In vitro Investigation of Antioxidant Potential of Stem Bark Extract of *Moringa oleifera* (Family: Moringaceae)

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Abstract

The present study is projected to evaluate the antioxidant potential of methanol and ethanol extracts of stem bark of Moringa oleifera (Family:Moringaceae). Moringa oleifera is a speedy growing and drought challenging plant inhabitant to the Indian subcontinent with several biological activities and therapeutic properties. Antioxidant activity was evaluated using total antioxidant capability, total flavonoid and total phenol contents determination assays. Moderate amounts of phenolics and flavonoids were found in the stem bark extracts, expressed as galic acid equivalent (GAE) and quercetin equivalent (QE) respectively. The methanol extract of Moringa oleifera (MEM) demonstrated highest flavonoid content (50.5±0.7 mg/gm QE) and phenolic content (32.5±1.18 mg/gm GAE) than ethanol extract of Moringa oleifera (EEM). In addition, the plant parts displayed good total antioxidant capacity articulated as ascorbic acid equivalent (AAE) with methanol extract of Moringa oleifera (MEM) being the highest one (54.17±3.54 mg/gm AAE). The results of present comprehensive analysis demonstrate that stem bark of Moringa oleifera possess moderate flavonoid, phenolic contents and prospective antioxidant activity and might be used as an excellent source of natural antioxidants and could be used for pharmacological test of various therapeutic activities.

Keywords: *Total antioxidant, Total phenol,* Total flavonoid, Moringa oleifera , Stem bark.

Introduction

Traditionally used medicine from plant extracts has been utilized widely which are clinically helpful and comparatively less toxic than available drugs in the market (Awaad et al., 2011). Type of solvent used in the extraction procedure plays an important role in the identification of biologically active molecules from plant material (Nahannu et al., 2018). A variety of solvents have been used in the extraction of plant to collect active materials from plants such as alcohols (ethanol or methanol), petroleum ether, diethyl ether, chloroform, ethyl acetate, n-butanol and water (Poulsen et al., 1998).

Free radicals are the main culprit of a number of diseases such as neurodegenerative problems, liver diseases, cancer, arteriosclerosis, and diabetes etc. Oxidative damage caused by oxidizing bio molecules such as free radicals and results in cell death and following tissue damage. As a result, free radical scavenge compounds have been used to reduce or inhibit the oxidative damage on the human body (Brighente et al., 2007). Many plant derived molecules have been used in therapeutics which shown a promising effect (Lokhande et al., 2007). A variety of medicinal plants are known as sources of natural antioxidants and widely used in chemo prevention of diseases and aging.

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Antioxidants are acted as shielding agents who are used in reducing damage which are caused by oxidative agent from ROS in the body and inhibiting the development of several chronic diseases. For this reason, there is a rising interest in components that show evidence of antioxidant activities, which are supplied to animals and humans as food supplements or as definite pharmaceuticals. Antioxidants are delaying the oxidation process of other molecules by preventing the propagation or initiation of oxidizing chain reactions (Köksal et al., 2008).

Moringa oleifera (Moringaceae; Bengali name:Sajne) is a rapid growing, drought challenging plant inhabitant to the Indian subcontinent and commonly cultivated in all region of Bangladesh as a popular vegetable for cooking purposes. All parts of this plant had been exhibited to have variously biological activities which are used in reducing hyperglycemia (Mbikay et al., 2012) and many others pharmacological activities (Farooq et al., 2012).

Antioxidant properties with sunflower oil, α-tocopherol and linoleic acid were found in *Moringa oleifera* leaves extracts (Arabshahi et al., 2007). The leaves of *Moringa oleifera* are suggested as supplement because of rich in nutrients for breastfeeding mothers and infant in Asia and Africa (Fuglie et al., 1999). Various chemical compounds are isolated from this plant such as Nitrile compounds, mustard oil glycosides, benzyl glycosides, phenolic glycosides, flavonoid glycosides, thiocarbamate glycosides and amino acid (Farooq et al., 2012). The purpose of the study is to identify the antioxidant properties of methanolic and ethanolic stem bark extract of *Moringa oleifera.*

Materials and Methods

Collection of plant material: The stem bark of this plant was collected from Gazipur city, Dhaka, Bangladesh, in March 2019 at the full-grown stage. Plant parts are cutting into small pieces and these cutting parts were dried in shade at temperature between 21ºC to 30ºC for around 20 days. The cutting pieces were crushed by a mechanical grinder and passed through a 60 Mesh sieve to get fine powder and stored into an air-tight container.

