Anti-oxidant activity of methanolic extract of aquatic flowering plant *Nymphaea capensis* leaf

Mohammad Alam (nazmulalam.pharm@gmail.com)
North South University and International Islamic University Chittagong
Md. Shahrear Biozid
North South University and International Islamic University Chittagong
Md. Faruk
North South University and International Islamic University Chittagong
Md. Jainul Abeden
International Islamic University Chittagong
Khandoker Usran Ferdous
North South University
Iffat Ara Nitul
North South University
Md. Rafikul Islam
International Islamic University Chittagong

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Abstract

Background: *Nymphaea capensis* is an aquatic flowering plant which is included in the family of Nymphaeaceae. Literature review on the plants of Nymphaeaceae family exhibited significant medicinal activities. Therefore, the objective of the present study is to evaluate possible anti-oxidant activity of crude methanol extract of *N. capensis* leaf.

Method: In antioxidant study, methanolic plant extract was evaluated for 1,1-diphenyl,2-picrylhydrazyl (DPPH) and reducing power capacity. Moreover, total phenolic and total flavonoid content of plant extracts were determined and expressed in mg of gallic acid equivalent per gram of dry sample (mg GAE/g dry weight).

Result: In the DPPH free radical scavenging assay, methanol extract showed concentration dependent inhibition of the free radicals. IC_{50} of Ascorbic acid was 14.84 µg/ml whereas *N. capensis* was 130.94 µg/ml. In case of reducing capacity, at conc. 62.5, 125, 250, 500, 1000 ug/ml, the absorbances of Ascorbic acid were 0.65, 1.12, 1.45, 1.78 and 1.89 respectively. In case of *N. capensis*, the absorbances were 0.46, 0.75, 1.04, 1.27 and 1.50 respectively. The extract displayed a concentration dependent increase in reducing power. In the case of total phenolic content, the methanol extract of *N. capensis* contained a considerable amount of phenolic contents which was 215±7 of GAE mg/g. In the case of total flavonoid content, methanol extract of *N. capensis* contained significant amount of flavonoid contents which was 184.75±6.78 of GAE mg/g.

Conclusion: These results suggested that the methanol extract of *N. capensis* possess considerable anti-oxidant activity.

Introduction

Nymphaea capensis is an aquatic flowering plant which family is Nymphaeaceae. It occurs in freshwater areas in temperate and tropical climates throughout the world. In Bangladesh, *N. capensis* is commonly known as Nil Sapla or Blue water-lily. It commonly found in lakes, ponds, canals and swamps of Bangladesh as well as other parts of the world such as Africa, Asia Australia and Northern America [1, 2]. In Madagascar, the rootstocks of *N. capensis* are eaten as food and supposedly are delicious in taste. In the Nymphaeaceae family, other plants such as the boiled or raw inner rhizomes of *N. jamesoniana* were eaten as vegetables in Argentina. In Asia and Africa, the rhizomes and tubers of N. lotus were also eaten as fruit which sweet in tastes. *N. odorata* leaves were consumed by North Americans which contains good amount of tannins, 40% crude protein. *N. tetragona* leaf buds and seeds are consumed as vegetables in Japan [2].

N. capensis also has good medicinal properties. The methanol extract of *N. capensis* leaves possess significant sedative and anxiolytic activities as well as mild cytotoxic activities on mice [3]. In the case of Nymphaeaceae family, other plants also has great medicinal properties and various types of compounds were isolated from them. Such as *N. jamesoniana* has been used as an astringent, *N. lotus* has been

consumed as a traditional medicine which produce analgesic, anti-inflammatory, astringent, and sedative effect. It also exhibited significant antibiotic and antioxidant effect. *N. Mexicana* also exhibited antioxidant, DNA protective properties and antiproliferative activity. *N. ampla* flowers and other parts possess narcotic properties, which contain aporphine and quinolizidine alkaloids [2, 4]. According to this traditional consumption and laboratory experiments, Nymphaeaceae family exhibited great medicinal properties. Therefore, this literature review on Nymphaeaceae family made us curious to find out the anti-oxidative properties of methanolic extract of *N. capensis* leaves.

