PharmacologyOnLine April 30, 2016

Archives • 2016 • vol.1 • 109-119

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FINGERPRINTING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOL LEAF EXTRACT OF *NAPOLEONA VOGELII* (LECYTHIDACEAE)

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Abstract

The use of plants for medicinal purposes has gained widespread acceptance over the years. The therapeutic activities of medicinal plants so used are not unconnected with their chemical composition. In the present study High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) was used to quantify the flavonoids, phenolic acids and carotenoids present in the methanol leaf extract of *Napoleona vogelii* (lecythidaceae) (MNV). Phytochemical composition of the extract was evaluated. Standard protocols were also used to estimate the total phenols and total flavonoids content of the extract. Nitric oxide (NO), hydroxyl radical (OH^-), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and 2,2-diphenyl -1-picrylhydrazyl (DPPH) radicals scavenging activity, inhibition of lipid peroxidation and the ability of MNV to chelate ferrous ions as well its reductive potential were also evaluated. The presence of flavonoids, saponins, cardiac glycosides and terpenoids were confirmed in the extract. Our results show that *Napoleona vogelii* has excellent $ABTS^{\cdot+}$ and DPPH radicals scavenging activity. The extract was found to compare favourably with ascorbic acid in the inhibition of lipid peroxidation. We also observed that the extract chelated ferrous ions and inhibited NO to an appreciable level, while showing a moderate reducing power and inhibition of OH^-. The inhibitory concentration (IC_{50}) values for the various radical scavenging assays were DPPH - 187.10 µg/ml, NO - 145.05 µg/ml, OH^- - 243.90 µg/ml and ABTS – 115.63 µg/ml. The HPLC-DAD analysis showed significant levels of flavonoids, phenolic acids and carotenoids. From the foregoing, we conclude that methanol extract of *Napoleona vogelii* could be considered relevant in the treatment modalities for a wide array of diseases because of the presence of several biologically active compounds which might be responsible for the observed antioxidant activities.

Keywords: Flavonoids, phytochemical, peroxidation, hydroxyl radical, analysis.

Introduction

Free radicals are highly reactive molecules or chemical species capable of independent existence. Generation of highly reactive oxygen species (ROS) is an integral feature of normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation, and fertilization [1]. The release of oxygen free radicals has also been reported during the recovery phases from many pathological noxious stimuli to the cerebral tissues [2]. Their production however, multiplies several folds during pathological conditions. Damage due to free radicals caused by ROS leads to several damaging effects as they can attack lipids, protein/ enzymes, carbohydrates, and DNA in cells and tissues. They induce undesirable oxidation, causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation [1]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [3]. The theory of oxygen-free radicals has been known about fifty years ago [4]. However, only within the last two decades, has there been an explosive discovery of their roles in the development of diseases, and also of the health protective effects of antioxidants [3]. Antioxidants have been known to play protective role in human body against deleterious effects of reactive free radicals and it has been defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [5]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants [6]. The use of plants as sources of remedies for the treatment of many diseases dates back to pre-history and people of all continents have this old tradition [7]. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known [8]. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties [9]. *Napoleona vogelii* Hook is one of the most potent species of lecythidaceae that is richly endowed with pigments. The fruits are green when unripe and reddish-orange when ripe. It was reported that extracts fron *Napoleona vogelii* could be used as an excellent source of acid-base indicators [10]. Francis *et al* (1966) defined it as a genus of about 125 species of trees native to the tropical rainforest of Africa, and are classified in the family of lecythidaceae, and subfamily of Vogelii Hook [11]. The plant is found mostly in rain forest and along the sea shores, extending from Sierra-Leone to Nigeria [12]. *Napoleona vogelii* leaves showed positive results for the treatment of cough and asthma [13]. The analgesic property of methanol and n-hexane leaf extracts of *Napoleona vogelii* were investigated using ethanol, indomethacin and hypothermic restraint-stress ulcer models [14]. The present study was to determine the phytochemical composition, i*n vitro* antioxidant activity and HPLC analysis of the methanol extract of *Napoleona vogelii.*

Materials and methods

Acetonitrile, formic acid, gallic acid, chlorogenic acid, ellagic acid and caffeic acid purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, isoquercitrin, rutin, catechin, e picatechin, kaempferol, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, ascorbic acid and Folin-Ciocalteau reagent were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software. All other chemicals and reagents used were of analytical grade.

