



## **Phytochemical Study and Antioxidant Properties of Aqueous Extracts of *Murraya paniculata* Leaf**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Sample collection, all laboratory experiments, data collection, graphical representation and drafting of manuscript were done by author TSBM. Authors SA and IJ helped to draft the manuscript. Study design and supervision, data analysis was done by author TH. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To investigate the chemical groups present, total phenolic compound, total flavonoid and to evaluate the possible antioxidant activity of aqueous extracts of *Murraya paniculata* leaf.

**Place and Duration of Study:** Department of Applied Chemistry and Chemical Engineering Department, Noakhali Science and Technology University, Bangladesh, in 2017

**Methodology:** The dry leaves were boiled and extracted with water. The extracts were concentrated and then investigated for their total phenolic compound, total flavonoid, evaluated the antioxidant activity. The preliminary screening of the various extracts was carried out using standard methods. Total phenolic content (TPC) was determined by the modified Folin-Ciocalteu method, total antioxidant capacity (TAC) and total flavonoid content were measured according to the Phosphomolybdate-method and Dowd-method respectively.

**Results:** The aqueous extract showed good antioxidant activity with good phenolic and flavonoid contents (400±0.44 mg ascorbic acid equivalent/g dried weight, 263±0.62 mg gallic acid equivalent/g dried weight, 63±0.19 mg quercetin equivalent/g dried weight respectively).

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**Conclusion:** The research work revealed the presence of various bioactive phytochemical compounds which show the medicinal importance of leaves *Murraya paniculata* through a facile extracting method.

**Keywords:** *Murraya paniculata*; phytochemicals; antioxidant activity; phenolic content; flavonoid content.

## 1. INTRODUCTION

Numerous diseases including neuro-degenerative, cancer, aging, cellular injury, cardiovascular and renal disorders are related to the antioxidant damage due to lone unpaired electron containing highly reactive oxygen species (ROS) [1-5]. In the medical science various antioxidant drugs are developed to treat the patients suffering from these diseases due to oxidative stress, however, their long term therapeutic can cause harmful side effects for human body. Phytochemicals can act as alternative to these synthetic products [6]. A medicinal plant consists of various bioactive substances which can be used as raw elements for chemotherapeutical semi synthesis and they have useful uses for therapeutic purposes [7-9]. Many people in the Indian subcontinent use traditional medicine from medicinal plants. Various medicinal plants are grown in Bangladesh. As medicinal plants have no side effects on human health and they have medicinal values, these plants materials should be investigated and characterized to use them in human health treatment [10].

*M. paniculata* is a common plant in Indian subcontinent also known as orange jasmine or Honey bush or Kamini belonging to the family *Rutace* [11]. This tree is found throughout India, Bangladesh, tropical Sri Lanka to Myanmar, southern China and Taiwan, Thailand and throughout the Malesian region to Northeastern Australia and Caledonia. This tree is small in size with a spreading crown and short trunk, leaves alternate, impercipient, 10-17 cm long; leaflets usually 3-5, mostly 3-7 cm long, ovate or elliptic-lanceolate or rhomboid, glossy and darker above, gland-dotted, base cuneate or rounded. The leaves are stimulant and astringent. They have successful uses to treat diarrhea and dysentery in many countries [11]. It is reported that these leaves are used to heal joint pain as paste with mustered oil. The leaves have anti-diabetic [12], anti-nociceptive and anti-inflammatory [13], anti-diarrheal, oxytocic, anti-fertility and in-vitro antioxidant properties [14].

It is found that many researches were conducted to explore antioxidant activity from petroleum ether, chloroform, methanol, ethanol, hexene extracts of various parts of *M. paniculata* plant [11,15], however in this study the leaves of *M. paniculata* were only boiled with water to get extracts which is an easy, early, and cost-effective method to investigate phytochemicals present in leaves of *M. paniculata*.

The aim and objective of the current study were to investigate the chemical groups present, total phenolic compound, total flavonoid and to evaluate the possible antioxidant activity of *M. paniculata* leaves by using a very simple method of boiling by water to justify its use in traditional treatments.

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation

The leaves of *M. paniculata* were collected from the Botanical Garden, Dhaka, Bangladesh and identified by a botanical expert. The leaves were separated from undesirable materials, washed with tap water. The leaves were boiled with water for 4-5 hours to extract the polar compounds. After that, the extracted sample was collected, filtered and then dried and stored for further investigation [16].