Nowadays people are prone to inflammation due to imbalance lifestyle and diet which later turn into chronic inflammation. This inflammation occurs mainly because of reactive oxygen species (ROS). Through autooxidation and lipid peroxidation inflammatory mediators such as cytokines, interleukins, chemokines, bioactive amines, bradykinin, and inflammatory lipids produce reactive oxygen species (ROS) [5]. Reactive oxygen species (ROS) are superoxide anion (O_2•-), hydroxyl radicals (OH•), singlet oxygen ($1O_2$) and hydrogen peroxide (H_2O_2) which causes great harm to the cells and are also responsible for various diseases such as cardiovascular diseases, ischemia, diabetes, inflammation, cancer, anemia, ageing and degenerative diseases [6,7]. Antioxidants plays pivotal role to neutralize the free radicals. Lots of drugs were isolated from natural sources such as anticancer drugs in clinical trials were also isolated from plant sources which contains significant amount of antioxidant activity [8, 9]. Henceforth, our experiment was designed to investigate the anti-oxidant activity of methanol extract of *Nymphaea capensis* leaves.

Materials And Methods

Extract preparation

The leaves were sun dried and ground. The ground (300 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Chemicals and drugs

DPPH (CAS 1898–66–4 Sigma Aldrich CHEMIE GmbH USA), Methanol was bought from SIGMAW (Sigma-AldrichW, St Louis, USA),Sodium carbonate (Na2CO3), ferric chloride (FeCl3),Aluminium chloride, Potassium acetate potassium ferricyanide [K_3Fe(CN)6], trichloroacetic acid (TCA), buffer and ascorbic acid were purchased from Merck (Darmstadt, Germany).Gallic acid (CAS 149–91–7 GmbH USA) and Folin Ciocalteu reagent (FCR) was purchased from Merck Co. (Germany). All chemicals in this investigation were of analytical reagent grade.

Antioxidant Activity

Antioxidant activity of *N. capensis* is determined by DPPH radical scavenging assay, reducing power capacity assay, total phenolic content and total flavonoid content.

DPPH radical scavenging assay

Free radical scavenging activity of methanolic extract of *N. capensis* leaf was determined according to the Brand-Willium [10] method with a slight modification. This activity was determined spectrophotometrically by taking absorbance of DPPH at 517 nm. Then the % of free radical inhibition was calculated using following equation:

Percentage of inhibition = [(Abs of control − Abs of sample) ÷ Abs of control] × 100

Lower the absorbance with high concentration of extract indicates potential antioxidant activity of test sample. Ascorbic acid was used as a reference standard.

Reducing power capacity

Reducing power capacity of the methanolic extract of *N. capensis* was determined according to Oyaizu (1986) method [11]. The amount of ferrous complex was determined spectrophotometrically by taking absorbance at 700 nm where plant extracts having excellent antioxidant property show greater absorbance with the higher concentration of extract solution. Ascorbic acid was used as a reference standard.

Estimation of total phenolic content

TPC in the *N. capensis* plant extract was determined by following the Folin-ciocalteu method [12]. TPC was determined spectrophotometrically by taking absorbance at 760 nm. Standard Gallic acid solution of different concentrations with same procedure were used to prepare a calibration curve by plotting the absorbance against their respective concentrations from where a standard equation was formulated to determine the unknown concentration of Gallic acid equivalent (GAE) phenolic concentration of the sample by putting the value of absorbance in the equation.

Total phenolic content was determined by following equation:

TPC = (C × V)/m (mg GAE/gm)

Determination of total flavonoid content

Total flavonoid of *N. capensis* was determined using Aluminum chloride colorimetric method with slight modification [13]. Briefly, 1.0 ml of each of the concentrations of *N. capensis* was mixed with 3.0 ml of methanol, 0.2 ml of 10% AlCl3, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water was added to the resulting mixture which was then incubated at room temperature for 30 min to complete the reaction. The reaction mixture was recorded for absorbance at 420 nm. Gallic acid was used as standard and the results were expressed as milligrams of gallic acid equivalents per gram of dried fraction.

Statistical analysis

The data was analyzed by Microsoft Excel 2010 (Roselle, IL, USA) and GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad Software Inc., San Diego, CA) were used for the statistical and graphical evaluations. Values were expressed as mean ± Standard error of mean (± SEM).

