



Received on 09 September, 2016; received in revised form, 02 November, 2016; accepted, 17 November, 2016; published 01 March, 2017

CHARACTERIZATION OF ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *AEGLE MARMELLOS* (L.)

Nusrat Jahan Bristy¹, A. H. M. Nazmul Hasan^{* 2}, Md. Nur Alam¹, Tania Binte Wahed¹, Panna Roy and K M Khairul Alam¹

Department of Pharmacy¹, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Department of Pharmacy², University of Asia Pacific, 74/A, Green Road, Dhaka-1215, Bangladesh.

Keywords:

Aegle marmelos(L.),
Antioxidant, Cytotoxicity,
DPPH, Flavonoids, Phenols

Correspondence to Author:

A. H. M. Nazmul Hasan


Lecturer, Department of Pharmacy
University of Asia Pacific, 74/A,
Green Road, Dhaka-1215,
Bangladesh.

E-mail: nazmul_0155@yahoo.com

ABSTRACT: *Aegle marmelos*(L.), family rutaceae is highly reputed medicinal tree commonly known as the bael. All parts of the plant have medicinal properties and extensively used by the traditional medicine practitioners of Bangladesh in different health ailments like Diabetes, Diarrhea, Jaundice, typhoid. The present study was dedicated to investigate phytochemical and pharmacological properties of ripe fruit, half-ripe fruit, leaf and seed of the plant extracted with methanol solvent. Initial phytochemical screening confirmed the presence of different phytoconstituents including alkaloid, flavonoids, carbohydrate, glycoside, saponin, tannin, glucoside and steroids in different extracts. Antioxidant potential was evaluated using total phenol and total flavonoid contents determination assays, total antioxidant capacity, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, NO radical scavenging assay, reducing power assessment, CUPRAC (Cupric Reducing Antioxidant Capacity), total alkaloid determination assay. All the plant parts were found to possess moderate amounts of phenolics and flavonoids, expressed as gallic acid equivalent (GAE) and quercetin equivalent (QE) respectively. The methanolic extract of half-ripe fruit demonstrated highest phenolic content (6.05 mg/gm GAE) while methanol extract of seed exhibited highest flavonoid content (4.29 mg/gm QE). In addition, the plant parts displayed total antioxidant capacity expressed as ascorbic acid equivalent (AAE) with methanol extract of leaf being the most potent one (10.31 mg/gm AAE). In DPPH radical and NO radical scavenging methods, a dose dependent scavenging of DPPH and NO radical was observed by all the extractives. Highest DPPH radical scavenging was demonstrated by methanol extract of half-ripe fruit with IC₅₀ value of 251.2 µg/ml whereas IC₅₀ value of standard ascorbic acid was noted as 18.4 µg/ml. In case of NO radical scavenging method, highest NO radical scavenging was also demonstrated by methanol extract of half-ripe fruit with IC₅₀ values of 46.364 µg/ml. However in reducing power and CUPRAC assays, methanol extract of half-ripe fruit and leaf were found to exhibit moderate but concentration dependent reducing power respectively. In total alkaloid content determination methanol extract of seed showed the highest alkaloid content expressed as atropine equivalent (4.86 mg/gm). In Brine Shrimp Lethality Bioassay, all the extracts produced dose dependent cytotoxicity effect to brine shrimp nauplii with methanol extract of seed exhibiting highest toxicity having LC₅₀ value 86.77 µg/ml where standard vincristine sulphate had the LC₅₀ value of 2.47 µg/ml.

INTRODUCTION: The science and practice of medicine plays significant role in identifying the new molecules from natural source and the history of drug from natural sources is very noteworthy and prominent.

Plants are considered as natural chemical factory and secondary metabolite obtained from plant sources have distinctive pharmacological effects^{1, 2, 3}. The vital cellular components undergo oxidative damage with excessive generation of reactive oxygen species and responsible for many chronic disease. Antioxidants are such chemical species that protect vital cellular components and there is been always an effort to search safe and effective antioxidant from natural sources as synthetic antioxidants possess many side effects^{3, 4}.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.8(3).1476-84</p> <hr/> <p>Article can be accessed online on: www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(3).1476-84</p>
---	--

Medicinal plants possess antioxidant activity due to the presence of phenolic compounds and they also have other phytoconstituents which are also responsible for various pharmacological effects⁵.

