INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 19, No. 2, p. 51-64, 2021

RESEARCH PAPER OPEN ACCESS

A comprehensive screening of bioactivities and phytochemicals of different extracts of *Moringa oleifera*

Shahin Alam, Tanvir Ahamed*, Priya Noor

Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Key words: *Moringa oleifera*, Bioactive compounds, Antioxidant, Antimicrobial activity.

http://dx.doi.org/10.12692/ijb/19.2.51-64 Article published on August 22, 2021

Abstract

Moringa oleifera Lam. (Sazna) is considered a miracle plant of nature possessing outstanding medicinal properties and nutritional values. Almost all the parts of *this* plantare edible and also have potential bioactivity.The leaves, flower, seeds have been used traditionally as folk remedies for the treatment of many diseases such as diabetes, constipation, gastritis, ulcerative colitis, etc. For the amazing nutritious and medicinal value, our study focused on the bioactivity and phytochemicals of *Moringa oleifera* extracts. Phytochemicals are the chemical compounds produced by plants, are involved in protection against fungi, plant viruses and bacterial infection. The preliminarily phytochemical screening revealed the extract richness of Alkaloid, carbohydrate, coumarin, flavonoid, glycoside, phenol, protein, vitamins, minerals etc. in both flower and leaf extracts. Quantitative analysis revealed that the highest amount of phenol, flavonoid, tannin, protein were found for Sazna flower ethanolic extract (SFM), Sazna flower methanolic extract (SFE), Sazna leaf ethanolic extracts (SLE) and SFM respectively.Antioxidant activity was determined by FRAP (Ferric oxide reducing power). The highest (202.18±0.087 µg/mL) ferric oxide reducing activity was found for SFE in comparison to other extracts. For the reducing power assay, SFM showed the highest amount of reducing sugar content (896.55±0.77 µg/mL). The highest Vitamin C content (6.81±0.007 µg/mL) was found for SFM at 1000 µg/mL. The results suggest that *M. oleifera* (Sazna) has outstanding antioxidant activity and could serve as a potential source of natural antioxidants. However, antimicrobial assays were done with flower and leaf extracts at different solvents at different concentrations.Findings from this study revealed that The maximum activity was observed for methanolic flower extract (14.5±0.5 mm) against gram-positive bacteria and methanolic leaf extract (10.2±0.25 mm) against gram-negative bacteria both at a concentration of 10 mg/disc thus suggests need to refine and standardize these extracts as an alternative source of antimicrobial medicines.The data obtained from this study on bioactivities and phytochemicals of *M. oleifera* will be useful for the further discovery of new drugs.

* **Corresponding Author:** Tanvir Ahamed ✉ tanvirahamed@juniv.edu

Introduction

From ancient times plants are always been considered a major source of medicine. According to the world health organization (WHO), medicinal plants are being used as natural remedies for multiple diseases and around 80% of the people around the world depend on indigenous medicine for their basic healthcare requirements (Valdez-Solana *et al.,* 2015; Duraipandiyan *et al.,* 2006). Usage of plants as indigenous medicine is increasing day by day as they are reported as safe, nontoxic, less expensive, rarely have side effects and are readily available. The presence of different bioactive compounds and elemental composition of the plant may be the main reasonfor using the plant as the source of medicine (Demiray *et al.,* 2009).As adverse effects and microbial resistance are developing due to using chemically synthesized drugs, scientists are turning to ethnomedicine. Scientists found a huge number of phytochemicals from plants that have been reported as a safe and potential alternative to chemically synthesized drugs with fewer adverse side effects (Sasidharan *et al.,* 2011).

A large number of medicinal plants are used of which the most promising one is *Moringa oleifera* Lam. (*Moringa*) belongs to the family moringaceae. *Moringa* species is widely distributed throughout India, Pakistan, Bangladesh, Afghanistan Africa, Saudi Arabia, Southeast Asia, the Caribbean Islands, and South America (Bamishaiye *et al.,* 2011). However, *Moringa oleifera* is commonly known as 'Drumstick' but it is also known as horseradish tree, ben oil tree, miracle tree and mother's best friend. It is a small plant having a height of 10-12m height but rich in different types of phytochemicals such as proteins, vitamins, minerals, folic acid and α-carotene **(**Dahiru *et al.,* 2016**)**. Besides, the foliage of *M. oleifera* is well-known not only as a rich source of phenolics and glucosinolates, minerals, tocopherols, carotenoids, folate, polyunsaturated fatty acids, and ascorbic acid (Saini *et al.,* 2016) but also contain vital minerals such aszinc, magnesium, manganese, iron, potassium, calcium, copper, etc (Paikra, 2016).

M.oleifera is being considered one of the world's most treasurers trees, as almost every part of the tree is edible or exhibits beneficial properties. Both leaf and young shoots are eaten as greens, in salads, in vegetable curries, and as pickles. The leafcan be eaten in multiple formats; fresh, cooked, or leaf can be stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Mahmood *et al.,* 2010).

For understanding the beneficial effect of this plant different type of study has been performed which reveals that one hundred grams of dry *M.oleifera* leaf contain nine times the vitamin A compared to carrots, fifteen times compared to the potassium of bananas, seventeen times compared to the calcium of milk, twelve times compared to the vitamin C of oranges and twenty-five times compared to the iron of spinach (Vaidya and Devasagayam, 2007)**.** The leaves and seeds of *M. oleifera Lam*. may protect against some effects of arsenic toxicity which is an especially important light of the news. One of the major global public health concerns is the contamination of the groundwater with the arsenic and *M. oleifera* seeds have been found to show extraordinary result for the water purification process (Gupta, 2010).

The different pharmacological studies revealed that different extracts of this plant exhibited multiple bioactivities. Studies showed that alcoholic extract of leaf of *M. oleifera* is highly functional against pain and therefore it can be concluded this plant has analgesic activity whereas the aqueous extract of *M.oleifera* roots also shows an impressive antifertility profile. *M. oleifera* is reported to exhibit a wide range of pharmacological effects that include Diuretic, antiulcer, hypotensive, hypolipidemic, hepatoprotective, antifungal, antibacterial, antitumor, antipyretic, antispasmodic and activities (Biswas *et al.,* 2012).

Based on the nutritional, biological, pharmacological activity of this plant the current study is designed to investigate the bioactivity, both qualitative and quantitative phytochemical analysis and the

antimicrobial potential of both flower and leaf extracts.