Plant material and extraction

Napoleona vogelii leaves were collected from a farmland in Iseyin, Oyo-state in South-Western Nigeria. The leaves were identified at Botany Department, University of Ibadan Nigeria. The leaves were air-dried for three weeks and pulverized. 700g of the pulverized sample was extracted in 80% methanol by maceration for 72 hours. The methanolic extract was concentrated in a rotary evaporator, lyophilized and preserved for further use.

Determination of total phenolic content

The total phenolic content of the extract was determined according to the method described by [15]. In this assay, the phenolic group present in plant extract interacts with Folin – Ciocalteau in

alkaline medium using Na_2CO_3 solution giving a blue colour, which has maximum absorption at 685nm and correlates with total phenolic content. 0.1ml of methanol extract of the plant of different concentrations was rapidly mixed with 0.1ml of Folin Ciocalteu reagent, followed by the addition of 0.3ml sodium carbonate (15% w/v) solution. The mixture was incubated in the dark for 30mins.The absorbance of the blue colour was read at 760 nm after 30mins on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using tannic acid (graded doses, 50 – 250ug/ml) as a standard phenol. Total phenol content of the extract was expressed as tannic acid equivalents (TAE) after calculation using the following equation: C= (cV)/m, where, C =total phenol contents, mg/g plant extract in TAE, c = concentration of tannic acid obtained from calibration curve (mg/ml), V =the volume of the sample solution (ml), m= weight of the sample (g). All tests were conducted in triplicate.

Determination of total flavonoid

Total flavonoid of the leaf extract was determined using the method described by Kumaran and Karunakaran [16] with slight modifications. Briefly, 0.5 ml of extract solution (1 mg/ml) and standard (quercetin) at different concentrations were taken in test tubes. 3.0 ml of methanol followed by 0.1 ml of 10% aluminum chloride solution was added into the test tubes. 200ml of 1M potassium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 2.8 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink colored solution was noted at 415 nm using a spectrophotometer against blank methanol. Total flavonoid content of the extract was expressed as quercetin equivalents (QE) after calculation using the following equation:

$$C= (cV)/m,$$

where C = total flavonoid content, mg/g plant extract in QE, c = concentration of quercetin obtained from calibration curve (mg/ml), V =the volume of the sample solution (ml), m= weight of the sample (g). All tests were conducted in triplicate.

Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) packed with 5µm diameter particles. The mobile phase contained 1% formic acid (A) and acetonitrile (B), and the composition gradient was: 13% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively [17] with slight modifications. *Napoleona vogelii* (leaves) methanolic extract was analyzed dissolved in ethanol at a concentration of 20 mg/ml. The presence of eleven antioxidants compounds was investigated, namely, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, epicatechin, quercetin, quercitrin, isoquercitrin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 400 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve [18].

DPPH (1, 1, Diphenyl 2-Picryl Hydrazyl) assay

The antioxidant activity by DPPH assay was assessed according to Molyneux method [19]. To 1 ml of various concentration of the extract 1 ml of DPPH 0.1mM was added in a test tube. Tannic acid was used as the standard for comparison. After incubation for 30 mins in dark at room temperature, absorbance was recorded at 517 nm. The percent DPPH radical scavenging was calculated with the equation:

% DPPH radical scavenging = [(Absorbance control – Absorbance sample)/ Absorbance control] × 100.

ABTS Radical scavenging activity

A TEAC assay was conducted based on the method of Ramos *et al* [20]. The ABTS aqueous solution (7 mM) was oxidized with potassium peroxodisulfate (2.45 mM) for 16 hours in the dark at room temperature. The $ABTS^{\cdot+}$ solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm. An aliquot (20 µL) of various concentrations of the extract was mixed with 180 µL $ABTS^{\cdot+}$ solution and the

absorbance was read at 734 nm after 1 min. Trolox was used as a reference standard. The percent $ABTS^{\cdot+}$ radical scavenging was calculated with the equation:

% $ABTS^{\cdot+}$ radical scavenging = [(Absorbance control – Absorbance sample)/ Absorbance control] × 100.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was evaluated according to the modified method of Sreejayan and Rao, [21]. Various concentrations of extract were prepared. Sodium nitroprusside (2.5 mL, 10 mM) in phosphate buffered saline (PBS) was added to 0.5 ml different concentrations of extracts. The reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml aliquot was removed and 0.5 ml of Griess reagent: (1% (w/v) sulfanilamide, 2% (v/v) H_3PO_4 and 0.1% (w/v) naphthylethylene diamine hydrochloride) was added. The absorbance was measured at 546 nm. Ascorbic acid was used as reference standard and was treated the same way as that of fractions. Sodium nitroprusside in PBS (2 ml) was used as control. The nitric oxide radicals scavenging activity of the extracts and ascorbic acid was calculated according to the following equation:

Percentage of inhibition = $[(A_0 – A_1) / A_0] \times 100$

where A_0 is the absorbance of sodium nitroprusside in PBS and A1 is the absorbance in the presence of the extracts and ascorbic acid.

Iron chelating ability

The in vitro Fe^{2+} chelating ability of plant extract was assayed according to the method of Minnoti and Aust with slight modification [22]. Briefly, 900 µl of aqueous $FeSO_4$ (500 µM) and 150µl of extract were incubated for 5min at room temperature, 78 µl of 1,10- phenanthroline (0.25 % w/v , aqueous) was added. The absorbance of the orange colour solution was read at 510nm with a spectrophotometer. The principle of the assay is based on distruption of o-phenanthroline - Fe^{2+} complex in the presence of chelating agent. Ethylenedaiamine tetraacetic acid (EDTA) was used as the standard. The in vitro Fe^{2+} chelating ability of the sample was calculated by using the following formula:

Chelating ability(%) = $(A_{control} - A_{sample})/ A_{control} \times 100$.

Where;

$A_{control}$ = The absorbance of the control (reaction mixture in the absence of sample) ie $FeSO_4$ alone.

A_{sample} = The absorbance of the reaction mixture (sample, $FeSO_4$ and 1,10-phenanthroline)

Reductive potential

Reducing power of the extracts were measured by the direct reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ and was determined by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+} [23]. Different concentrations of extracts in 0.5ml of distilled water were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) the mixture was incubated at 50 °C for 20 min. After 20 min incubation, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%). Finally, 0.5 ml of $FeCl_3$ (0.1%) was added to this solution and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates greater reduction capability.

Hydroxyl radical scavenging activity

The hydroxyl radical ($^{\cdot}OH$) scavenging activity was measured by the method of Jin *et al* [24]. The hydroxyl radical was generated in a mixture of 1.0 ml of 0.75mM 1,10-phenanthroline, 2.0 ml of 0.2M sodium phosphate buffer (pH 7.4), 1.0 ml of 0.75 mM $FeSO_4$ and 1.0 ml of H_2O_2 (0.01%, v/v). After addition of 1.0 ml sample solution, the mixture was incubated at 37°C for 30 min. Then, the absorbance of the mixture at 536 nm was measured. Deionized water and VC were used as the blank and positive control respectively. The scavenging activity on $^{\cdot}OH$ was calculated by the following equation:

Scavenging activity (%) $= (Abs_{sample} – Abs_{blank})/ (Abs0 – Abs_{blank}) *100$

where Abs0 is the absorbance of the deionized water instead of H_2O_2 and sample in the assay system.

In-vitro inhibition of lipid peroxidation

In vitro inhibition of lipid peroxidation was estimated according to the method of Ruberto and Baratta [25]. In this assay, egg yolk homogenate served as lipid rich medium, and $FeS0_4$ acts as initiator of lipid peroxidation .Briefly, 50µl of plant extract was mixed with 0.25ml 10% egg yolk .This was followed by the addition of 10µl FeS04 (0.07M aqueous).The mixture was incubated at room temperature for 30min.This was followed by the addition of 0.75ml of glacial acetic acid (5% v/v aqueous) and 0.75ml of thiobarbituric acid 0.8% in Sodium dodecylsulphate

(SDS) 1.1%. The mixture was incubated in a water bath (90^0C) for 60 min, cooled and centrifuged at 3000rpm. 1ml of the pink colour supernatant was read at 532 nm on a spectrophotometer.
In vitro inhibition of lipid peroxidation =

[(Acontol – Asample)/Acontrol] X 100

Statistical analysis
Results are expressed as mean± standard deviation. Tukey test at $p < 0.05$ and independent sample t-test were used for data analysis.