Bangladesh is a land rich of medicinal plants and there is still many plants remained unexplored for their medicinal properties. *Aegle marmelos* (L.), commonly known as Bael belonging to the family Rutaceae is widely used in Bangladesh particularly to make juice of its fruit pulp and also used in the sub continent for its various medicinal properties⁶. This plant is although native to northern parts of India, but also widely distributed throughout the Bangladesh, Myanmar, Ceylon, Thailand and Indochina⁷. *A. marmelos* is a slow-growing, medium sized tree, up to 12 to 15 m tall with short trunk, thick, soft, flaking bark and the fruit is round, pyriform, oval, or oblong, 5 to 20 cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, gray-green until the fruit is fully ripe, when it turns yellowish⁸. Both ripe and unripe fruit, as well as the roots, leaves and branches have all been used in traditional medicine system in Bangladesh. The present study was designed to investigate different phytochemical groups, antioxidant potential and cytotoxic activity of methanolic extracts of leaves, ripe fruit, unripe fruit and seeds of *Aegle marmelos*.

MATERIALS AND METHODS:

Study design: The present study was designed to investigate the presence of various phytoconstituents, antioxidant and cytotoxic potential of the methanolic extracts of ripe fruit, half ripe fruit, seeds and leaves of *Aegle marmelos*.

Collection and identification of plant parts: The whole plant was collected from Jahangirnagar University, Savar, Dhaka, Bangladesh and identified by the taxonomist of the department of botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Chemicals and Reagents: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, sodium nitroprusside, sodium phosphate, sulphanilamide, phosphoric acid and naphthylethylenediamine were obtained from SD Fine Chem. Ltd, India. Quercetin, Gallic acid and Folin-Ciocalteu reagent (FCR), Vincristine, Di-methyl sulphoxide

(DMSO), ammonium molybdate was obtained from Merck, Germany. Ferric chloride and neocapriner were obtained from Sigma Chemical Co.

Extraction process: The plant parts were collected in fresh condition and sun-dried first and then, dried in an oven at reduced temperature (< 70°C) to make suitable for grinding. The powdered plant materials were submerged in sufficient volume of methanol in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The extracts were then filtered and dried on electrical water bath^{2,9}.

Evaluation of phytoconstituents: The crude extracts of different parts of the plant were qualitatively tested for the presence of phytoconstituents and these were identified by characteristic color changes using standard procedures¹⁰.

Determination of Total Phenol: Total phenolic content was determined by Folin-Ciocalteu Reagent (FCR)¹¹. The FCR actually measures a sample's reducing capacity. 1.0 mL of each plant extracts or standard of different concentration solution were taken in test tubes and 5 mL of Folin - ciocalteu (Diluted 10 fold) reagent solution was added to the test tubes. 4 mL of Sodium carbonate solution was added into the test tubes. The test tubes were incubated for 30 minutes at 20°C to complete the reaction (Only for standard). The test tube was incubated for 1 hour at 200°C to complete the reaction (Only for extract). The absorbances of the solutions were measured at 765 nm using a spectrophotometer against blank. Standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of Gallic acid and total phenol values was expressed in terms of Gallic acid equivalent, which is a common reference compound.

Determination of Flavonoid content: Total flavonoid was determined by aluminum chloride colorimetric method described by Chang et al¹². 1 mL of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer.

The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/mL of sample.

Determination of Total Antioxidant Capacity:

The phosphomolybdenum method of antioxidant capacity determination is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH¹³. Sample extracts (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against blank. Total antioxidant capacity of the extract was measured from the regression equation prepared from the concentration versus optical density of ascorbic acid.

DPPH free radical scavenging assay: DPPH scavenging activity of the plant was measured by the method developed by Manzocco et al¹⁴. The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated from the measured absorbance data. Ascorbic acid was used as a reference or standard antioxidant in this assay method. The percentage (%) inhibition activity was calculated from the following equation:

$$\frac{(A_0 - A_1)/A_0}{100} \times 100$$

Where, A₀ is the absorbance of the control, and A₁ is the absorbance of the extract/standard.