Extraction of Plant Material: A fraction of crushed powder (1 kg) was extracted sequentially with methanol and ethanol (2 L of each) by cold extraction process. The sample was stirred constantly and was set aside for nearly 72 hours in each fraction. The entire extracts were filtered off and evaporated to dryness (45ºC) under reduced pressure through rotary evaporator. The amounts of yields of the ethanol and methanol extracts were 2.6 g (0.26%) and 2.3 g (0.23%) respectively. As a final point the extracts were defatted through refrigeration at 4° C temperature.

Determination of Total Phenolic Content: The phenol content of extracts was identified by using Folin-Ciocalteu method (Singleton et al., 1999). The extracts were neutralized with sodium carbonate and were oxidized with Folin-Ciocalteu reagent. The absorbance of the resultant blue color was determined at the wavelength of 760 nm after 60 min. where gallic acid used as standard total phenol content was expressed as mg GA equivalent/gm of extract.

Determination of Total Flavonoids Content: The flavonoids content was measured according to Kumaran and Karunakaran (Kumaran et al., 2007) using quercetin as a reference compound. 1ml aluminium trichloride in Ethanol was mixed with 1 mg of plant extract in methanol (20 mg/ml) and one drop of acetic acid, and finally diluted with Ethanol to 25 ml. The absorption at 415 nm of wavelength was read after 40 min. Blank samples were prepared from plant extract (1 mg) and one drop of acetic acid, and finally diluted to 25 ml with ethanol. The absorption of standard solution (0.5 mg/ml) (quercetin) in methanol was determined under the similar conditions.

Determination of Total Antioxidant Capacity: The antioxidant property of the extracts of Moringa oleifera was determined by the phosphomolybdenum method according to the technique of Prieto et al. (Prieto *et al.,* 1999). The test is based on the reduction of Mo (VI) - Mo (V) by subsequent formation of a green phosphate/Mo (V) complex and the extract at acidic pH. 0.3 ml plant extract was mixed with 28m M sodium phosphate, 4mM ammonium molybdate and 3 ml of reagent solution (0.6 M sulfuric acid). The reaction solution was incubated at 95º C for 90 min. After that the absorbance of the solution was determined at 695 nm of wavelength using a spectrophotometer against blank later than cooling to room temperature. Methanol (0.3 ml) is used as the blank in the place of extract. The antioxidant property is expressed as the number of equivalents of ascorbic acid.

Results and Discussion

Total Antioxidant Capacity

Total antioxidant capacity of the different extracts of stem bark of Moringa oleifera were analyzed by the phosphomolybdenum method and was expressed as equivalents of ascorbic acid (AAE) per gram of plant extract. Total antioxidant property of the samples was calculated using the ascorbic acid standard curve ($y = 0.006x + 0.101$; $R^2 = 0.991$) (Figure 1). Methanolic extract of *Moringa oleifera* (MEM) was observed to possess the highest total antioxidant property (Table 1 & Figure 4).

Total Phenol Content Determination

Total phenolic content of the different extracts of stem bark of *Moringa oleifera* were evaluated by using a reagent named Folin-Ciocalteu and were expressed as equivalents of Gallic acid (GAE) per gram of plant extract. The total phenol contents of the test samples were calculated using the Gallic acid standard curve ($y = 0.009x + 0.058$; $R^2 = 0.997$) (Figure 2). Methanolic extract of Moringa oleifera (MEM) was observed to posses the highest amount of phenol content (Table 1 & Figure 4).

