts in methanol (10 mg/ml) was mixed with 100 l of 20 % aluminium trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100 l of plant extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$$Flavonoid\ content = \frac{A \times m_o}{A_o \times m}$$

Where A is the absorption of plant extract solution, A_o is the absorption of standard rutin solution, m is the weight of plant extract, mg and m_o is the weight of rutin in the solution, mg. The flavonoid content is expressed in mg rutin equivalents/mg plant extract.

The content of flavonols was also determined as described by Kumaran and Karunakaran[30] with slight modifications. Briefly, 1 ml of each methanolic plant extracts (10 mg/ml) was mixed with 1 ml aluminium trichloride (20 mg/ ml) and 3 ml sodium acetate (50 mg/ ml). The absorbance at 440 nm was read after 2.5 h. The absorption of standard rutin solution (0.5 mg/ml) in methanol was also measured under the same conditions. The amount of flavonols in plant extracts in rutin equivalents (RE) was calculated by the same formula for flavonoids.

Determination of total anthocyanin contents: Monomeric anthocyanin contents of the plant extracts were measured using a spectrophotometric pH differential protocol described by Giusti and Wrolstad[31] with slight modifications. Monomeric anthocyanins undergo reversible structural transformation with a change in pH manifesting different absorbance spectra. The pH differentiation method is based on the difference between the coloured oxonium form (predominant at pH 1) and the colourless hemiketal form (pH 4.5) and permits accurate and rapid measurement of the total anthocyanin content. The plant extracts were mixed thoroughly with 0.025 M potassium chloride buffer (pH 1.0) in 1:8 ratio of extract to buffer. The mixtures were vortexed and allowed to stand for 15 min. The absorbance values of the mixtures were then measured at 515 and 700 nm against a distilled water blank. Similarly, the extracts were then combined with sodium acetate buffer (pH 4.5), mixed by vortexing, allowed to stand for 15 min and the absorbance values of the solutions were measured at the same wavelengths. The anthocyanin content was calculated as follows:

$$Total\ monomeric\ anthocyanin\ \left(\frac{mg}{100g}of\ dried\ sample\right) = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times C)}$$

where A is absorbance = $(A_{515} - A_{700})_{\text{pH 1.0}} - (A_{515} - A_{700})_{\text{pH 4.5}}$; MW is the molecular weight for cyanidin 3-glucoside = 449.2; εis the molar absorptivity of cyanidin 3-glucoside = 26 900; and C is the concentration of the buffer in mg/ml. Monomeric anthocyanin content was expressed as mg of cyanidin 3-glucoside equivalents per 100 g of dried plant extracts.

In vitro anti-oxidant assays

Qualitative DPPH radical-scavenging assay using thin-layer chromatography: Qualitative screening for anti-oxidant activity was done using the DPPH radical according to the method of Takao *et al.*[32]. Briefly a thin layer chromatogram of the extract on silica gel plates (Merck) was developed using methanol–ethyl acetate (50:50,v/v) as mobile phase. DPPH radical test was performed directly on thin

layer chromatography (TLC) plates by spraying with DPPH (0.2 % (w/v) in methanol to reveal the anti-oxidant activity of the extracts.

Quantitative DPPH radical-scavenging assay: Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by Gyamfi *et al.*[33] with slight modifications[24]. Briefly, a 2.0 ml solution of the extract at different concentrations diluted two-fold (2–250 µg/ml) in methanol was mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. L-ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with an Agilent 8453E UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\%\ Inhibition\ = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of the extract that caused 50 % inhibition of DPPH radical and was calculated by linear regression of plots, where the abscissa represented the concentration of tested sample and the ordinate the average percent of inhibitory activity from three replicates.