### 2.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening of the aqueous extract of *M. paniculata* was carried out according to the methods described in Dev et al. 2015 [17], to investigate the presence of alkaloid, reducing sugar, flavonoids, saponins, phenolic compounds, tannins, amino acids and proteins, glycosides.

### 2.3 Antioxidant Activity

In order to investigate the antioxidant properties of the examined extract, reducing power assay, total antioxidant capacity, total phenolic content,

total flavonoids and reduction of ferric ions by ortho-phenanthroline color method were performed [18]

## 2.4 Reducing Power Assay

Reducing Power Assay of the extract was measured based on the conversion from Fe (III) to Fe (II) which was indicated by the formation of Perl's Prussian blue and it was observed at 700 nm [19]. 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferricyanide (1%) were added to 2 mL of various concentration of sample. After incubation at 50°C for 20 min, the addition of 2 mL of trichloroacetic acid (10%) was followed. Then the mixture was centrifuged at 3000 rpm for 10 min and upper portion of the solution was collected. 2 mL of distilled water and 0.4 mL of 0.1% (w/v) fresh ferric chloride was added with 2 mL of sample mixture. After 10 min reaction, the absorbance was measured at 700 nm. In this method, higher the absorbance shows higher the reducing power and BHT was used as reference standard. Increased absorbance of the reaction mixture showed enhanced reducing power.

## 2.5 Total Antioxidant Capacity

The total antioxidant capacity of the extracts was determined by phosphomolybdate method where ascorbic acid was used as a standard [20]. 1 mL of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate) was added with 0.1 mL of sample solution. The mixture in capped tubes was incubated in a water bath at 35°C for 90 min. Then the mixture was cooled at room temperature and finally the absorbance of the mixture was measured at 765 nm against a blank. Ascorbic acid was used as the standard and Total Antioxidant Capacity was measured as equivalents of Ascorbic acid. The total antioxidant capacity is concentration-dependent.

## 2.6 Reduction of Ferric Ions by Ortho-phenanthroline Color Method

2 mL of extracts of various concentration as mixed with a reaction mixture containing 1 mL ortho-phenanthroline (5 mg in 10 mL methanol), 2 mL ferric chloride 0.2 mm (3.24 mg in 100 mL distilled water). Then the mixture was kept in room temperature for 10 minutes. Finally, the absorbance was measured at 510 nm. Gallic acid was used as reference standards [18,21].

## 2.7 Total Phenolic Content

The total phenolic content of the extracts was measured by following Folin-Ciocalteu method [18]. A reaction mixture was prepared by adding 0.5 mL of each extract (1 mg/mL), 5 mL Folin-Ciocalteu reagent (1:10 v/v in distilled water) and 4 mL of 7.5% sodium carbonate with each other. The mixture was vortexed for 15 s and kept for 30 min at 40°C for color development. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. The standard curve was prepared using a various concentration of solutions of gallic acid in 50% methanol. The result was expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

## 2.8 Flavonoid Contents

The flavonoid contents of extracts were measured according to Dowd-method [22]. 0.5 mL of extract solution in methanol was added with 0.1 mL of 10% (w/v) AlCl<sub>3</sub> solution, 0.1 mL (1 M) potassium acetate and 2.8 mL distilled water. The mixture was allowed to stand for 30 min at room temperature and measured at absorbance at 415 nm against the blank. Quercetin was used as standard. The result was expressed as quercetin equivalents in milligrams per gram (QE/g) of dry extract.

## 2.9 Statistical Analysis

All data are presented as mean  $\pm$  standard deviation (SD) for at least three replications for each experiment. All statistical analysis and graphs were drawn using MS-Excel.

## 3. RESULTS

Phytochemical screening of the extract stated the presence of alkaloids, flavonoids, saponins, phenolic compounds and tannins, glycosides however reducing sugar and amino acid and protein were absent.

### 3.1 Reducing Power Assay

The reducing capacity of a compound may act as an important property which reflects its potential antioxidant activity. Fig. 1 shows the dose response curves for the reducing powers of all concentrations of extracts. The aqueous extract of leaves of *M. paniculata* showed significant reducing power (0.836 $\pm$ 2) comparing to standard BHT (1.099 $\pm$ 1.4) at 500  $\mu$ g/mL.