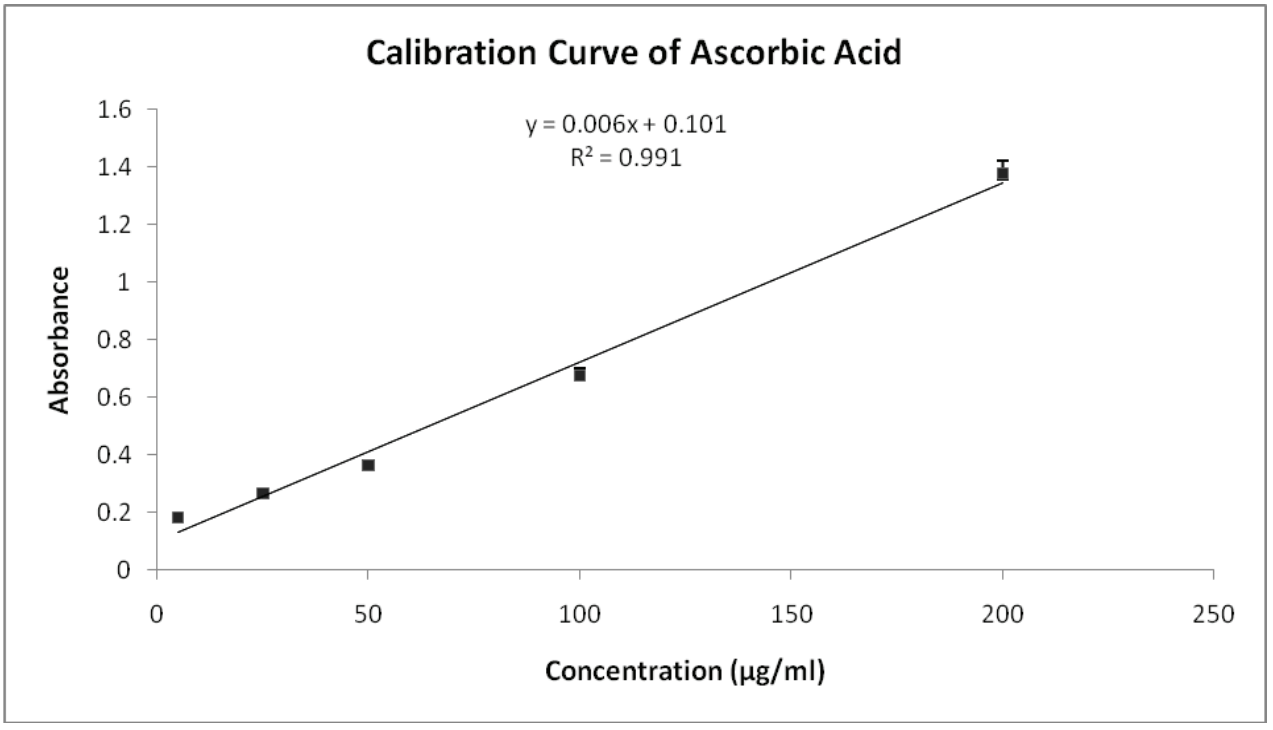

Figure 1: Calibration curve of ascorbic acid.