Results

Antioxidant Activity

DPPH Free radical scavenging activity

In the DPPH free radical scavenging assay, methanol extract showed concentration dependent inhibition of the free radicals as shown in Figure 1. Here the methanol extract of *N. capensis* showed significant activity. At conc. 31.25, 62.5, 125, 250, 500ug/ml, the percentages of inhibition of ascorbic acid were 38.37, 53.57, 73.78, 81.08 and 92.16 whereas the percentages of *N. capensis* were 25.54, 43.64, 57.83, 72.83 and 87.16 respectively. IC_{50} of Ascorbic acid was 14.84 µg/ml whereas *N. capensis* was 130.94 µg/ml.

Table 1: Percentage of DPPH radical scavenging activity of ascorbic acid.

Concentration (µg/ml)	Absorbance	% of Scavenging	IC_{50} (µg/ml)
500	0.058	92.16	
250	0.140	81.08	
125	0.194	73.78	14.84
62.5	0.345	53.57	
31.25	0.456	38.37	

Table 2: Percentage of DPPH radical scavenging activity of N. Capensis.

Concentration (µg/ml)	Absorbance	% of Scavenging	IC_{50} (µg/ml)
500	0.095	87.16	
250	0.201	72.83	
125	0.312	57.83	130.94
62.5	0.417	43.64	
31.25	0.561	25.54	

Ferric reducing capacity

The reducing capacity of a compound indicates its potential antioxidant activity. Figure 2 shows the dose response curves for the reducing power of methanol extract of *N. capensis.* At conc. 62.5, 125, 250, 500, 1000 ug/ml, the absorbances of Ascorbic acid were 0.65, 1.12, 1.45, 1.78 and 1.89 respectively. In case of *N. capensis,* the absorbances were 0.46, 0.75, 1.04, 1.27 and 1.50 respectively. The extract displayed a concentration dependent increase in reducing power. The reducing power increased with increasing amount of the extracts. Higher absorbance of the reaction mixture indicates a higher reducing power. Thus, the present results showed that higher reducing power was evident in methanol extract of *N. capensis.*

Table 3: Reducing power of N. Capensis with standard Ascorbic acid.

Concentration (µg/ml)	Ascorbic acid	*N. capensis*
1000	1.89	1.50
500	1.78	1.27
250	1.45	1.04
125	1.12	0.75
62.5	0.65	0.46

Determination of total phenolic content

Total phenolic content was estimated by gallic acid (Figure 3) and expressed as milligrams of gallic acid equivalent (GAE). The methanol extracts of *N. capensis* contained a considerable amount of phenolic contents of 215±7 of GAE mg/g of extract (Table 4).

Table 4: Total phenol content of *N. Capensis* with necessary data.

Concentration(µg/ml)	Absorbance	m (gm)	c (mg/ml)	A= (c×v)/m	Mean (mg/g)
200	0.295	0.0002	0.043	215	
200	0.305	0.0002	0.044	222	215±7
200	0.285	0.0002	0.041	208	

Determination of total flavonoid content

The total flavonoid content of the *N. capensis* leaves were estimated by using aluminium chloride colorimetric technique and found that the *N. capensis* extract contained significant amount of flavonoid contents of 184.75±6.78 of GAE mg/g of extract. (Table: 5).

Table 5*: Total flavonoid content of N. Capensis with necessary data.*

Concentration(µg/ml)	Absorbance	m (gm)	c (mg/ml)	A= (c×v)/m	Mean (mg/g)
200	0.348	0.0002	0.035	177.85	
200	0.358	0.0002	0.037	185	184.75±6.78
200	0.367	0.0002	0.038	191.42	

Discussion

Free radicals such as Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are undoubtedly produced in our body which is a part of normal physiological process [14].Various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing occurs due to free radicals [15,16]. Antioxidants are the compounds which are accountable to sabotage the free radicals and protect our body from various free radical associated diseases. The mechanism is

associated with free radical mediated oxidative process through initiation, propagation and termination [17]. Production of antioxidant can be occurred inside the body as well as naturally in many foods [18]. In this way, endogenous antioxidants such as superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins as well as exogenous antioxidants like vitamin E, vitamin C, β-carotene, vitamin E, flavonoids protect physiological system from free radicals by destroying them [19].