Then % inhibitions were plotted against log concentration and from the graph IC₅₀ was calculated.

Nitric oxide scavenging capacity assay: Nitric oxide radical scavenging capacity was estimated on the basis of Griess-Illosvoy reaction¹⁵. In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). 4.0 ml of each fraction and standard (ascorbic acid) was added into 1.0 ml of Sodium nitroprusside (5 mM)

solution and incubated for 2 hours at 30°C to complete reaction. Then 2.0 ml solution was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent and absorbance of the solution was measured at 550 nm using a spectrophotometer (Shimadzu UV PC-1600) against blank. Percentage scavenging activity was calculated from

$$[(A_0 - A_1)/A_0] \times 100$$

Where A₀ is the absorbance of the control, and A₁ is the absorbance of the extract/ standard. The inhibition curves were prepared and IC₅₀ values were calculated.

Reducing power capacity assessment: Reducing power capacity assessment of the plant extracts was determined using the method described by Oyaizu et al¹⁶. 2.0 ml of each plant extracts or standard of different concentration solutions were taken and 2.5 ml of potassium ferricyanide [K₃Fe(CN)₆], 1% solution was added into each test tubes. The test tubes were incubated for 10 min at 50° C and 2.5 ml of trichloroacetic acid, 10% solution was added. The resultant mixtures were centrifuged at 3000 rpm for 10 min and 2.5 ml supernatant solution were withdrawn from each of the mixtures and mixed with 2.5 ml of distilled water. Then 0.5 ml of ferric chloride (FeCl₃), 0.1% solution was added. The absorbance's of the solutions were measured at 700 nm using a spectrometer against a typical blank solution.

Cupric Reducing Antioxidant Capacity: This study was based on the method described by Resat et al¹⁷. 500 µl of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes. 1.0 ml of 0.01 M CuCl₂.2H₂O solution and 1.0 ml of ammonium acetate buffer (pH 7.0) was added into the test tubes. Then 1.0 ml of 0.0075 ml of Neocaproin solution and 600 µl of distilled water was added into the test tubes. The total mixture was incubated for 1 hour at room temperature then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank.

Total alkaloid Content Determination: Total alkaloid content was determined by slightly modified method described by Fazel et al¹⁸. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered.

The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean \pm S.D.

Brine Shrimp lethality bioassay for cytotoxic activity: Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts¹⁹. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared by using sea salt 38 g/L and adjusted pH 8.5) under constant aeration for 48h.

Sample solutions are prepared by dissolving the test materials in pre-calculated amount of DMSO (Di-methyl sulphoxide). Ten nauplii are taken in vials containing 5 ml of simulated seawater. The samples of different concentrations are added. Survivors are counted after 24 hours. The median lethal concentration, LC₅₀ values of the test samples after 24 hours are obtained by a plot of percentage of dead Shrimps against the logarithm of the sample concentration using Microsoft Excel. Vincristine sulphate is usually used as the reference cytotoxic drug.

RESULTS:

Phytochemical screening: Preliminary phytochemical screening of the crude extracts of different parts of *Aegle marmelos* (L.) revealed the presence of different kind of chemical groups that are summarized in **Table 1**.

TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS

SI	Name of the tests	Methanol extracts of ripe fruit	Methanol extracts of half ripe fruit	Methanol extracts of leaf	Methanol extracts of seed
1.	Test for carbohydrate	+	+	-	+
2.	Test for Glycoside	+	+	-	+
3.	Test for steroid	-	+	-	+
4.	Test for alkaloid				
❖	Mayer's Reagent	+	+	+	-
❖	Hager's Reagent	+	-	-	+
❖	Wagner's reagent	+	+	+	+
❖	Dragendroff's reagent	+	+	-	-
5.	Test for saponin	-	-	+	+
6.	Test for tannin	-	+	+	-
7.	Test for glucoside	+	+	-	+
8.	Test for flavonoid	+	+	-	-

“+” indicates presence, “-” indicates absence.