Materials and methods

Study location and plant materials

The experiments were conducted at the Cell Genetics and Plant Biotechnology Laboratory (CGPBL), Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh (23°53′14′′ N 90°15′56′′ E). The leaves and flowers of *M. oleifera* were collected from the germplasm centre of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1342, Bangladesh.

Preparation of extracts

The collected leaf and flower of *M. oleifera* were washed thoroughly using distilled water. After washing, samples were sun-dried for seven days and then dried in a hot air oven (JSR, Korea) at 50°C for 72 h. Both leaf and flower were ground separately in a mechanical grinder to get a uniform coarse powder. The powdered materials were taken in separated conical flasks with 70% ethanol and 70% methanol as the solvent and kept in an orbital shaker for three days at room temperature. The extracts were filtered using Whatman no. 1 (pore size 11 µm) filter paper and the filtrates were concentrated using an evaporator at 45°C, and finally, stock solutions of the extracts (100 mg/mL) were prepared using 0.1 N NaCl.

Determination of extraction yield

After the preparation of the stock solution of extracts, the extraction yield was determined. The yield coefficient is the ratio of the percentage of the weight of extract after evaporation per weight of dry powder taken (Polash *et al.*, 2017).

$$\text{Extraction yield} = \frac{\text{weight of extract agter evaporation}}{\text{weight of dry powder taken}} \times 100$$

Qualitative screening of phytochemicals

The phytochemical screening was carried out in terms of qualitative and quantitative analysis using different standard methods. Different extracts of *M. oleifera* were screened for the presence and absence ofalkaloid, carbohydrate, coumarin, flavonoid, glycoside, phenol, protein, resin, saponin, tannin and terpenoid.

Test for Alkaloid (Wagner's Test)

1 mL of different extracts of *M. oleifera* were taken into test tubes. Then 3-4 drops of Wagner's reagent (1.27 g iodine +2 g KI_2 in 100 mL distilled water) were added to the test tubes. After mixing, the solutions were heated at 60°C for 30 minutes in the heated water bath. The appearance of a radish brown color indicated the presence of alkaloids (Rizk, 1982).

Test for Carbohydrates (Fehling's Test)

An equal volume of Fehling's A (copper sulfate in distilled water) and Fehling's B (Na-K tartarate and sodium hydroxide in distilled water) reagents were taken into test tubes and mixed thoroughly. The tubes were boiled (65°C) in the water bath for a minute. Then an equal volume of different extracts of *M. oleifera* was added to the reagent mixture and boiled for 10-15 min. A primarily yellow color appeared and finally, a brick-red precipitate of cuprous oxide was obtained and indicated the presence of reducing sugars (Patel *et al.*, 2014).

Test for Coumarin

2 mL of different sample extracts were taken into test tubes. Then 2.5 mL of 10 % NaOH were added to each tube. The yellow color of the mixture indicated the presence of coumarin (Ugochukwu *et al.*, 2013).

Test for Flavonoids (Alkaline Reagent Test)

Few drops of 10 % NaOH were added to test tubes each containing 1 mL of sample extracts. The addition of NaOH to sample solution initiated the formation of intense yellow color and then the solution became colorless upon addition of few drops of diluted acid HCL. This indicates the presence of flavonoids (Mondal *et al.*, 2017).

Test for Glycosides

1 mL of glacial acetic acid were added to test tubes each containing 1 mL of sample extracts and then a

few drops of ferric chloride ($FeCl_3$) solution was added in each tube. The appearance of a brown color ring at the top indicated the presence of glycosides (Ahamed *et al.*, 2017).

Test for Phenols (Ferric Chloride Test)

1 mL of different sample extracts were mixed with 1 mL of distilled water or ethanol into test tubes. Then few drops of ferric chloride ($FeCl_3$) were added to the solution. The plant extracts containing phenolic compounds showed red, blue, green and purple color (Soloway *et al.*, 1952).

Test for Proteins (Xanthoprotic Test)

1 mL of each extract of *M. oleifera* wastaken into test tubes. 2-6 drops of concentrated HNO_3 were added to each tube. Then concentrated NaOH solution was added to neutralize the solution. The appearance of yellow or orange colour indicates the presence of protein and amino acids in the sample extracts (Mondal *et al.*, 2017).

Test for Resins

1 mL of different sample extracts was taken into test tubes. In each tube, few drops of acetic anhydride were added. Then 1 mL of concentrated sulphuric acid was added by the side of the test tubes. The presence of yellow to orange color confirmed the presence of resins in the extract samples (Iqbal *et al.*, 2015).

Test for Saponins (Foam Test)

1ml of the different extracts of *M. oleifera* taken in the test tubes containing 2ml of water and shaken for 5 minutes. The formation of a 1 cm thick layer of foam for 10 minutes indicated the presence of saponins (Kumar *et al.*, 2015).

Test for Tannins

1 mL of distilled water were added to test tubes containing 0.5 mL of different sample extracts and then 1-2 drops of ferric chloride ($FeCl_3$) solution were added in each tube. The blue color was appeared indicating the presence of gallic tannin and the green black color was appeared indicating the presence of catecholic tannin (Patel *et al.*, 2014).

T*est for Terpenoids (Salkowski test)*

5 mL of different sample extracts were taken into test tubes and mixed properly with 2 mL of chloroform. Then 3 mL of concentrated sulphuric acid was added to each tube. The appearance of the reddish-brown coloration at the interface indicates the presence of terpenoids (Sheel *et al.,* 2014).

Determination of total phenolic content

The total phenol present in the different extracts of *M. oleifera* was determined using the method of (Shohael *et al.,* 2006a). Briefly, 0.1 mL of each extract and standards were mixed with 2.5 mLdistilled water which is followed by the addition of 0.1 mL (2 N) Folin–Ciocalteu reagent. After vigorous mixing, the mixture was kept at room temperature for 6 min. Following that, 0.5 mL of 20% sodium carbonate (Na_2CO_3) solution was added. After all test tubes containing the respective mixture were incubated for 30 min at room temperature in the dark and then color changed was observed.

The absorbance was measured at 760 nm in a UV–visible spectrophotometer (T60 UV–Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). Total phenolic content was expressed as microgram of Gallic Acid ($C_7H_6O_5$) Equivalent (µg GAE)/ml of extract.