DPPH radical scavenging assay is extensively used system for the detection of antioxidant activity in plant extracts. In this assay, at the time of receiving an electron or hydrogen, DPPH radical go through a color change from purple to yellow. At the absorbance of 517 nm, the activity of experimented antioxidant agents can be determined [20]. Degree of color change is proportional to the concentration and potency of antioxidant agents. Scavenging activity of antioxidant agent is determined by the degree of discoloration [21]. In this assay, IC_{50} of Ascorbic acid was 14.84 µg/ml whereas *N. capensis* was 130.94 µg/ml. Lower IC_{50} value denotes an extract with greater scavenging activity.

In this reducing power assay, due to the presence of the reductants in the solution, the reduction occurs and ferric (Fe3+) form converts to ferrous (Fe2+) form. As a result, at an absorbance of 700 nm, ferrous (Fe2+) ion can be observed. The reduction of ferric ion is an indicator of electron donating activity. It is an important mechanism of antioxidant action. Due to antioxidants, free radicals are destroyed. [22,23]. According to the figure 2, *N. capensis* exhibited increased absorbance's in increased concentrations which showed the considerable antioxidant activity of this methanolic plant extract.

Phenolic compounds have extensive antioxidant activity due to their ability to scavenge free radicals. It contains aromatic ring bearing one or more hydroxyl groups [24]. Phenolic are the largest group of phytochemicals for most of the antioxidant activity in plant or plant extracts [25, 26]. As a result, this test is widely used for the determination of antioxidant activity in plant extracts. In this test, Total phenolic content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). The methanol extracts of *N. capensis* contained a substantial amount of phenolic contents of 215±7 of GAE mg/g of extract.

Flavonoids generally exhibit significant antioxidant activity by radical scavenging, ion chelating and lipid peroxidation inhibiting properties [27, 28]. Among naturally occurring phenolic compounds, flavonoids are the largest group. It contains various biological activities such as ntimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer and so on [29]. Flavones and flavonols are the most extensively found in all the phenolics. Flavonoids have two benzene rings separated by a propane unit [30]. Antioxidant activity of flavonoids can sabotage free radicals such as super oxide and hydroxyl radicals [31].

In this test, Total flavonoid content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). The methanol extract of *N. capensis* contained a substantial amount of flavonoid contents of 184.75±6.78 of GAE mg/g of extract.

Conclusion

The results presented in this experiment indicated that crude methanol extract of *Nymphaea capensis* leaf possess considerable amount of anti-oxidant activity. This study also supports the traditional use of this plant as a therapeutic agent. Further studies are required to unfold the underlying mechanism and major constituents of this aquatic plant.

Declarations

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors read and approved the final content of this manuscript for publication.