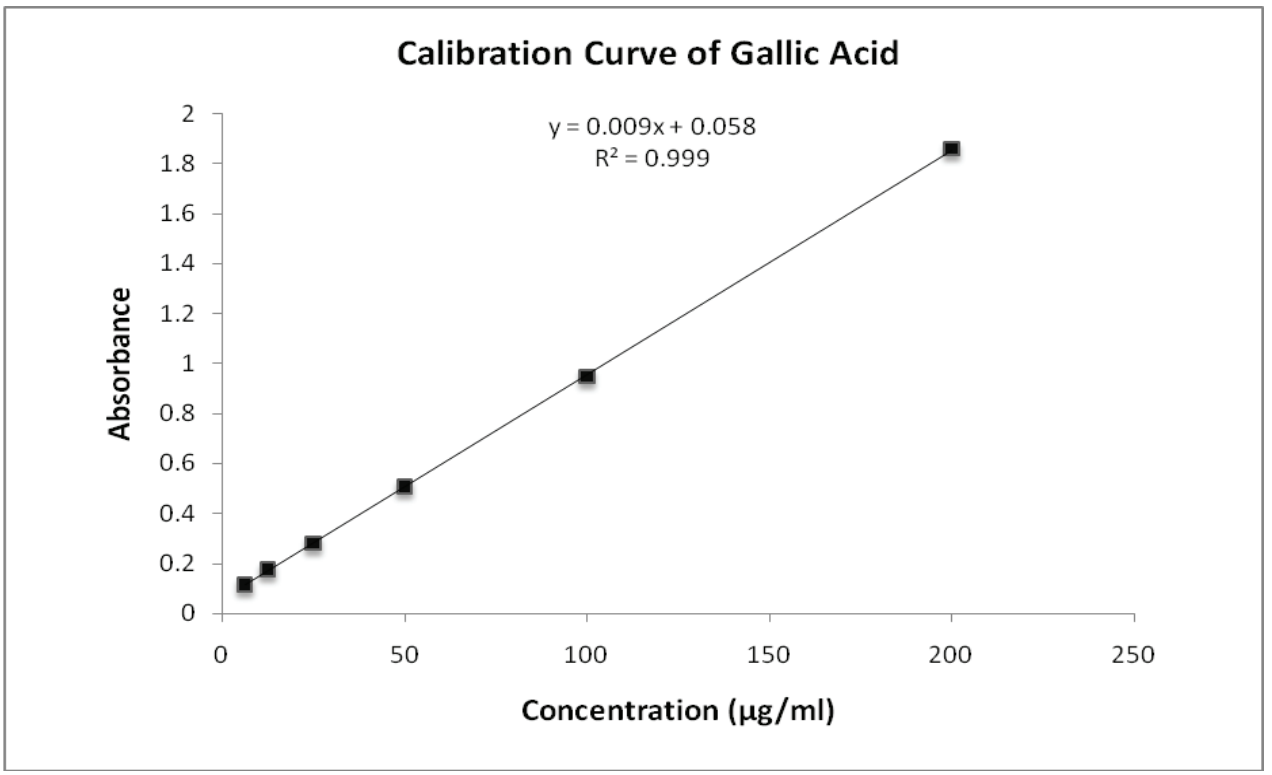

Figure 2: Calibration Curve of Gallic Acid.

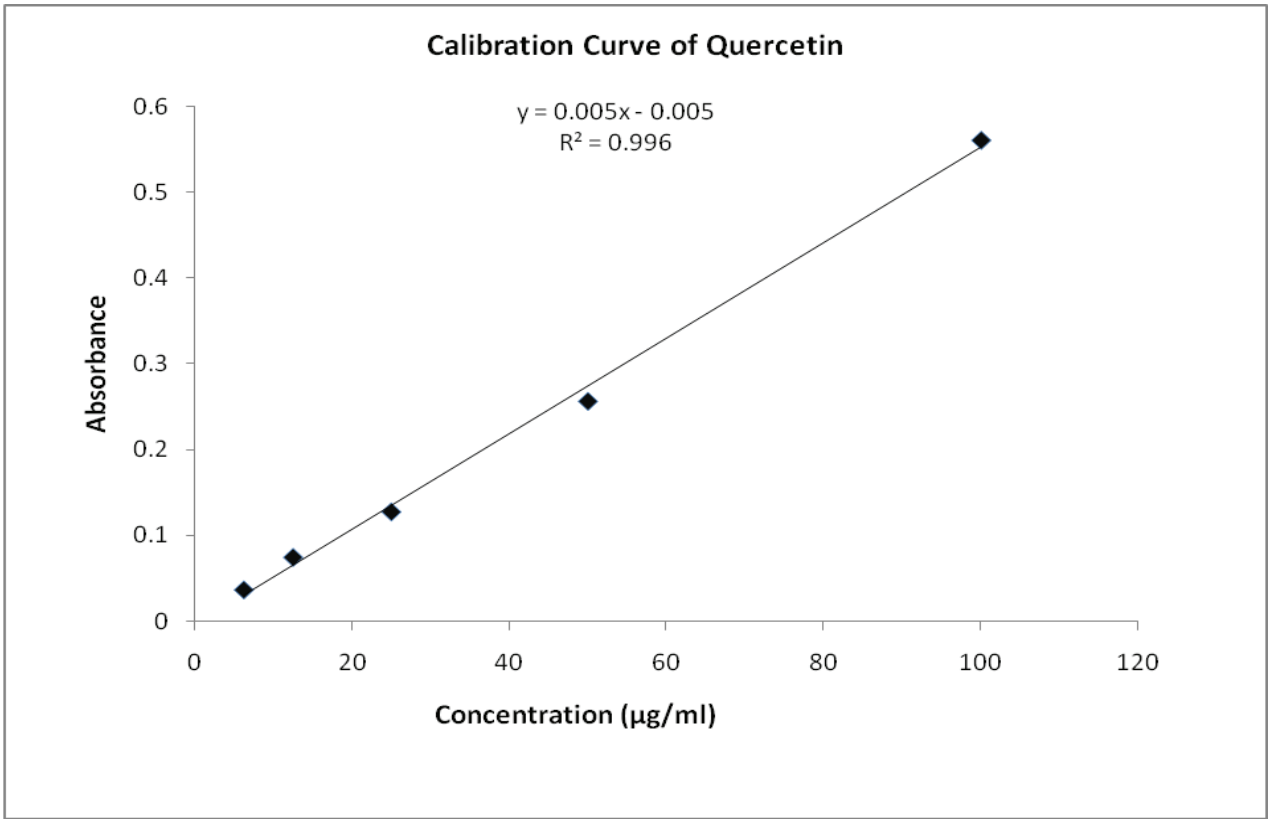

Figure 3: Calibration Curve of Quercetin.