Discussion

Several methods have been developed to appraise the antioxidant capacity and the ability of plants to scavenge free radicals. Phytochemical analysis is one of the preliminary routine means of evaluating the medicinal relevance of plants. The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretory products in it. These usually consist of the phenolic compounds like alkaloids, tannins, saponins, carbohydrates, glycosid es, flavonoids, steroids etc [26]. In the present study as shown in table 1, MNV tested positive for the presence of flavonoids, terpenoids, saponins and cardiac glycosides. This is a confirmation of an earlier work [27]. It is generally believed that there is a direct correlation between total phenol content and antioxidant activity [28]. Phenolics have the capacity to adsorb and neutralize the free radicals generated during oxidative stress [29]. The flavonoids which are widespread in plants, naturally contributes to the free radical scavenging activity together with other phenolics [30]. In our results in table 2 the total phenol and total flavonoid contents of MNV were shown to be 55.88 mg/gTAE and 13.10 mg/gQE respectively.
HPLC is a chromatographic technique which is used to separate, identify, quantify and purify individual components of a mixture. HPLC fingerprinting has become an important quality control tool for herbal samples. It provides quantitative and qualitative information to researchers as well as enables the screening of samples for the presence of new compounds [31]. In our results presented in tables 3 and 4, the various phenolic acids, flavonoids and carotenoids present in the extract were identified and their relative quantities estimated. The phenolic acids identified are gallic acid, chlorogenic acid, caffeic acid, and ellagic acid and their respective quantities are 4.39 mg/g, 11.57 mg/g, 10.96 mg/g, and 27.41 mg/g. The flavonoids and their relative quantities are catechin-4.85 mg/g, rutin-25,09 mg/g, quercetin-18.63 mg/g, quercitrin-4.15 mg/g and kaempferol-21.78 mg/g. Other compounds present and their relative quantities are tocopherol-0.68 mg/g, β-carotene-1.83 mg/g, and lycopene-0.57 mg/g. Shahidi (2000) proposed that antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acid, peptides, proteins, flavonoids and other phenolic compounds might play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage [32]. MNV may therefore be considered useful in preventing/ameliorating oxidative damage which is a key feature of many chronic diseases. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity [33]. The transformation of Fe^{3+} to Fe^{2+} in the presence of either the extract or the standard (ascorbic acid) is a measure of reducing capability [34]. Increased absorbance of the reaction mixture correlates with greater reducing power. Our result in fig 1 showed that MNV has a moderate reducing power with an absorbance of 0.902 at 400 µg/ml while quercetin has an absorbance of 1.796 at the same concentration. The reducing power of the extract however increased in a concentration dependent manner. The reducing power of a compound is mainly due to the availability of hydrogen atoms that could be donated to a free radical and convert it to a more stable product. Through this, the chain reaction initiated by the free radical could be effectively terminated. Iron is an important transition metal necessary for several life processes including respiration, oxygen transport etc. It occurs naturally in either ferric (Fe^{3+}) or ferrous (Fe^{2+}) form. Despite its importance in biological systems, iron is the most important lipid oxidation pro-oxidant among transition metals due to its high reactivity [35, 36]. Though both forms of iron are involved in formation of free radicals, ferric ion is tenfold less active than the ferrous form [37]. Therefore compounds that could chelate Fe^{2+} would be important in counteracting the generation of free radicals and oxidative damage of biological molecules arising from the progression of Fenton reaction in which Fe^{2+} is an important mediator. The iron chelating activity of MNV is presented in fig 2. Our result shows that MNV has an appreciable iron chelating activity when compared with ethylenediamine tetraacetic acid (EDTA). At the highest concentration tested (400 µg/ml) MNV has a percentage iron chelating activity of 41.55% while EDTA has a percentage iron chelating activity of