Superoxide radical ($O_2^{\cdot-}$)-scavenging assay: This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich,[34] and the method used by Martinez *et al.*[35] to determine superoxide dismutase with slight modifications. Briefly, each 3.0 ml reaction mixture contained 0.05 M phosphate buffered saline (PBS) (pH 7.8), 13 mM methionine, 2 M riboflavin, 100 M EDTA, NBT (75 M) and 1.0 ml of test sample solutions (10–250 g/ml). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 560 nm after 20 min. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes containing reaction mixtures were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was estimated by comparing the absorbance of the control and those of the reaction mixture containing test sample as per the equation:

$$\%\ Inhibition\ = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

Nitric oxide radical ($NO^{\cdot}$) scavenging assay: Nitric oxide ($NO^{\cdot}$) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.*[36] with slight modifications [24]. Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25 ^{o}C for 180 min in front of a visible polychromatic light source

(25 W tungsten lamp). The $NO^{\cdot}$ radical thus generated interacted with oxygen to produce the nitrite ion (NO_2^-) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1 % sulphanilamide in 5 % phosphoric acid and 0.1 % naphthylethylenediamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with

naphthylethylenediamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

RESULTS

Analyses for Secondary Metabolites (Phytochemicals)

Qualitative Analysis on Phytochemical Constituents: Qualitative analysis carried out on plant root extract showed the presence of important phytochemical constituents as summarized in Table I. Phenolic tannins and saponins were the major phytochemical constituents present in relatively high amount.

Table- I: Phytochemical constituents in plants

Phytochemical constituents (secondary metabolites)	Relative amount
Flavonoids	+
Tannins	++
Alkaloids	+
Steroids	+
Glycoside	+
Saponins	+++
Resin	+
Protein	+
Carbohydrates	+

+ = present in trace amount;++ = present in moderate amount;+++ = present in high amount

Quantitative Analysis on Phytochemical Constituents: Flavonoids and flavonlos compounds were a major class of bioactive components in the extract. The amount of total phenolics was 0.059 ± 0.020 to 1.165 ± 0.027 mg gallic acid equivalents (GAE)/mg of dry plant extract (Table II), non-tannins was 0.139±0.036, tannins 0.080±0.048 total flavonols, 0.70 ± 0.058, and total flavonoids 0.615± 0.008 mg quercetin equivalent (QUE)/g of dry plant extract.

Table II: Quantitative phytochemical constituent

Extract	Phenolic contents *			Total flavonols ‡	Total flavonoids ‡
	Total Phenols	Non-tannins	Tannins		
Napoleona imperialis	0.059 ± 0.020	0.139 ± 0.036	0.080 ± 0.048	0.70 ± 0.058	0.615± 0.008

Data represented as Mean ± SD (n = 3); * Expressed as mg gallic acid equivalents (GAE) / mg dry weight plant extract; ++ Expressed as mg quecertin equivalents (QUE)/g dry weight plant extract

Free Radical Scavenging Activity

Inhibitory Effect of Plant Extracts on DPPH Radicals: Extracts showed significant dose-dependent DPPH radical scavenging capacity. *N. imperialis* was most efficient at a concentration of 2500 g/ml, inhibiting 88.36 ± 4.72 % of DPPH radical compared to 250µg/ml ascorbic acid which inhibited 84.7 ± 10.43 %.

Inhibitory Effect of Extracts on Superoxide ($O_2^{\cdot-}$) anion radical: The plant extracts inhibited the formation of reduced NBT in a dose-related manner. As shown in Table IV,*N. imperialis* showed the maximal $O_2^{\cdot-}$ anion inhibitory activity of 46.24 ± 7.60E-01 % at the concentration of 2500 g/ml,compared to Quercetin (68.23 ± 0.41 %, at 250 g/ml). The $O_2^{\cdot-}$ scavenging effect of the extracts could culminate in the prevention of $^{\cdot}OH$ radical formation since $O_2^{\cdot-}$ and H_2O_2 are required for $^{\cdot}OH$ radical generation.