Determination of Total Flavonoid Content

The total flavonoid contents of the different extracts of stem bark of Moringa oleifera was evaluated by Aluminium chloride colorimetric method. The total flavonoid content was calculated using the quercetin standard curve ($y = 0.005x - 0.005$; $R^2 = 0.996$) (Figure 3) and was expressed as equivalents of quercetin (QE) per gram of the plant extract. Methanolic extract of Moringa oleifera (MEM) was observed to have the highest amount of flavonoid content (Table 1 & Figure 4).

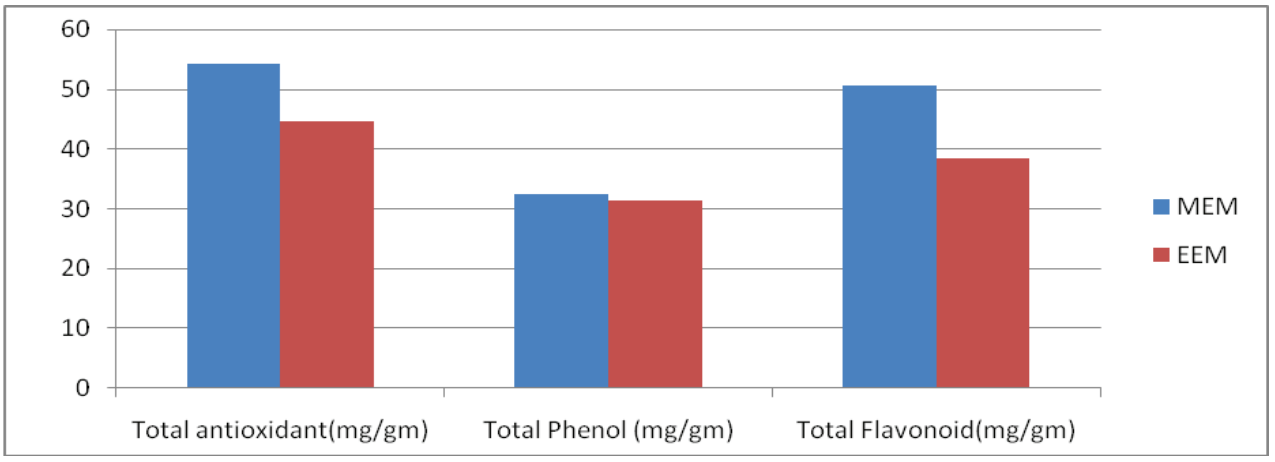

Figure 4: Total antioxidant capacity, total phenol and total flavonoid contents of Methanol extract of Moringa oleifera (MEM) and Ethanol extract of Moringa oleifera (EEM)

Extract	Total antioxidant (in mg/gm, Ascorbic acid equivalents)	Total Phenol (in mg/gm, Gallic acid equivalents)	Total Flavonoid (in mg/gm, Quercetin equivalents)
MEM	54.17±3.54	32.5±1.18	50.5±0.7
EEM	44.58±4.12	31.39±1.9	38.5±3.54

Table 1: Total antioxidant capacity, total phenol and total flavonoid contents of Methanol extract of Moringa oleifera (MEM) and Ethanol extract of Moringa oleifera (EEM). All values are expressed as mean ± SD.

Conclusion

From the study, it can be understood by taking into consideration the results that the stem bark extract of Moringa oleifera have moderate antioxidant activity which relates conventional use in a variety of diseases as well as with different recognized research reports. However, the experiment conducted is only a preliminary work and there is profusion of scopes for further thorough research for better perceptive of its precise pharmacological activities, mechanism of action as well as the active compound(s) responsible for these actions.

Declaration of Competing Interest

None declared.

Ethical Approval

Not required.

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