60.52%. In the same vein, the IC_{50} values for extract and standard were 287.76 µg/ml and 153.85 µg/ml respectively. DPPH is a stable free radical, the reduction of which is routinely used to screen medicinal plants for their radical scavenging activity. The reduction of DPPH demonstrated by its decolourization from its characteristic violet colour in ethanol solution to yellow colour is an indication of the presence of phenolic compounds, and the degree of the reduction is suggestive of the antioxidant capacity of such compounds. The DPPH radical scavenging activity of MNV is presented in fig 3. MNV compared favourably with tannic acid in a concentration dependent manner in the DPPH radical scavenging assay. MNV and tannic acid showed percentage DPPH radical scavenging activity of 78.81% and 93.53% respectively. The IC_{50} values for MNV was 187.10 µg/ml while that of tannic acid was 139.45 µg/ml. The ABTS assay is a decolourization assay and the extent of decolourization is a hint to the radical scavenging activity of an antioxidant [38]. Our result presented in fig 4 shows that MNV has a better ABTS radical scavenging activity than trolox which was the standard scavenger. At the highest concentration tested MNV showed a percentage radical scavenging activity of 87.99% while trolox showed 83.18% at the same concentration. We also observed that MNV had an IC_{50} of 115.63 µg/ml compared with trolox whose IC_{50} was 125.52 µg/ml. Nitric oxide is an important cell mediator that regulates a number of functions in biological systems. It is known to have vasodilatory, anti-inflammatory, anti-proliferative and cardiovascular activities. Despite these, excess levels of NO can produce harmful effect in organisms because it is a reactive oxygen specie [39]. In the present study as shown in figure 5, MNV and ascorbic acid showed percentage nitric oxide scavenging activies of 55.42% and 88.27% respectively at the highest concentration tested. Our result also showed that MNV had an IC_{50} value of 145 µg/ml whereas the IC_{50} for ascorbic acid which was used as standard was 115.21 µg/ml. Hydroxyl radicals are reactive oxygen species that initiate peroxidation of lipid membranes [40]. $^{\bullet}OH$ is one of the most damaging free radicals in the body and can be important mediator of damage to cell structures, nucleic acids, lipids and proteins [4]. The present study revealed that MNV had a moderate hydroxyl radical scavenging activity. Our result in fig 6 shows that at 400 µg/ml the percentage inhibition of hydroxyl radical by both MNV and mannitol are 40.84% and 80.56% respectively. IC_{50} values for extract and standard were 243.90 µg/ml and 195.39 µg/ml respectively. Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generate a number of degradation products. Malondialdehyde, one of the products of lipid peroxidation has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress [41]. From our result presented in fig 7, it was observed that MNV compared favourably in a concentration dependent manner with ascorbic acid in the inhibition of lipid peroxidation assay. At 400 µg/ml, MNV and ascorbic acid showed percentage inhibition of lipid peroxidation of 79.96% and 85.42% respectively.

Conclusion

Our study established that methanol extract of Napoleona vogelii contains various flavonoids and phenolic acids in appreciable quantities. It was also observed that the extract possesses concentration dependent scavenging activities which ranged from moderate to excellent against the radicals used in the study. It is therefore apposite to conduct further in vivo research on the extract. This will help to delineate the mechanisms of action of the extract in living systems, and also the specific oxidative damage associated diseases for which it could be safely applied.

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Table 1. Phytochemical screening of *Napoleona vogelii* leaves

Phytochemical	**Presence**
Saponins	+ve
Tannins	-ve
Flavonoids	+ve
Alkaloids	-ve
Terpenoids	+ve
Anthraquinones	-ve
Cardiac glycosides	+ve
Steroids	-ve

Table 2. Total phenol and total flavonoids content of methanol extract of *Napoleona vogelii* leaves

Total Phenol	55.88 mg/gTAE
Total Flavonoids	13.10 mg/gQE

Table 3. Phenolic acids and flavonoid composition of *Napoleona vogelli* (leaves) methanolic extract.

Compounds	**mg/g**	**%**
Gallic acid	4.39 ± 0.01[a]	0.43
Catechin	4.85 ± 0.02[a]	0.48
Chlorogenic acid	11.57 ± 0.01[b]	1.15
Caffeic acid	10.96 ± 0.03[b]	1.09
Ellagic acid	27.41 ± 0.01[d]	2.74
Rutin	25.09 ± 0.03[d]	2.50
Quercitrin	4.15 ± 0.02[a]	0.41
Quercetin	18.63 ± 0.01[c]	1.86
Kaempferol	21.78 ± 0.01[c]	2.17

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$

Table 4. Carotenoids compositions of *Napoleona vogelli* (leaves) methanolic extract.