### 3.2 Total Antioxidant Capacity

Total antioxidant capacity of the aqueous extract of *M. paniculata* leaves was determined by phosphomolybdate method [17-18]. Fig. 2 shows the total antioxidant capacity for all concentrations of aqueous extract of *M. paniculata* leaves. The total antioxidant capacity of aqueous extract of leaves of *M. paniculata* was found ( $400 \pm 0.44$  AAE/g) which was closed to standard ascorbic acid that proved the strong antioxidants in this extract.

### 3.3 Ferric Reducing Antioxidant Potential

The ferric ion reduction is broadly utilized to assess antioxidant activity. In this strategy,  $Fe^{2+}$  responds quickly with *o*-phenanthroline and form a red colour complex which is outstandingly stable. This complex is visible in absorption at wavelength of 510 nm. The aqueous extract of *M. paniculata* leaves showed significantly antioxidant activity and the values ( $1.337 \pm 0.42$ ) which is higher than standard antioxidants gallic acid ( $1.140 \pm 0.23$ ) at 500  $\mu\text{g/mL}$  (Fig. 3).

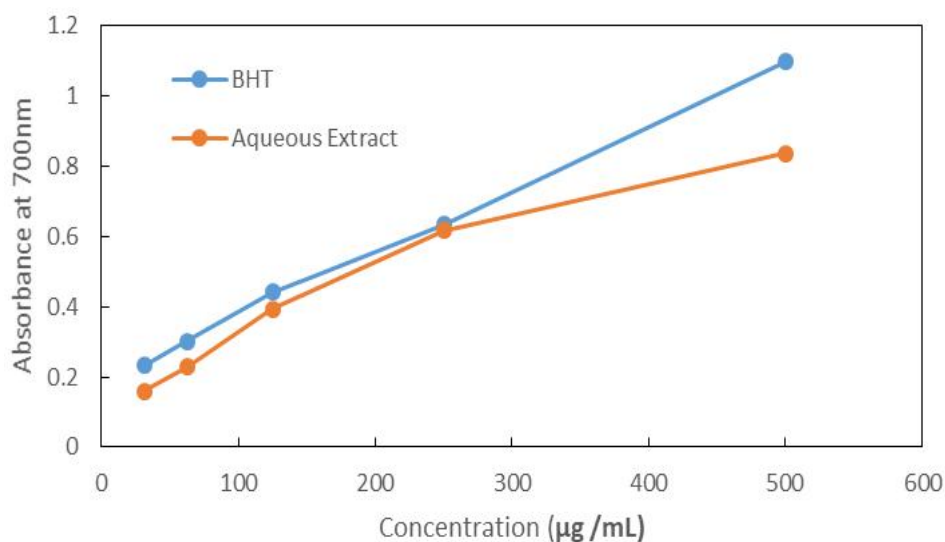


Fig. 1. Comparative analysis of aqueous extract of *M. paniculata* leaves with standard BHT for reducing power assay

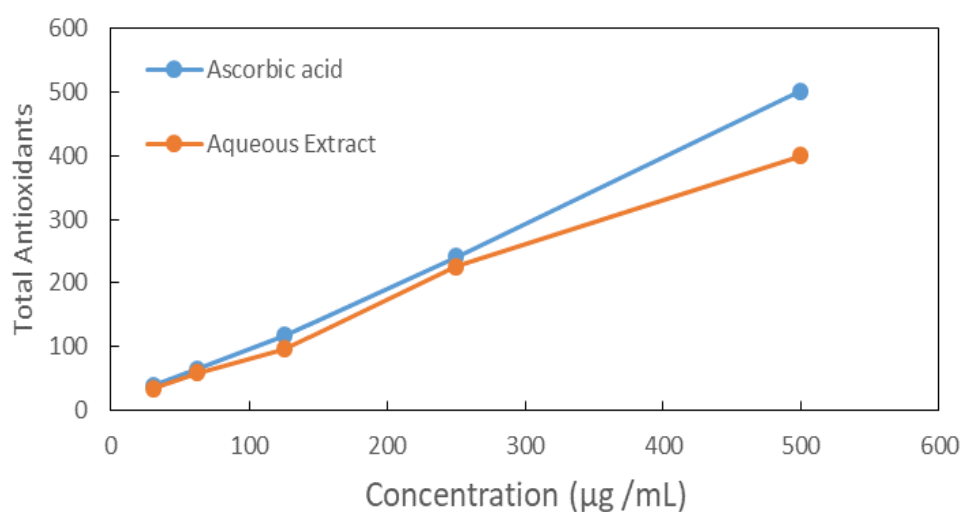


Fig. 2. Comparative analysis of aqueous extract of *M. paniculata* leaves with standard ascorbic acid for total antioxidant capacity