Total phenol content: The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid ($y = 0.009x + 0.098$, $R^2 = 0.983$). Methanolic extract of half-ripe fruit of *Aegle marmelos* (L.) was found to contain the highest amount of phenols (6.05 ± 0.113 mg/g) and all other test fractions were found to contain almost equal amount of phenolic compounds (**Table 2**)

Flavanoid content determination: Flavanoid content of the plant extracts were calculated from the regression equation of the standard curve ($y = 0.009x - 0.036$, $R^2 = 0.972$) and expressed as quercetin equivalents (QE).

In our present study, we found that all the test fractions under consideration contain almost equal quantity of flavonoids (**Table 2**).

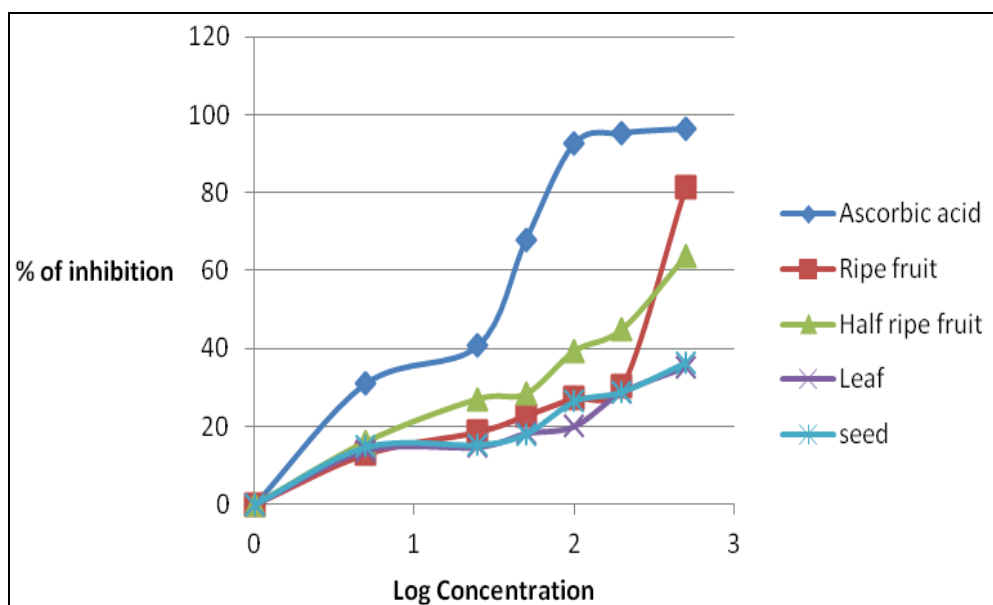
Total antioxidant capacity assay: Total antioxidant capacity is expressed as ascorbic acid equivalents (AAE) per gram of plant extracts and calculated using the calibration curve of standard ascorbic acid ($y = 0.002x + 0.204$; $R^2 = 0.946$). According to our present study, methanolic extract of ripe fruit and leaf possessed highest amount of total antioxidant potential followed by half ripe fruit (9.55 ± 0.1393 mg/g) and seed (7.37 ± 0.041719 mg/g) (**Table 2**).

TABLE 2: RESULT OF ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF AERIAL PARTS OF AGELE MARMELOS. VALUES ARE PRESENTED AS MEAN±SD (STANDARD DEVIATION).

Extracts	Total phenol(mg/g, Gallic acid equivalent)	Total flavonoid(mg/g, Quercetin equivalent)	Total antioxidant(mg/g, Ascorbic acid equivalent)	Total alkaloid(mg/g, Atropine equivalent)
Ripe fruit	5.93±0.044	3.37±0.079903	10.12±0.002828	3.91±0.007778
Half ripe fruit	6.05±0.113	3.44±0.007071	9.55±0.1393	3.42±0.000707
Leaf	5.45±0.0007	4.22±0.016263	10.31±0.068589	2.47±0.000707
Seed	5.13±0.062	4.29±0.014142	7.37±0.041719	4.86±0.001414

DPPH free radical scavenging activity: When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorised, which can be quantitatively measured from the changes in absorbance at 517 nm. The IC₅₀ values of the different extracts of *Aegle marmelos* (L.) are presented in the **Table 3**. Percent (%) of DPPH radical scavenging activity was found to rise with

increasing log concentration of the different extracts with highest scavenging displayed by methanolic extract of half-ripe fruit of the plant (**Fig. 1**). DPPH radical scavenging capacity of the extracts was found to decrease in the following order: Half-ripe fruit> Ripe fruit> Seed> Leaf.