Carotenoids	***N. vogelii***
	mg/g
Tocopherol	0.68 ± 0.01[a]
β-Carotene	1.83 ± 0.01[b]
Lycopene	0.57 ± 0.02[a]

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$

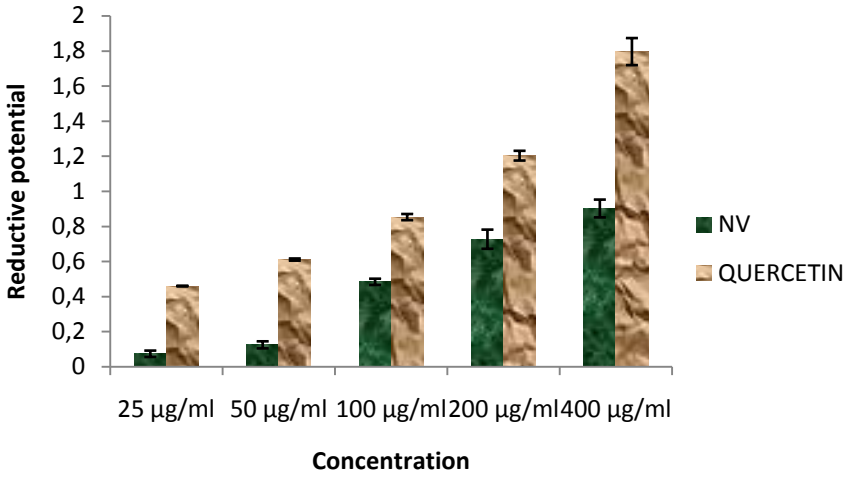

Figure 1. Reductive potential of methanolic leaf extract of MNV

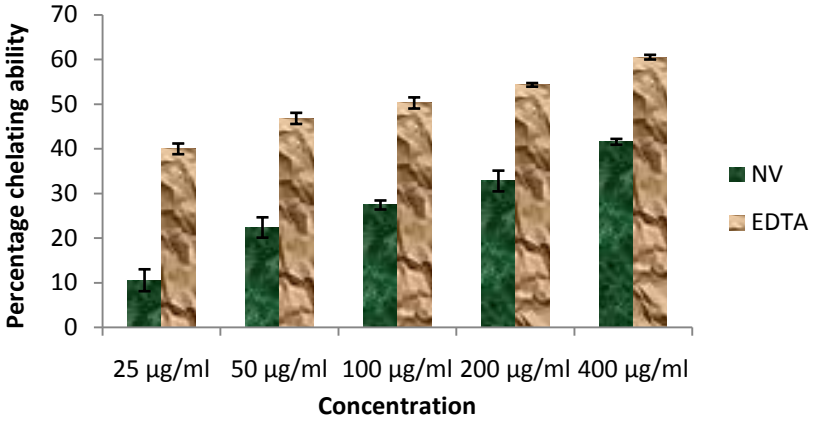

Figure 2. Percentage iron chelating activity of methanolic leaf extract of MNV