### 3.4 Total Phenolic and Flavonoids Contents

Fig. 4 shows the total phenolic content and total flavonoids of aqueous extract of *M. paniculata* leaves at 500 µg/mL. The phenolic content was measured spectrophotometrically at 765 nm. Flavonoid contents in selected plant extracts were determined using aluminum chloride calorimetric method. The results were derived from the calibration curve ( $y = 0.0124 + 0.1013x$ ,  $R^2 = 0.9963$ ) of quercetin (QE) (0-500 µg/mL). The total phenolic content of the aqueous extract of *M. paniculata* leaves were measured in terms of gallic acid equivalent (GAE) (mg/g). The total

phenolic content of the aqueous extract was  $263 \pm 0.62$  (mg GAE/g). The total flavinoid content of the aqueous extract was  $63 \pm 0.19$  (mg QE /g).

### 4. DISCUSSION

Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, phenolic compounds and tannins. Secondary metabolites are very important for the plant [23, 18]. The presence of alkaloids proves the antimalarial, antiseptic, antibacterial properties of the plant materials [24] whereas the presence of flavinoids have the potential of anti-inflammatory,

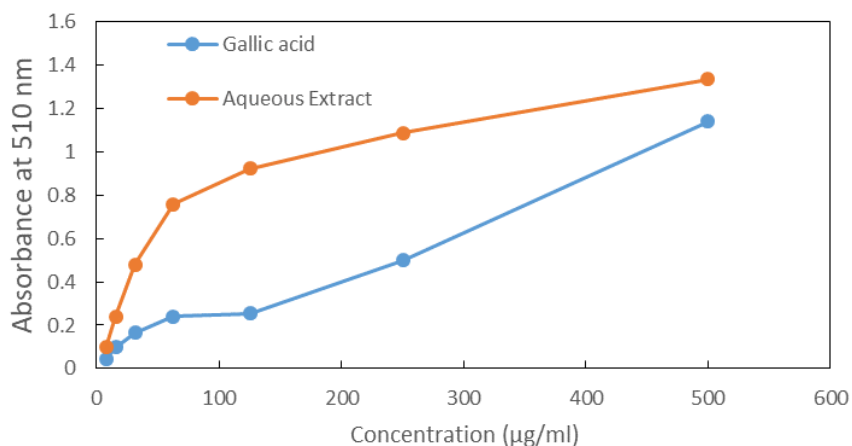


Fig. 3. Comparative analysis of aqueous extract of *M. paniculata* leaves with standard gallic acid for reduction of ferric ions by ortho-phenanthroline color method