Determination of total flavonoid content

The total flavonoid content of the different sample extracts was determined by the method of (Shohael *et al.,* 2006b). 0.25 mL of each extract/standard was mixed with 0.25 mL distilled water in test tubes. Then, 75 µL of 5% sodium nitrate solution was mixed extract/standard. After 6 min, 0.15 mL of 10% aluminum chloride ($AlCl_3$) solution was added, and the mixture was allowed to stand for a further 5 min and then 0.5 mL of 1 M sodium hydroxide (NaOH) was added. Again, 2.5 mL distilled water was mixed properly. The absorbance was measured instantly at 510 nm in a T60 UV–visible spectrophotometer. The concentration of flavonoids was expressed as microgram of Catechin ($C_{15}H_{14}O_6$) Equivalent (µg CE)/ml of extract.

Determination of total tannin content

The tannins were determined by the Folin-Ciocalteu method. At first 0.1 mL of each extract was added to a test tube (10 mL) containing 7.5 mL of distilled water. Then 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35 % Na_2CO_3 solution were added to each test tube. Finally, the volume was adjusted to 10 mL using distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV-Visible spectrophotometer. The tannin content was expressed in terms of µg of TAE/ml of extract. This protocol of total tannin estimation was adopted from (Tambe *et al.,* 2014) with some modifications.

Determination of total protein content

To determine total protein content bovine serum albumin (BSA) was used as a positive control. At first, 0.2 mL of each extract was taken in test tubes. After that, 2 mL of alkaline copper sulphate reagent (analytical grade reagent) was added to the tubes and mixed the solutions properly by vortex mixer. Subsequently, the mixture was incubated at room temperature for 10 min. Then 0.2 mL of Folin-Ciocalteau reagent was added to each tube and these were incubated for 30 min. The optical density was measured at 660 nm with a UV-vis spectrophotometer. This method was adopted from (Peterson, 1977) and is also known as the Lowery method.

Antioxidant activity

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay of *M. oleifera* leaf and flower extracts was performed according to (Tanvir *et al.,* 2017) with little modification. Different concentrations (62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL and 2000 µg/mL) of standard or sample extracts were prepared and placed into different test tubes. From prepared samples, 0.2 mL of the different concentrations of standard and sample extracts were taken into different test tubes.Then 1.5 mL of FRAP reagent was added to each tube. Then the mixtures were incubated for 4 min at 37 °C in the heated water bath. After that time, the absorbance of the mixtures was measured at 593 nm using UV–visible spectrophotometer. The absorbance of the standard was plotted against concentration to prepare a standard curve. The absorbance of the sample extracts was expressed as microgram of Green vitriol ($FeSO_4.7H_2O$) equivalent per millilitre (µgFE/mL).

Estimation of total Vitamin-C content

Total vitamin-C content is estimated by following the (Omaye *et al.,* 1979) colorimetric method with slight modification. Different concentrations (125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL) of sample extracts and standard were prepared of which 500 µL of sample and standard were taken in each test tube. Then 100 µL 2,4- dinitrophenylhydrazine thiourea-copper (DTC) solution was added to each tube. The solutions were mixed properly and incubated for 3 hours at 37°C. After that, 750 µL of ice-cold 65% sulphuric acid was added to each tube and mixed thoroughly. Then, tubes were kept at room temperature for 30 minutes. The absorbance of the standards and samples were measured at 530 nm using a UV-Visible spectrophotometer. The ascorbic acid content of sample extracts of various dilutions was expressed as microgram of ascorbic acid per mL of sample extracts (µg AA/mL).

Estimation of Total Reducing Sugar Content

The total reducing sugar (carbohydrates) content of *M. oleifera* flower and leaf extracts was determined by the Nelson-Somogyi method with some modification (Somogyi, 1952). Different dilutions of standards and sample solutions were prepared in different falcon tubes or test tubes. 1 mL of different dilutions of standards and sample solution was pipetted out to different falcon tubes. 1 mL of copper reagent was added to each tube and mixed properly by vortex mixer. These solutions were boiled at 90°C for 15 minutes in the water bath and then cooled. After cooling, 1 mL of arsenomolybdate (color reagent) was added to the mixture and the solutions were mixed well. The optical density was measured at 520 nm with a UV-vis spectrophotometer against a blank containing reagent and distilled water, without

any sample or standard solution, which was also prepared through the above procedure. The absorbance of glucose solutions was plotted against concentration to get a standard calibration curve. Total glucose content present in each sample extract was expressed as microgram of glucose equivalent per mL of sample extracts (µg Glucose/mL).

Screening of Antimicrobial Activity

Antibacterial activity of ethanolic and methanolic extracts of *M. oleifera* leaf and flower extracts was determined by the disc diffusion method according to the protocol described (Polash *et al.,* 2017).

The bacterial strains used for the experiment were collected as pure cultures from the Dept. of Biotechnology and Genetic Engineering, Jahangirnagar University. Both gram-positive and gram-negative organisms were used for this test.The organisms used in this research were *Bacillus subtillis and Escherichia coli.* All isolates were sub-cultured onto selected culturing media to ensure purity and confirm their identification. The test organisms were cultured overnight at 37°C before being used in the antibacterial assay described below.

Four different concentrations of ethanolic and methanolic extracts of *M. oleifera* leaf and flower extracts were used in this antibacterial screening. These were 10 mg/ml, 7.5 mg/ml, 5 mg/ml and 2.5 mg/ml prepared by dissolving each 10 mg, 7.5mg, 5 mg and 2.5 mg of 3 solvent extracts of leaf and flower in 1 ml of 0.9 % NaCl solution respectively. The mixtures were prepared in centrifuge tubes. Prepared concentrations of each extract were tested against the two types of bacterial strain indicated above.

To perform an antibacterial activity, discs were prepared from Whatman no. 3 filter paper. The diameter of a disc was 5.55 mm. Standard discs (positive control) were prepared by soaking the filter paper discs with tetracycline solution (10 mg/ml).Blank discs were used as negative controls which were prepared by soaking the filter paper discs with 0.9% NaCl solution. Sample discs were prepared by soaking the filter paper discs with the ethanolic and methanolic extracts of *M. oleifera* flower and leafextracts. The zone of inhibition around the sample discs indicated the antibacterial activity of *Moringa oleifera* leaf and flower extracts. At first, the Petri plates were marked properly. The soaked filter paper discs were air-dried in a laminar airflow hood and placed on the LB agar medium that was pre-inoculated with the test organisms by sterile forceps.