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Figures

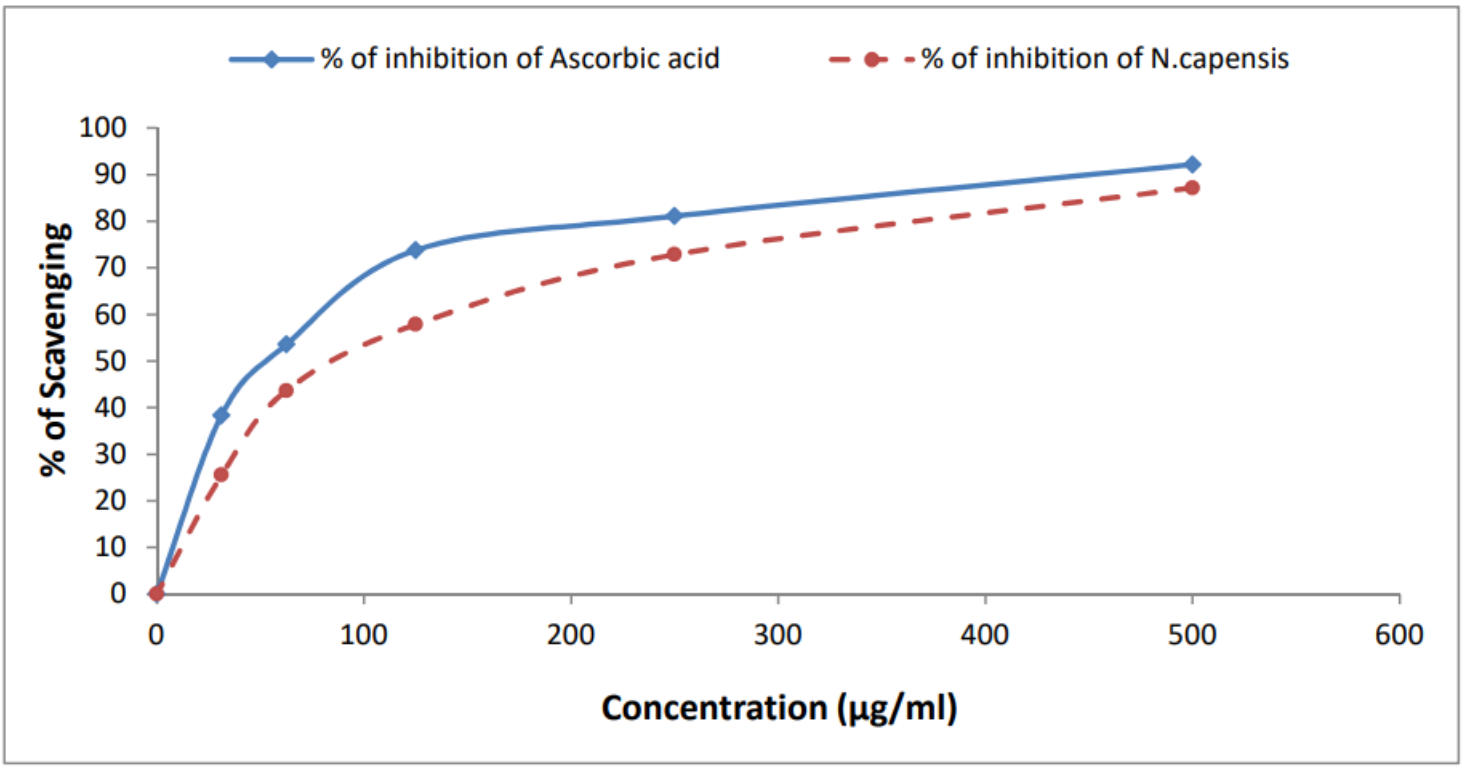

Figure 1

DPPH of N. Capensis with standard Ascorbic acid.