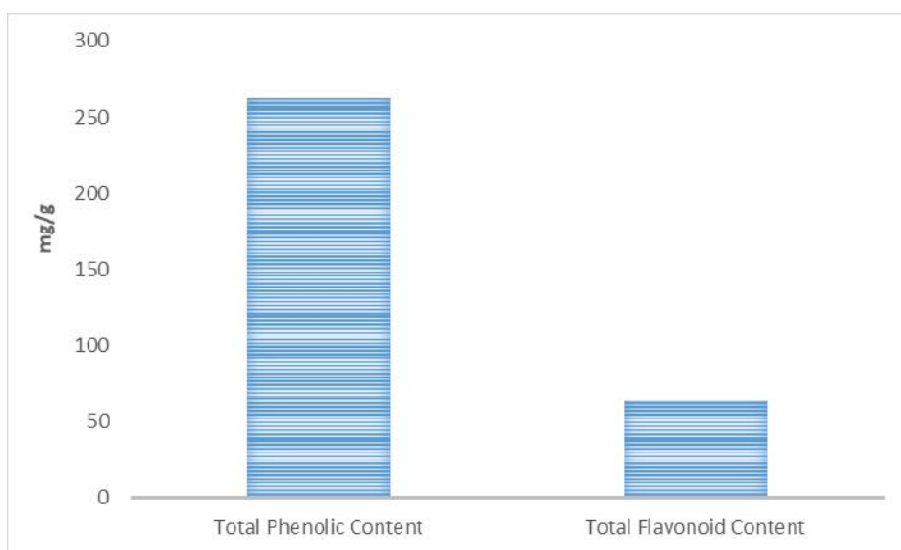


Fig. 4. Total phenolic compound and flavonoid aqueous extract of *M. paniculata*

anti-microbial, anti-cancerous, anti-allergic activity [25]. Phenolic constituents and saponins, present in the plant materials, exert immunomodulatory activity and antibacterial and antifungal properties [26]. The presence of tannins in the extracts may be utilized for accelerating wound and inflamed mucous membrane healing [27]. Finally, the presence of these phytochemicals shows the medicinal efficacy of the leaves extracts of *M. paniculata*.

In the Reducing Power Assay, total antioxidant action is an indicator of the entire capacity to withstand the negative impact of stretch actuated by free radical formation [28]. The reduction of Fe (III) is often used as an indicator of electron donor activity. The reduction of the Fe<sup>3+</sup>/ferricyanide complex to the ferrous form caused due to the presence of the antioxidants [23,29]. Fe<sup>2+</sup> can be examined by absorbance measurement at 700 nm. In case of known amount of sample, the higher absorbance indicates the better reducing power. The antioxidants present in the extracts of *M. paniculata* caused their reduction of Fe<sup>3+</sup> /ferricyanide complex to the ferrous form, and thus proved the reducing power [30].

Total antioxidant capacity of the aqueous extract of *M. paniculata* leaves was determined by phosphomolybdate method. In this method, formation of a green phosphomolybdate (V) complex is the reduction of molybdenum (VI) to molybdenum (V) in the presence of antioxidant, which can be measured spectrophotometrically at 765 nm. Total antioxidant activity focus on the thermodynamic conversion and measures the number of electrons or radicals donated or quenched by a given antioxidant molecule and measure the capacity of biological samples under defined conditions. High antioxidant capacity of the extract might be caused for the polarity of water as solvent [18,31]. The polar solvents have a much stronger ability to dissolve and hence extract polar phytochemicals [18].

Ortho-substituted phenolic compounds interact with iron and may assert pro-oxidant effects. o-phenanthroline quantitatively forms complexes with ferric ion and the complexes get smashed in the presence of chelating agents [13]. The extracts interfered with the formation of ferrous-o-phenanthroline complex, which may give the information about the metal chelating activity of extracts [17,32-33]. Here ferric ion is reduced to ferrus ion. The Ferric Reducing Antioxidant Potential is linearly concentration-dependent

[22]. Phenolic compounds are plant constituents having antioxidant activity due to redox properties [33]. Under the basic reaction conditions, a phenol loses an H<sup>+</sup> ion to produce a phenolate ion, which reduces Folic-Ciocalteu reagent [34,35]. The high level of phenolic compounds is the main reason for the enhanced antioxidant activity of polar extract. The elevated level of phenolic compound is the most reason for the enhanced antioxidant activity of polar extracts [36]. The main role of phenolic compounds as free radical scavengers has been detailed in many papers [37-38]. The phenolic contents act as hydrogen donors thus as an efficient antioxidant activity [34]. Flavonoids are secondary metabolites with antioxidant activity, the capacity of which depends on the number and position of free OH groups [22,39].

## 5. CONCLUSION

The research work revealed the presence of various bioactive phytochemical compounds which showed the medicinal importance of leaves of *M. paniculata* through a very simple and cost-effective method. Like other solvent extraction, water boiling method also provides antioxidant rich extracts though there are no uses of chemical solvent or Soxhlet. Moreover, this method can be practicable in home and be an easy method to use the leaves of *M. paniculata* as herbal medicine in healing of various diseases. The aqueous extract of leaves of *M. paniculata* is a good source of antioxidant and phenolic compounds which are very adjuvant for medicinal purpose. Further investigation to study the mechanism of action of antioxidants is necessary to better understand their ability to control diseases that have a significant impact on human health.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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