The discs were placed separately on the medium. After placing the discs on the previously marked zone on the agar medium, the plates were kept upside down in the incubator at 37 °C to let the bacteria grow. The zone of inhibition was measured at each hour. In this manner, the zone of inhibition was measured for 8 hours. After 24 hours, the zone of inhibition was also measured.

Results

Extraction yield

The extraction yield of each sample in different solvents are summarized in Table 1. However, results showed a significant difference in the extraction yield using different solvents. Among solvents tested, the highest extraction yield (12.8%) was found in ethanol (leaf) which is followed by methanol leaf (12%), methanol flower (11.7%), and ethanol flower (7.1%).

Table 1. The percentage yield of *M. oleifera* leaf and flower powder in different solvents.

Solvent used	Extraction yield (%)
Methanol (flower)	11.7
Ethanol (flower)	10.9
Methanol (leaf)	12
Ethanol (leaf)	12.8

Qualitative screening of phytochemicals

Phytochemical screening was done for both flower and leaf extracts in two solvents (ethanol and methanol). The obtained result is presented in Table 2. From the qualitative findings, it is observed that both extracts of Sazna (*Moringa oleifera)* confirmed

the presence of alkaloids, carbohydrate, flavonoids, coumarin, flavanoids, glycosides, phenols, proteins, saponins, tannins, terpenoids and. Surprisingly, resin was only found for SLM (sazna methanolic leaf) extract. Terpinoids were present in SLE and SLM extracts. Unfortunately, Terpinoids was absent in SFE and SFM. However, alkaloids, coumarin, flavonoids, phenol, protein,saponin and tanninwere present in SFE (Sazna ethanolic flower), SFM (Sazna methanolic flower), SLE (Sazna ethanolic flower) and SLM (sazna methanolic leaf) extracts.

Table 2. Qualitative screening of bioactive compounds of ethanolic and methanolic extracts of flower and leaf from *M. oleifera*.

Phytochemicals	SFE	SFM	SLE	SLM
Alkaloid	+++	+++	+	++
Carbohydrate	+	-	+++	+
Coumarin	+++	+++	+++	+++
Flavonoid	+	+	+++	+++
Glycoside	+++	+++	++	+
Phenol	+++	+++	+++	+++
Protein	+++	+++	+++	+++
Resin	-	-	-	+
Saponin	+++	+++	++	+++
Tannin	+++	+++	+++	+++
Terpenoids	-	-	+	++

Indication: +++ = High; ++ = Moderate; + = Low; - = Not detected.

Quantitative determination of total phenolic, flavonoid, tannin and protein content

The presence of these phytochemicals can be confirmed preliminary by qualitative screening. However, to determine the amount it is necessary to perform quantitative analysis. In the present study, the quantitative analysis was performed for phenol, flavonoid, tannin and protein content.

The quantitative determination of phytochemicals present in ethanolic and methanolic extracts of *M. oleifera* flower and leaf is presented in Fig. 1.

Table 3. Inhibition zone (mm±S.E.M) of crude extracts of *M. oleifera against B. Subtilis*.

Solvent	Parts of extracts	Concentration of crude extracts of *M. oleifera against B. subtilis*			
		10	7.5	5	2.5 (mg/disc)
		Inhibition zone (mm)			
Ethanol	Flower	7.2±0.49	7.1±0.90	6.2±0.25	5.6±0.52
	Leaf	10.5±0.50	8.73±0.64	7.5±0.50	7.43±0.50
Methanol	Flower	14.5±0.5	13.03±0.55	12.5±0.76	11±0.52
	leaf	11.4±0.52	9.8±0.76	8.26±0.25	7.2±0.25
Tetracycline (30µg/ mL)		17-22			

The content of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent. From the figure 1A, it is clear that, higher amount phenol present in SMF extract at a concentration 100 µg/mL. For total phenol content (Fig. 1A), can be interpreted as SFM (100µg/mL) > SFE (100µg/mL) >SLM (100µg/mL) > SLE (100µg/mL) > SFM (50µg/mL) > SFE (50µg/mL) > SLM (50µg/mL) > SLE (50µg/mL) extract. Figure 1B clearly indicated that the highest amount of flavonoid

is reported in SFE whereas least amount was observed in SLE extract (Fig. 1B).For flavonoid content the amount present in the both flower and leaf extracts can be interpreted as SFE (2000µg/mL) > SFM(2000µg/mL) > SLE(2000µg/mL) > SLM(2000µg/mL) > SFM(1000µg/mL) > SLM(1000µg/mL) > SFE(1000µg/mL) > SLE(1000µg/mL).

Table 4. Inhibition zone (mm±S.E.M) of crude extracts of *M. oleifera against E. coli.*

Solvent	Parts of extracts	Concentration of crude extracts of *M. oleifera against E. coli*			
		10	7.5	5	2.5 (mg/disc)
		Inhibition zone (mm)			
Ethanol	Flower	6.06±0.45	8.34±0.56	7.44±0.45	5.50±0.43
	Leaf	9.5±0.68	8.73±0.64	8.3±0.50	6.43±0.50
Methanol	Flower	9.5±0.50	7.96±0.45	6.9±0.60	6.04±0.45
	leaf	10.2±0.25	8.26±0.25	7.8±0.80	6.4±0.59
Tetracycline (30µg/ mL)			17-22		

The result of total tannin is presented in Fig. 1(C). Based on the result, the highest amount of tannin was observed for SLE at a concentration (100µg/mL). Unfortunately, SFE extract contains the lowest amount of tannin at a concentration (50µg/mL).The data revealed that all the *Moringaoleifera*extracts showed a significant amount of total protein content in a dose-dependent manner.

The total protein content has been expressed as BSAEµg/mL (Bovine Serum Albumin equivalent) as presented in Fig. 1(D). Total protein content present in both flower and leaf can be interpreted as SFM (1000µg/mL) > SFE (1000µg/mL) > SLE (1000µg/mL) > SLM (1000µg/mL) > SFM (800µg/mL) > SFE (800µg/mL) > SLE (800µg/mL) > SLM (800µg/mL).