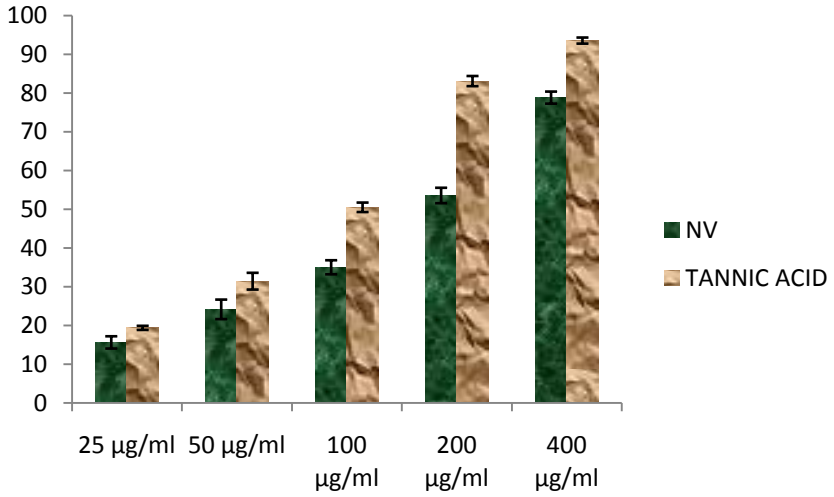

Figure 3. Percentage DPPH radical inhibition of methanolic leaf extract of MNV

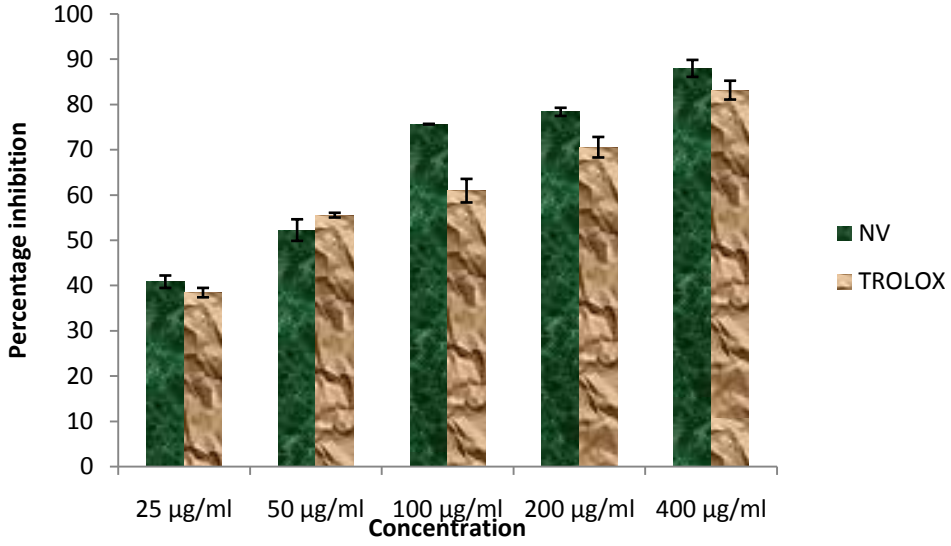

Figure 4. Percentage ABTS radical inhibition of methanolic leaf extract of MNV

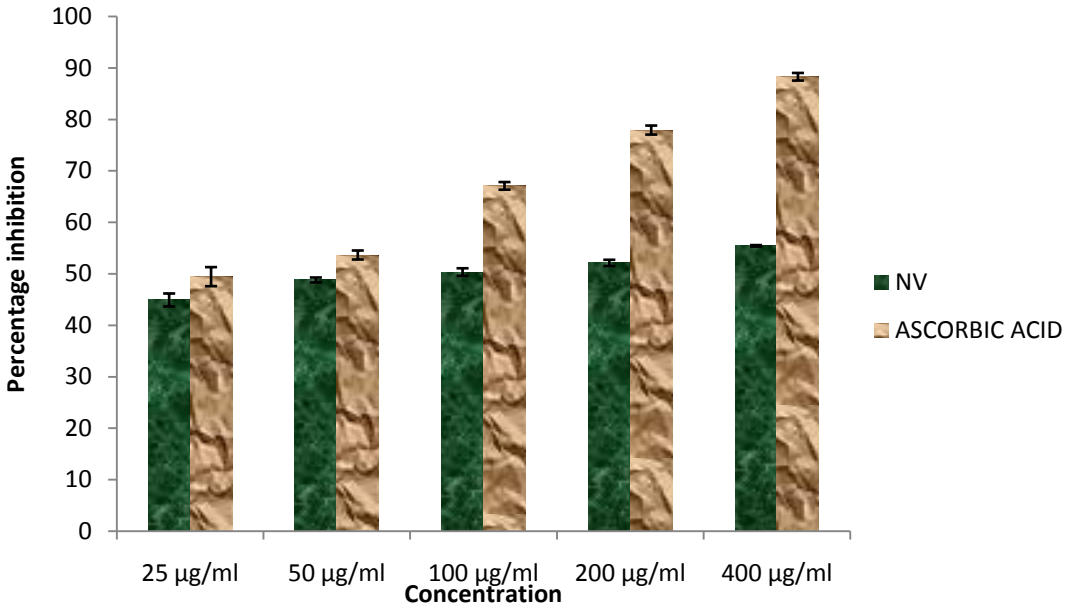

Figure 5. Percentage nitric oxide scavenging activity of methanolic leaf extract of MNV