Table III: DPPH Radical scavenging activity of *N. imperialis*root extract

Extract Concentration (µg/mL)	Inhibition (%)	Ascorbate Concentration (µg/mL)	Inhibition (%)
10000	61.58±11.73	1000	89.10 ± 3.72
5000	79.84±10.55	500	88.88 ± 2.66
2500	88.36±4.72	250	84.68 ± 10.4
1250	83.43±8.22	125	80.74 ± 4.14
625	77.08±1.80	62.5	65.56 ± 10.9
312.5	66.50±4.04	31.25	59.76 ± 8.57
156.25	59.85±13.64	15.625	53.26 ± 10.6
78.125	39.06±16.88	7.8125	42.65 ± 1.92
39.0625	36.46±23.25	3.90625	38.66 ± 1.53
19.53125	22.65±1.04	1.953125	35.41 ± 4.30
IC_{50}	117.33		11.41

Data represented as mean ± SEM (n = 3)

Table IV: Superoxide anion radical scavenging activity of *N. imperialis*

Extract Concentration (µg/mL)	% Inhibition	Quercetin Concentration (µg/mL)	% Inhibition
5000	40.46±1.04	500	68.25 ± 4.77
2500	46.24±7.60	250	68.23 ± 0.41
1250	46.23±1.72	125	66.40 ± 4.41
625	42.37±3.99	62.5	60.31 ± 1.21
312.5	36.43±4.54	31.25	55.04 ± 4.32
156.25	33.65±3.80	15.625	52.89 ± 1.70
78.125	25.20±1.53	7.8125	47.03 ± 0.04
39.0625	16.13±6.13	3.90625	39.37 ± 2.89
IC50	1472.65		17.018

Data represented as mean ± SEM (n = 3)

IC_{50} for free radical inhibition: The concentration of the extracts that inhibited 50 % of the DPPH and Superoxide anion radicals (IC_{50}) was used to determine the potency of the extracts. The lower the IC_{50} value the better the drug (extract) potency. As shown in Table V below, the plant extracts were not efficient inhibitors of different free radicals compared to standard anti-oxidants. The IC_{50} values for DPPH radical inhibition was 117.33 g/ml compared to ascorbate which was 11.41 g/ml; while superoxide anion radical inhibition was 1472.65 g/ml compared to quecertin which was 17.018.

Effect of extracts on nitric oxide ($NO^{\cdot}$) radical production: Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong Oxidizing character which can alter the structure and function of many cellular components. This study showed that the phenol rich extacts in SNP solution decreased levels of nitrite, a stable oxidation product of $NO^{\cdot}$ Liberated from SNP (Fig. 2). The extracts exhibited strong $NO^{\cdot}$ Radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The $NO^{\cdot}$ Scavenging capacity was concentration dependent with 250 µg/ml of the extracts scavenging most efficiently compared to α-tocopherol (Figure I)

Table V: Free radical and lipid peroxidation inhibitory potency (IC_{50})

	IC_{50} value for inhibitory potential		
Extract	DPPH radical(g/ml)		Superoxide anion($O_2^{\cdot-}$) (g/ml)
N.imperialis	117.33		1472.65
Ascorbate Quercetin	11.41 ----		---- 17.018

Data represented as mean ± SEM (n = 3); * compared to ascorbic acid; β compared to quecertin.