**FIG. 1: COMPARATIVE DPPH SCAVENGING ACTIVITY OF DIFFERENT PARTS OF AGELE MARMELOS.****TABLE 3: IC₅₀ AND LC₅₀ VALUES OF DIFFERENT EXTRACTS IN DPPH, NITRIC OXIDE, BRINE SHRIMP LETHALITY BIOASSAY**

Extracts	DPPH free radical scavenging assay, IC ₅₀ (µg/ml)	Nitric oxide scavenging assay, IC ₅₀ (µg/ml)	Brine shrimp lethality bioassay, LC ₅₀ (µg/ml)
Ripe fruit	3981.1	161.935	255.38
Half ripe fruit	251.2	46.364	126.19
Leaf	24316.3	330.536	294.97
Seed	15848.9	275.729	86.77
Ascorbic acid	18.4	4.79	--
Vincristine	--	--	2.47

Nitric oxide (NO) scavenging capacity assay: Scavenging of NO was determined by the decrease in its absorbance at 550 nm, induced by antioxidants. All the extractives showed a dose dependent scavenging of NO similar to the reference antioxidant ascorbic acid (**Fig. 2**). However, maximum scavenging of NO was found

with methanolic extract of Half-ripe fruit of *Aegle marmelos* (L.) with an IC₅₀ value of 46.364µg/ml; the result is comparable to ascorbic acid which was taken as the standard (4.79µg/ml). NO scavenging activity decreased in the following order: Half-ripe fruit> Ripe fruit> Seed> Leaf (**Table 3**).

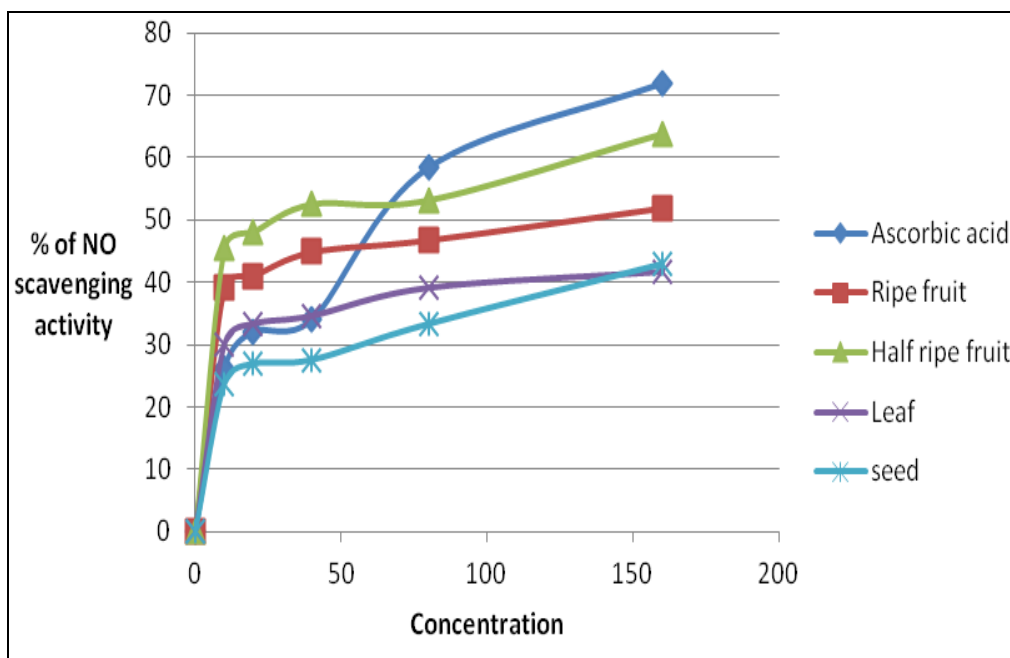


FIG. 2: NITRIC OXIDE SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF AGELE MARMELOS.