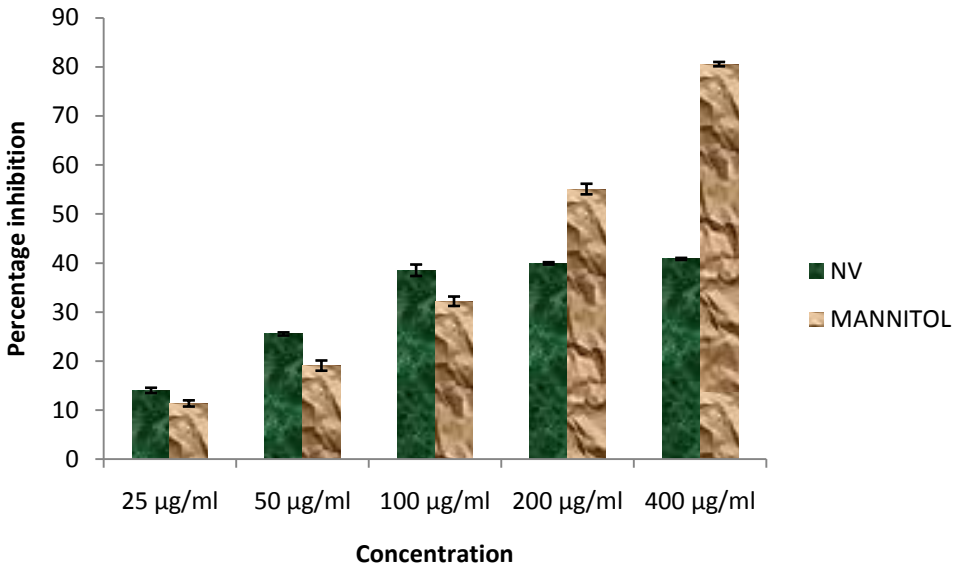

Figure 6. Percentage hydroxyl radical scavenging activity of methanolic leaf extract of MNV

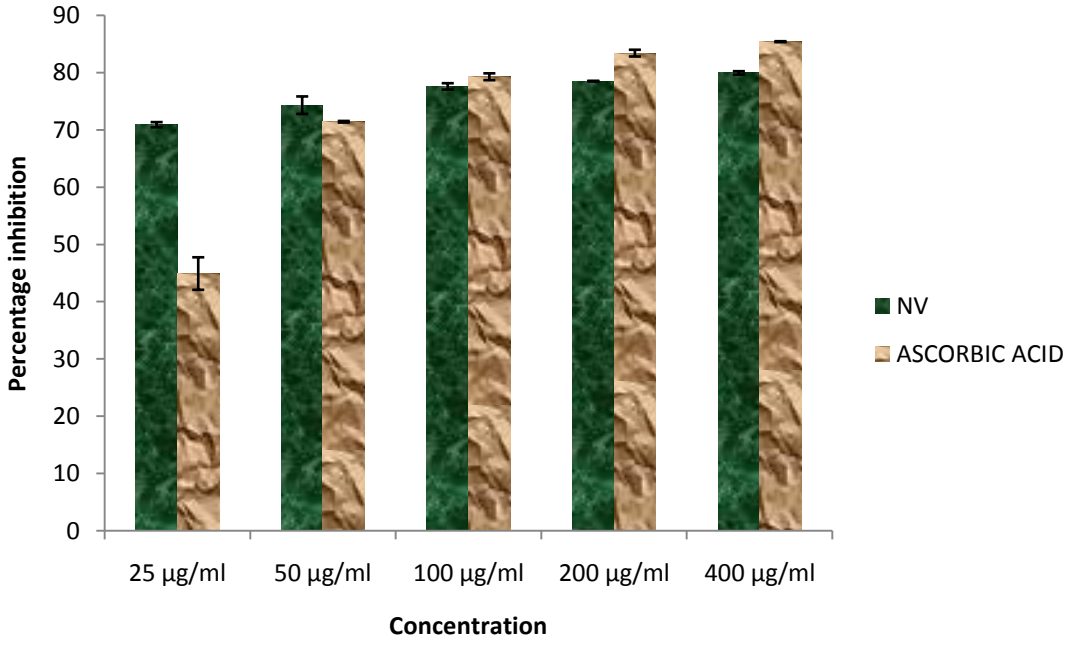

Figure 7. Percentage *in-vitro* inhibition of lipid peroxidation activity of methanolic leaf extract of MNV