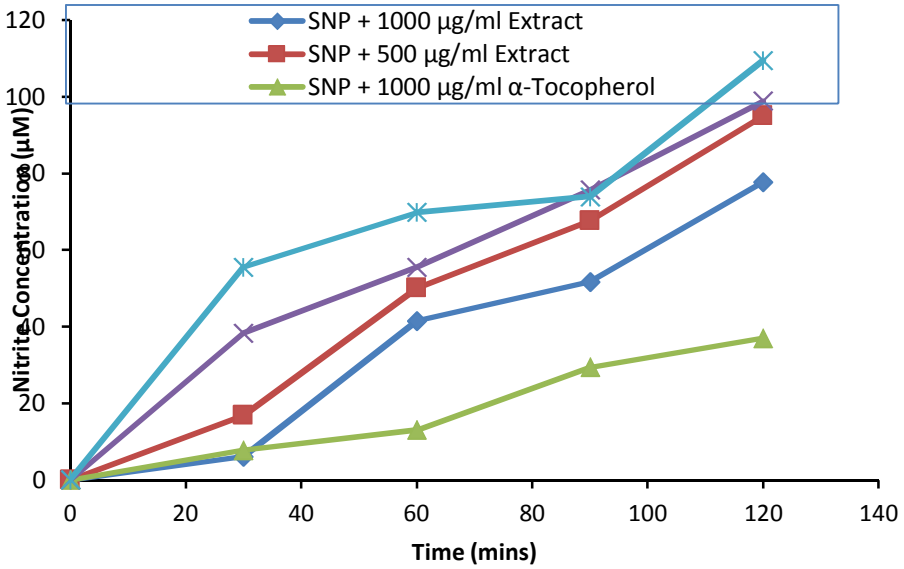

Figure I: Nitric Oxide ($NO^{\cdot}$) Radical scavenging activity of *N.imperialis* root extract.

DISCUSSION

Phytochemicals are chemicals derived from plants, these plant produce secondary metabolites in order to prevent themselves from insect attack and plant disease which in turn provides protective role or mechanism for human when consume in the case of oxidative stress cause by free radicals.

Phenolic content from plant extracts have been found to correlate with radical scavenging activity[37,38]. This is because polyphenolics have high redox potentials which allow them acts as reducing agents, hydrogen donors and singlet oxygen quenchers [39]

Phenolic compounds, flavonoids and flavonols which are known to possess good medicinal values[40], were assayed for in this extract. These phyto-chemicals have a lot of pharmacological properties as proved by earlier studies[41]. The observed presence of tannins could be of great medicinal importance since tannins serve as a good antioxidant [42]. Therefore *N.imperialis* root extracts are good source of antioxidants, which are widely believed to be an important line defense against oxidative stress leading to a lot of diseases like insomnia, diabetes etc.

Phenolic tannins and saponins were the major phytochemical constituents present in relatively high amount from the result as summarized in table I. Flavonoid and flavonol compounds were a major class of bioactive components in the extract. The amount of total phenolics was 0.059 ± 0.020 to 1.165 ± 0.027 mg gallic acid equivalents (GAE)/mg of dry plant extract (Table 2), non-tannins was 0.139±0.036, tannins 0.080±0.048 total flavonols, 0.70 ± 0.058, and total flavonoids 0.615± 0.008 mg quercetin equivalent (QUE)/g of dry plant extract.

The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in vitro* antioxidant activity of pure compounds as well as plant extracts [43, 44]. This is an extreme configuration and radicals quickly react with other molecules or radicals to achieve the stable configuration of four pairs of electrons in their outermost shells [45]. DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers[46, 47]. The extract maximal DPPH free radical inhibition was found to be 88.36 ±4.72 at a concentration of 2500µg/ml compared to 250µg/ml of standard ascorbic acid as shown in table III .The maximal superoxide anion inhibition was found to be 46.24±7.60E-0.1 at a concentration of 2500µg/ml compared to250µg/ml quecertin. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. All the fractions showed significantly higher inhibition percentage (stronger hydrogen –donating ability) and positively correlated with total phenolic content.

The IC_{50} for DPPH free radical inhibition was 117.33µg/ml compared to ascorbate 11.41.the IC50 0f superoxide anion radical inhibition was 1472.65µg/ml compared to quecertin 17.018µg/ml. the IC50 is the amount of extract capable of inhibiting 50% of the free radical, the lower the IC50 the better the potency of the extract.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [48].Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong Oxidizing character which can alter

the structure and function of many cellular components. The extracts exhibited strong $NO^{\cdot}$ radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The $NO^{\cdot}$ Scavenging capacity was concentration dependent with 250 µg/ml of the extracts scavenging most efficiently compared to α-tocopherol. The ability of the extract to quench NO could be highly useful in preventing the formation of the very harmful peroxynitrite between superoxide anion and nitric oxide and these could go a long way to suppress oxidative stress implicated by most diseases.

CONCLUSION

The hydromethanolic root extract of *Napoleona imperialis* exhibited significant antioxidant activity compared to ascorbic acid and the activity owing to the significant presence of bioactive secondary metabolites. These result therefore justify that the wound healing potential and antihypertensive effect of the plant extract may be associated with its free radical scavenging potential, hence its ethno pharmacological affect mechanism which may indirectly be by stimulating the patient's immune system.

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