Reducing power: The reducing power of the plant extracts were measured based on the Fe^{3+} / ferricyanide to ferrous reduction at 700 nm at different concentrations. Reducing capability is determined spectrophotometrically from the formation of Perl’s Prussian blue coloured complex. The extracts were found to display

moderate reducing power. Reducing power was found to increase with increasing concentration of the extracts in all cases but was not comparable to the standard (Ascorbic Acid) (Fig. 3). Among the extract the methanolic extract of half-ripe fruit *Aegle marmelos* (L.) exhibited the most reducing power.

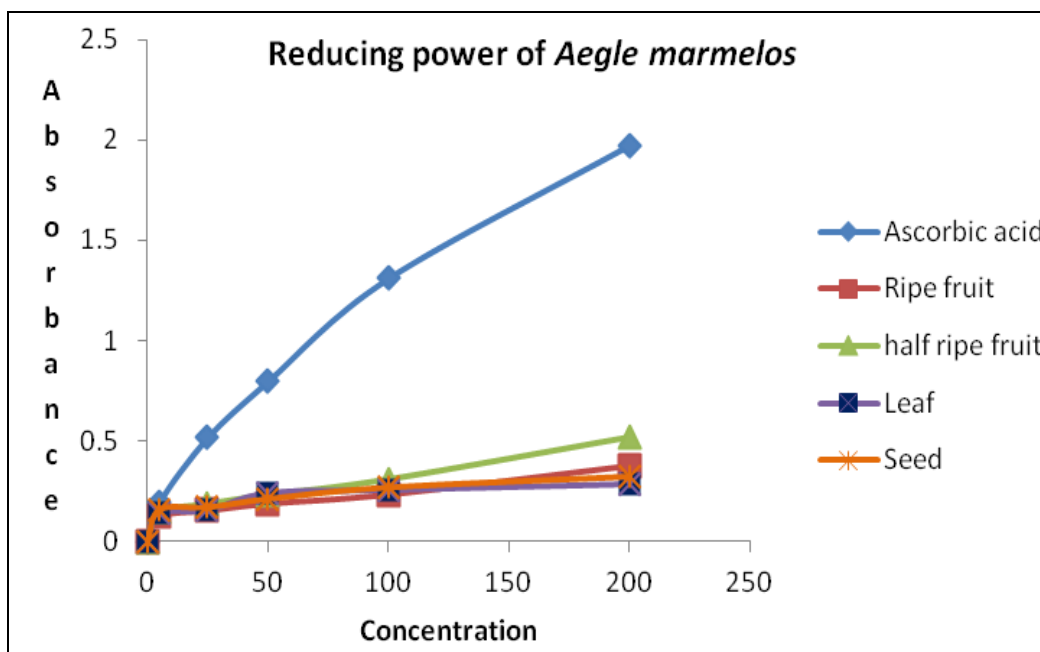


FIG. 3: REDUCING POWER OF DIFFERENT PARTS OF AEGLE MARMELOS (L.)

Cupric Reducing Antioxidant Capacity (CUPRAC): Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing concentrations of the different extracts. The standard ascorbic acid

showed highest reducing capacity. Among the extracts the methanolic extract of the leaf of *Aegle marmelos* showed maximum reducing capacity that is comparable to ascorbic acid (Fig. 4).