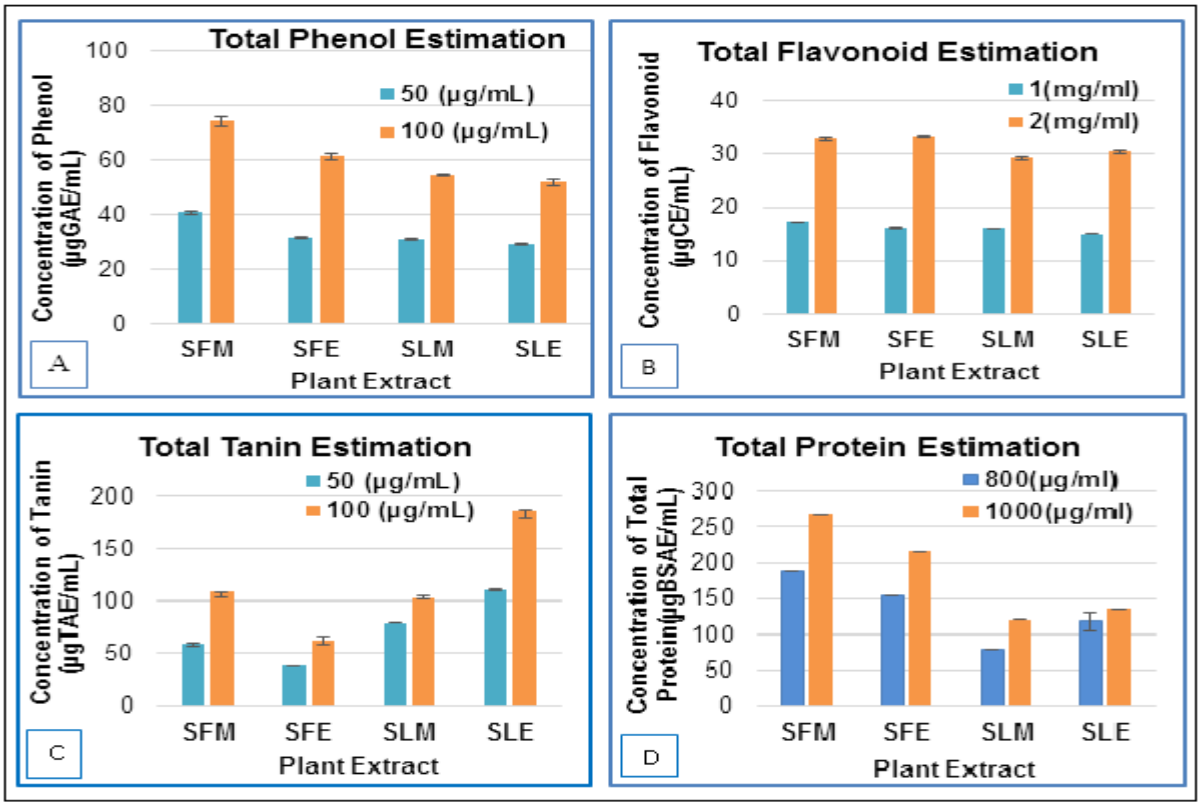

Fig. 1. Quantitative determination of (A) total phenolic content; (B) total flavonoid content and (C) total tannin content in methanolic and ethanolic extracts of flowers and leaf from Sazna (Moringa *oleifera).*

Determination of antioxidant activity

Antioxidant activity of *M. Oleifera* was evaluated in terms of reducing sugar test and Ferric reducing antioxidant activity both are widely used. The obtained result is depicted in Fig. 2**.**

The data revealed that all the *M.oleifera* extracts showed a significant amount of antioxidant activity in a dose-dependent manner. However, the ferric reducing antioxidant power assay (FRAP) is measured as GVEµg/ml (Green vitriol equivalent). Result reveals that the ferric reducing antioxidant power of the extracts was in the increasing trend with the concentrations.The Ethanolic extract of flower (SFE; $P < 0.0001$) showed all the three activities with the highest efficiencies (202.18±0.087 µg/mL) followed by the other three extracts (SFM, SLM, and SLE).

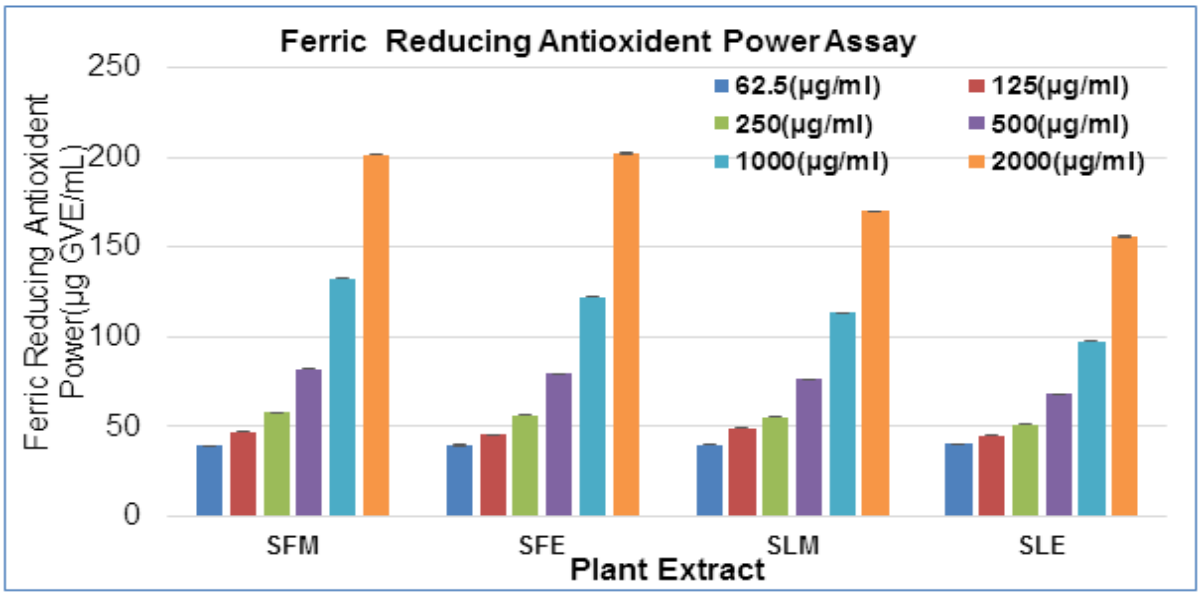

Fig. 2. Antioxidant activity of all extracts with respect to $Fe_2SO_4.7H_2O$. SLM= Sazna Leaf Methanol, SLE= Sazna leaf Ethanol, SFM= Sazna Flower Methanol, SFE= Sazna Flower Ethanol, GVE= Green Vitriol Equivalent.