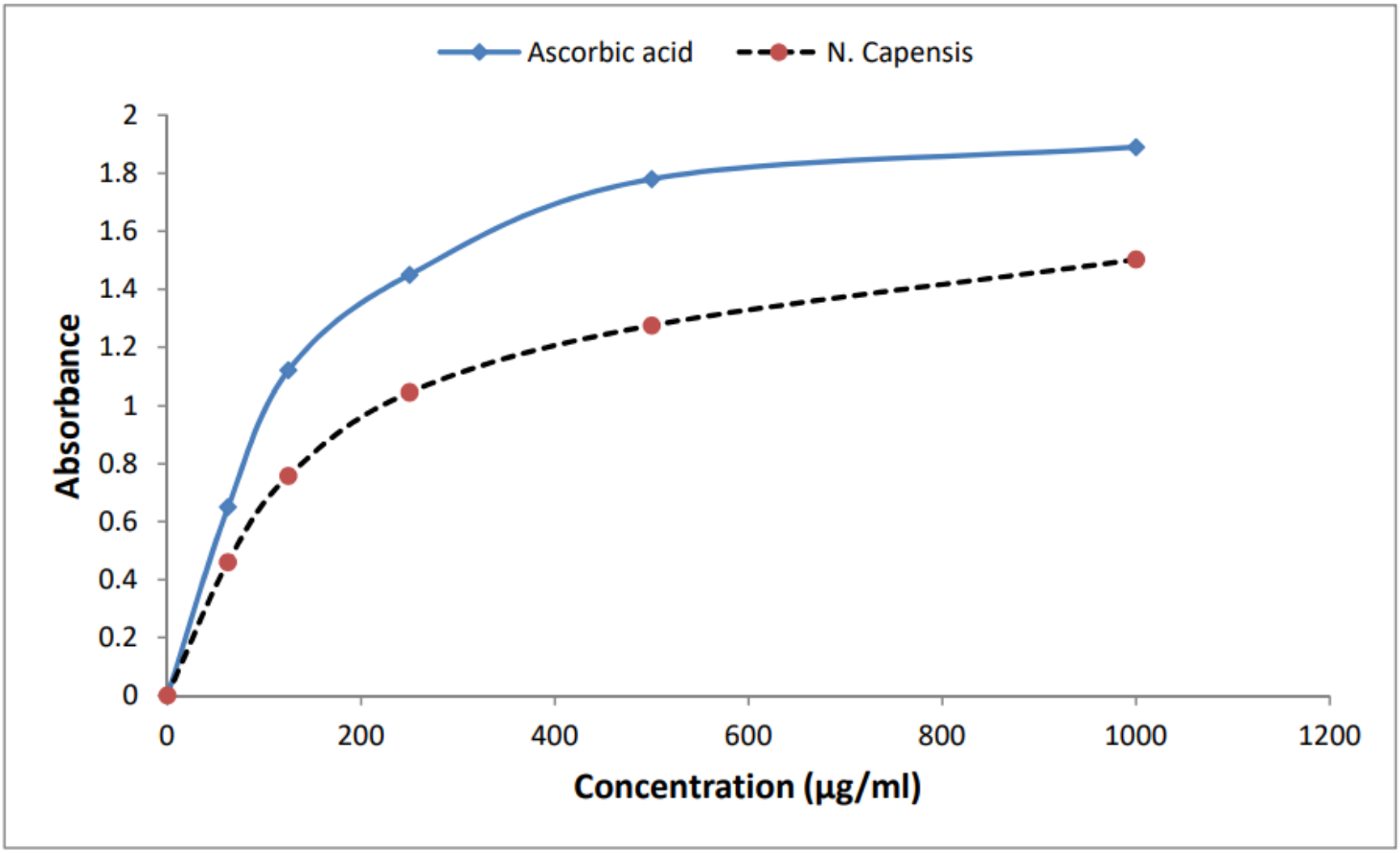

Figure 2

Reducing power of N. Capensis with standard Ascorbic acid

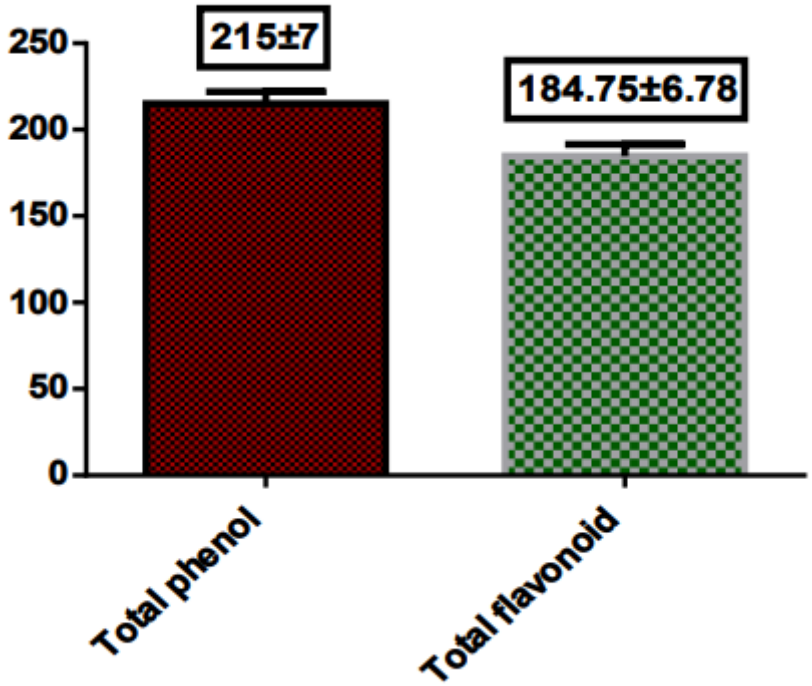

Figure 3

Total phenol and Total flavonoid