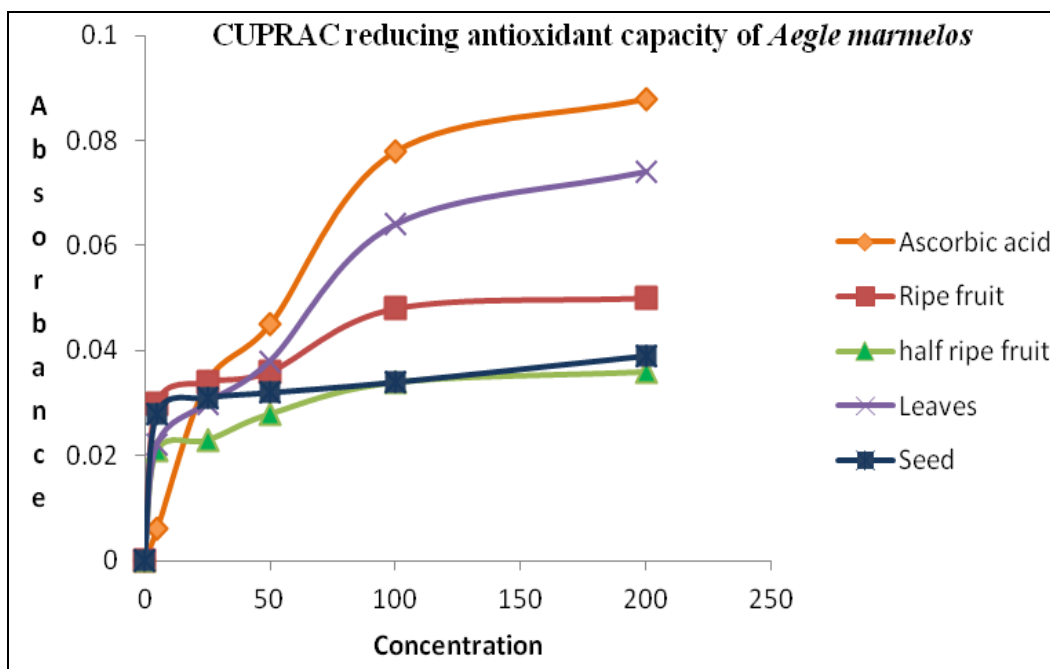


FIG. 4: CUPRIC REDUCING ANTIOXIDANT CAPACITY OF DIFFERENT PARTS OF AEGLE MARMELOS (L.).

Determination of total alkaloid content: The amount of total alkaloid of different extracts of *Aegle marmelos* (L.) was calculated using the equation ($y=0.085x-0.048$, $R^2=0.812$) and expressed as atropine equivalent (AE). Methanolic extract of seed of *Aegle marmelos* (L.) was found to possess the highest total alkaloid capacity (Table 2).

Brine Shrimp Lethality Bioassay for Cytotoxic Activity: All the extracts were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol extract of seed of *Aegle marmelos* (L.) was found to be the most toxic to Brine Shrimp nauplii, with LC_{50} of $86.77\mu\text{g/ml}$ whereas anticancer drug vincristine sulphate showed LC_{50} value $2.47\mu\text{g/ml}$. On the other hand, all the other extracts showed moderate to low toxicity (Table 3). The order at which cytotoxic potential of the test samples decreased was as follows: Vincristine sulphate > Methanol extract of seed > Methanol extract of Half-ripe fruit > Methanol extract of ripe fruit > Methanol extract of Leaf.

DISCUSSION: Preliminary phytochemical screening of the methanol extracts of ripe fruit, half-ripe fruit, leaf and seed of the plant *Aegle marmelos* (L.) revealed the presence of alkaloids and flavonoids. Glucosides, saponins, Glycosides, tannins and carbohydrates are also present in the

extracts but in a lesser amount and not in all extracts (Table 1). The presence of tannins, flavonoids, saponins and alkaloids are reported in the ethanolic extracts of leaves and fruits^{20, 21}. The presence of coumarins are also reported in the crude extracts of roots²¹. Various phytoconstituents like tannic acid, marmelosin, auraptin are isolated from this plant extracts^{7, 21}.

In our present study we did methanolic extraction of different parts of the plant and result summarized in the table also similar with the phytoconstituents reported in different phytochemistry report. Since the chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties, the constituents of the plant which are detected during this investigation should have some direct relationship with local medicinal uses.

Phenolic compounds obtained from plant source have the potential of acting as antioxidants or free radical scavengers². They possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions²².

The results strongly suggest that phenolics are important components of the tested plant extracts. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health—they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities.

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known reactive oxygen species²³.

In our current study, we found that the plant parts under investigation have been shown to possess mild amount of flavonoids. The total antioxidant activity of different extracts of *Aegle marmelos* (L.) was estimated from their ability to reduce Phosphate/Mo (VI) complex to Phosphate/Mo (V). It is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid and there is highly positive relationship between phenolic compounds and antioxidant activity. DPPH radical scavenging is a popular and reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of plant extracts. DPPH accepts an electron donated by an antioxidant compound and become decolorized, which can be quantitatively measured from the changes in absorbance. In our present study, we found that the plant under investigation possess moderate DPPH scavenging potential and showed dose dependent scavenging of DPPH potential similar to standard ascorbic acid (Fig. 1).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various biochemical processes. Excess generation and accumulation of nitric oxide are implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, Alzheimer's, and arthritis³. Based on these we can speculate that nitric oxide scavenging activity of *Aegle marmelos* (L.) may have great relevance in the prevention and control of disorders where NO is thought to play a

key role as plant fractions under this study showed promising NO scavenging potential (Fig. 2, Table: 3). There is strong correlation between antioxidant potential and reducing power of plant extracts. The extracts were found to display excellent reducing power and reducing power was found to increase with increasing concentration of the extracts in all cases and was comparable to the standard (Ascorbic Acid) (Fig. 3).