Reducing power assay

The total reducing sugar content has been expressed as GE µg /mL (Glucose Equivalent). The data revealed that all the *M. oleifera* extracts showed a significant amount of total reducing sugar content in a dose-dependent manner as represented in Fig. 3. Among the different extracts, SFM showed the highest amount of reducing sugar content was found for (896.55±0.77 µg/mL) at a concentration of 1000 µg/mL showed the and the lowest amount among them is found for SLE (150.73±0.288 µg/mL) at a concentration of 250 µg/mL.

Total Vitamin C content

The total Vitamin-C content has been expressed as AAE µg /mL (Ascorbic acid equivalent). The data revealed that all the extracts *M. oleifera* showed a moderate amount of Vitamin-C content in a dose-dependent manner as presented inFig. 4. Among the flower and leaf extracts of *M. oleifera* highest Vit-C content (6.81±0.007 µg/mL) was obtained for SFM at a concentration of 1000µg /mL. However, the lowest amount among them is found in SLE (1.13±0.007µg/mL) at a concentration of 125µg /mL.

Antibacterial activity

The antibacterial activity of ethanolic and methanolic flower and leaf extracts of *M. oleifera* are shown in Tables 3 and 4. Antibacterial activity was measured by determining the diameter of the zone of inhibition in millimetres against both bacteria.

These data revealed that almost all the ethanolic, methanolic flower and leaf extracts of *M. oleifera* have antibacterial activity against both gram-positive and gram-negative bacteria and the activity is

concentration-dependent. The maximum activity was observed for methanolic flower extract (14.5±0.5 mm) against gram-positive bacteria at a concentration of 10 mg/disc (Table 3). Maximum antibacterial activity was found for the methanolic leaf extract (10.2±0.25) against gram-negative bacteria (Table 4) at a concentration of 10 mg/disc. Against gram-positive bacteria,the lowest activity was obtained for ethanolic flower extract (5.6±0.52 mm) at a concentration of 2.5 mg/disc. The lowest activity against gram-negative bacteria was observed for ethanolic flower extract (5.50±0.43 mm) at a concentration of 2.5 mg/disc. Tetracycline and saline were used as the positive and negative control, respectively. Tetracycline showed the highest zone of inhibition (17-22 mm) and saline showed no zone of inhibition.

Discussion

Wide ranges of secondary metabolites (also known as phytochemicals) are found in plants that are ubiquitously present.

These phytochemicals are not directly associated with plant growth and development but they might play role in reproduction and survival (Böttger *et al.,* 2018).

These phytochemicals are widely distributed among leaves, stems and roots, flower, fruits and so on. The amount of the phytochemicals depend not only on the different parts of plants but also on the solvent used for the extraction procedure (Yadav and Agarwala, 2011).

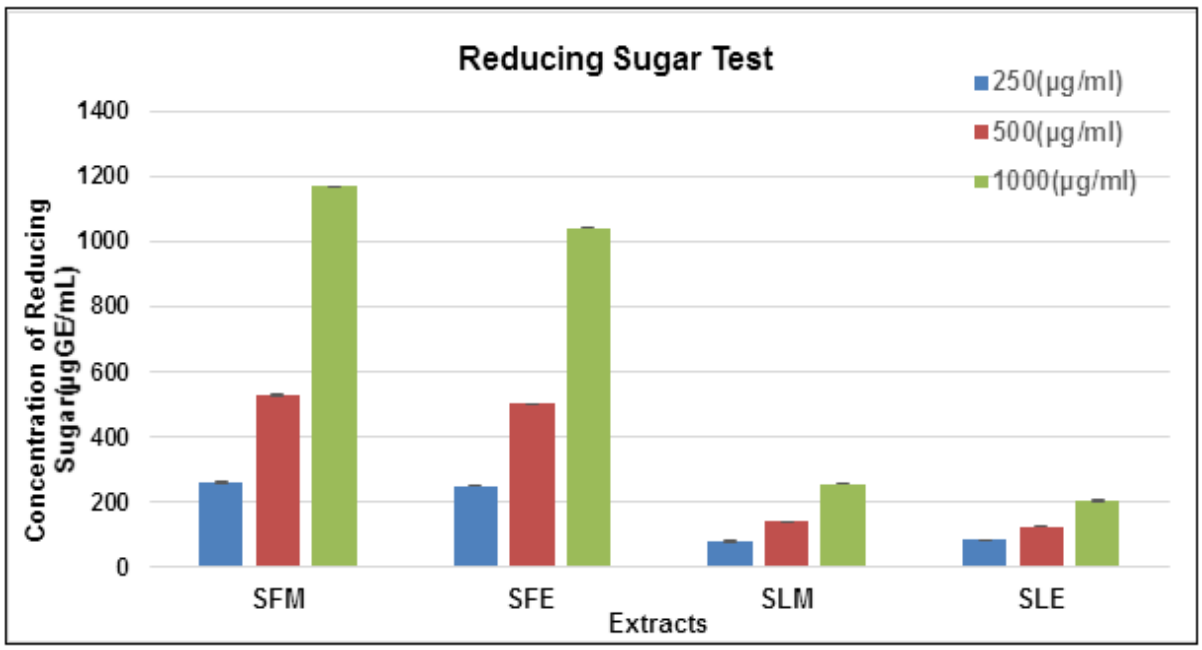

Fig. 3. Total reducing sugar content of all extracts with respect to glucose.SLM= Sazna Leaf Methanol, SLE= Sazna leaf Ethanol, SFM= Sazna Flower Methanol, SFE= Sazna Flower Ethanol, Extracts, GE=Glucose Equivalent.

The qualitative screening of the present study revealed that this plant contains different types of phytochemicals such as alkaloids, carbohydrate, flavonoids, coumarin, flavanoids, glycosides, phenols, proteins, saponins, tannins, terpenoids in both flower and leaf extracts. Whereas quantitative study also showed the presence of a significant amount of phenol, flavonoids, tannin and protein in both leaf and flower extracts. Current findings are similar to the result previously performed by (Adline and Devi, 2014 and Fahal *et al.,* 2018). From a previous study, it was found that phytochemicals such alkaloids, terpenoids, tannin have antitumors, antituberculosis, antimicrobial, antidiarrheal antiviral, antibacterial, antimalarial, anti-inflammatory activities **(**Das and Gezici, 2018). This investigation confirms different phytochemicals which in turn encouraged an investigationof pharmacological activity *in vivo*

model. Unstable chemical species are produced continuously by the free radicals are in the human body. For the oxidation of biomolecules, such as protein, lipid, amino acids and DNA, free radicals act as primary and main initiators which will lead to cell injury and can induce numerous diseases (Hsu *et al.,* 2003). However, the imbalance between antioxidants and reactive oxygen species results in oxidative stress and this oxidative stress is highly responsible for cellular damage and this is considered as the main cause of several diseases: cancer, cataracts, age-related diseases and Parkinson's disease (Erkan *et al.,* 2008).

The presence of flavonoids and phenolic compounds are responsible for antioxidant activity in the plant (Kähkönen *et al.,* 1999). The present study showed that both leaf and flower extracts exhibited good antioxidant activity in FRAP assay, therefore this plant can be used in antioxidant-related disease remedies. A previous study conducted by Luqman *et al.,* 2012 also showed that both ethanolic and aqueous extract of *Moringa* fruit and leaf has significant antioxidant activity.

The presence of phenolic and flavonoids compounds of *M. oleifera* may be contributed to its antioxidant activity.The present study showed significant antibacterial activity of *M. oleifera* against both gram-positive and gram-negative bacteria.

A similar antibacterial activity was reported by Singh and Tafida, 2014 and they showed the antibacterial activity against both gram-positive and gram-negative bacteria on three different *M. oleifera* extracts (methanol, ethanol and water).

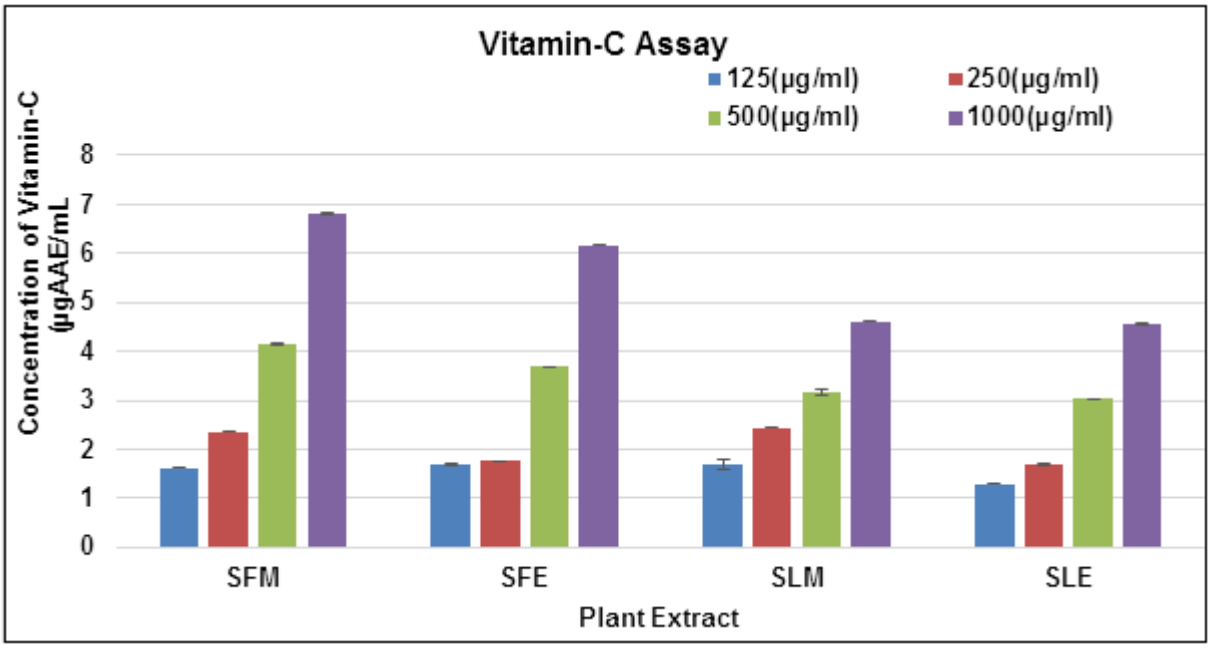

Fig. 4. Vitamin-C content determination of all extracts with respect to Ascorbic acid. SLM= Sazna Leaf Methanol, SLE= Sazna leaf Ethanol, SFM= Sazna Flower Methanol, SFE= Sazna Flower Ethanol.

Conclusion

According to the results found in this study, it can be summarized that phytochemicals present in the flower and leaf extracts can be used as a major source of therapeutic compounds. Both (leaf and Flower) extracts of *M. Oleifera* exhibited antioxidant activity which may be correlated with the presence of high phenolic compounds in the extracts. Total phenolic content had a positive correlation with antioxidant capacity. The plant extract contains bioactive principles which are active in the inhibition of free radicals. This study, therefore, supports the use of the extract *M. oleifera* is a medicinal plant. An extensive investigation needs to be carried out on the antioxidant properties and toxicity of the plant.Both extracts showed significant antimicrobial activity. To

elucidate the potential mechanism of action of these extracts, further investigations are highly recommended.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors are grateful to Cell Genetics and Plant Biotechnology Laboratory, Department of Biotechnology and Genetic engineering, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh for providing the financial support and instrumental facilities to carry out the research.

References

Adline J, Devi A. 2014. A study on phytochemical screening and antibacterial activity of *Moringa oleifera*. International Journal of Research in Applied, Natural and Social Sciences **2,** 169-176.

Ahamed T, Rahman SM, Shohael AM. 2017. Thin layer chromatographic profiling and phytochemical screening of six medicinal plants in Bangladesh. International Journal of Biosciences **11,** 131-140. http://dx.doi.org/10.12692/ijb/11.1.131-140.

Bamishaiye EI, Olayemi FF, Awagu EF, Bamshaiye OM. 2011. Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Advance Journal of Food Science and Technology **3,** 233-237.

Biswas SK, Chowdhury A, Das J, Roy A, Hosen SZ. 2012. Pharmacological potentials of *Moringa oleifera* Lam.: a review. International Journal of Pharmaceutical Sciences and Research **3,** 305.

Böttger A, Vothknecht U, Bolle C, Wolf A. 2018. Plant secondary metabolites and their general function in plants. Lessons on caffeine, cannabis & co, 3-17.

Dahiru D, Onubiyi JA, Umaru HA. 2006. Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aqueous leaf extract. African Journal of Traditional, Complementary and Alternative Medicines **3,** 70-75.

Das K, Gezici S. 2018. Secondary plant metabolites, their separation and identification, and role in human disease prevention. Annals of Phytomedicine **7,** 13-24. https://doi.org/10.21276/ap.2018.7.2.3

Demiray S, Pintado ME, Castro PML. 2009. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tiliaargentea, Crataegi folium leaves and Polygonumbistorta roots. World Academy of Science, Engineering and Technology **54,** 312-317.

Duraipandiyan V, Ayyanar M, Ignacimuthu S. 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine **6,** 1-7. https://doi.org/10.1186/1472-6882-6-35.

Erkan N, Ayranci G, Ayranci E. 2008. Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food chemistry **110,** 76-82. https://doi.org/10.1016/j.

Fahal ME, Rani BMA, Aklakur MD, Chanu TI, Saharan N. 2018. Qualitative and quantitative phytochemical analysis of *Moringa oleifera* (Lam) Pods. International Journal of Current Microbiology and Applied Sciences **7,** 657-665. https://doi.org/10.20546/ijcmas.2018.705.080.

Gupta RK. 2010. Medicinal and aromatic plants. CBS publishers and distributors **234,** 499.

Hsu CL, Chen W, Weng YM, Tseng CY. 2003. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by

different drying methods. Food chemistry **83**, 85-92. https://doi.org/10.1016/S0308-8146(03)00053-0.

Iqbal E, Salim KA, Lim LB. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. Journal of King Saud University-Science **27,** 224-232. https://doi.org/10.1016/j.jksus.2015.02.003.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry **47**, 3954-3962.

Kumar GP, Anilakumar KR, Naveen S. 2015. Phytochemicals Having Neuroprotective Properties from Dietary Sources and Medicinal Herbs. Pharmacognosy Journal **7,** 1-17.

Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. 2012. Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using in vitro and in vivo assays. Evidence-Based Complementary and Alternative Medicine **2012,** 1-12.

Mahmood KT, Mugal T, Haq IU. 2010. *Moringa oleifera*: a natural gift-A review. Journal of Pharmaceutical Sciences and Research **2,** 775.

Mondal R, Sarker RC, Banik PC. 2017. Knowledge attitude and behavior towards dietary salt intake among Bangladeshi medical and nonmedical undergraduate students. International Journal of Perceptions in Public Health **2,** 31-7.

Omaye ST, Turnbull JD, Sauberlich HE. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. In Methods in enzymology **62,** 3-11. https://doi.org/10.1016/0076-6879(79)62181-X.

Paikra BK. 2017. Phytochemistry and pharmacology of *Moringa oleifera* Lam. Journal of pharmacopuncture **20,** 194.

Patel NA, Patel PL, Patel DA, Desai SV, Meshram DY. 2014. Phytochemical analysis and antibacterial activity of *Moringa oleifera*. International Journal of Medicine and Pharmaceutical Sciences **4,** pp.27-34.

Peterson GL. 1977. A simplification of the protein assay method of Lowry *et al*. which is more generally applicable. Analytical biochemistry **83,** 346-356. https://doi.org/10.1016/0003-2697(77)90043-4

Polash SA, Saha T, Hossain MS, Sarker SR. 2017. Investigation of the phytochemicals, antioxidant, and antimicrobial activity of the *Andrographis paniculata* leaf and stem extracts. Advances in Bioscience and Biotechnology **8,** 149. https://doi.org/10.4236/abb.2017.85012

Rizk AM. 1982. Constituents of plants growing in Quatar. 1. A chemical survey of sixty plants. Fitoterapia **53,** 35-44.

Saini RK, Sivanesan I, Keum YS. 2016. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. Biotech **6,** 1-14.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines **8,** 1-10. https://doi.org/10.4314/ajtcam.v8i1.60483

Sheel R, Nisha K, Kumar J. 2014. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR Journal of Applied Chemistry **7,** 10-13.

Shohael AM, Ali MB, Yu KW, Hahn EJ, Paek KY. 2006a. Effect of temperature on secondary

metabolites production and antioxidant enzyme activities in *Eleutherococcus senticosus* somatic embryos. Plant Cell, Tissue and Organ Culture **85,** 219-228. https://doi.org/10.1007/s11240-005-9075-x.

Shohael AM, Ali MB, Yu KW, Hahn EJ, Islam R, Paek KY. 2006b. Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. Process Biochemistry **41,** 1179-1185. https://doi.org/10.1016/j.procbio.2005.12.015.

Singh K, Tafida GM. 2014. Antibacterial activity of *Moringa oleifera* (Lam) leaves extracts against some selected bacteria. International Jouranl of Pharmacy and Pharmaceutical Sciences **6,** 52-54.

Somogyi M. 1952. Notes on sugar determination. Journal of biological chemistry **195,** 19-23.

Tambe VD, Bhambar RS. 2014. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. Journal of pharmacognosy and phytochemistry **2,** 41-47.

Tanvir EM, Hossen M, Hossain M, Afroz R, Gan SH, Khalil M, Karim N. 2017. Antioxidant properties of popular turmeric (*Curcuma longa*) varieties from Bangladesh. Journal of Food Quality. https://doi.org/10.1155/2017/8471785

Ugochukwu SC, Uche A, Ifeanyi O. 2013. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian Journal of Plant Science and Research **3,** 10-13.

Vaidya AD, Devasagayam TP. 2007. Current status of herbal drugs in India: an overview. Journal of clinical biochemistry and nutrition **41,** 1-11. https://doi.org/10.3164/jcbn.2007001

Valdez-Solana MA, Mejia-Garcia VY, Téllez-Valencia A, Garcia-Arenas G, Salas-Pacheco J, Alba-Romero JJ, Sierra-Campos E. 2015. Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. Journal of Chemistry**5, 1-10.** https://doi.org/10.1155/2015/860381

Yadav RNS, Agarwala M. 2011. Phytochemical analysis of some medicinal plants. Journal of phytology **3,** 10-14.