The brine shrimp bioassay has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound as well as plant products¹. In the brine shrimp lethality, bioassay all the extracts showed considerable lethality and seed extract was found most potent. There is also many article claimed the anticancer, antibacterial and antiviral properties of this plant and this become more authentic from this study^{7, 8, 20}. The observed cytotoxic action may be due to the presence of various phytochemicals present in the plant.

CONCLUSION: Fruits of *Aegle marmelos* are commonly used by the people of Bangladesh to prepare juice and such delicious juice are commonly taken as tonic or to reduce thirst in summer. The medicinal properties of different parts of this plant are already known to the traditional medicine practitioners of Bangladesh and also in the subcontinent. Results from this study confirmed that different extracts possess mild to moderate antioxidant cytotoxic potential. However, further studies are suggested to understand the underlying mechanism of observed activities and to isolate and characterize active compounds that are responsible for its different bioactivity. Our current study suggests that, this plant could be a source of novel antioxidant and anticancer compounds.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

ACKNOWLEDGEMENT: We are greatly thankful from the deepest core of the heart to the department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh to provide sufficient laboratory support to conduct this research work. We are also very thankful to Mrs. Tania Binte Wahed and Md. Nur Alam, Assistant Professor, Department of Pharmacy, Jahangirnagar

University, Savar, Dhaka, Bangladesh, for their valuable guidance throughout this research work.

REFERENCES:

- Hossain MS, Uddin N, Islam AM, Hasan AN, Hossain MM, Hasan MR, Khalik MF and Rana MS: Evaluation of in vitro antioxidant and brine shrimp lethality activities of different stem extracts of *Zizyphus rugosa* Lam. Journal of Food Measurement and Characterization 2015; 9:454-462.
- Dhar KS, Wahed TB, Hasan AN and Wahed SB: In vitro antioxidant activities and cytotoxicity study of the methanolic extract of barks of *Syzygium cymosum*. International Journal of Pharmaceutical Sciences and Research 2016; 7(3): 1021-1025.
- Hasan AN, Roy P, Bristy NJ, Paul SK, Wahed TB and Alam MN: Evaluation of in vitro antioxidant and brine shrimp lethality bioassay of different extracts of *Polygonum plebeium* R. Br. International Journal of Advanced Research 2015; 3(12): 97-107.
- Urmi KF, Chowdhury MA, Diba F, Rahman KA, Habib MR and Hamid K: Evaluation of antioxidant and cytotoxic potential of different extracts from the Leaves of *Aegle marmelos* L. Research Journal of Pharmacy and Technology 2013; 6(4): 384-387.
- Rajan S, Gokila M, Brindha P and Sujatha RK: Antioxidant and phytochemical properties of *Aegle marmelos* fruit pulp. International Journal of Current Pharmaceutical Research 2010; 3(2): 65-70.
- Rahman S and Parvin R: Therapeutic potential of *Aegle marmelos* (L.)-An overview. Asian Pacific Journal of Tropical Disease 2014; 4(1): 71-77.
- Gupta D, John P, Pankaj K, Kaushik R and Yadav R: Pharmacological review of *Aegle marmelos* CORR. fruits. International Journal of Pharmaceutical Sciences and Research 2011; 2(8): 2031-2036.
- Dhankhar S, Ruhil S, Balhara M, Dhankhar S and Chhillar A: *Aegle marmelos* (Linn.) Correa: A potential source of Phytomedicine. Journal of Medical Plant Research 2011; 5(9): 1497-1507.
- Cannel RJP: How to approach the isolation of a natural product, In: Natural products isolation, 1st edition (Humana press, New Jersey) 1998: pp. 1-55.
- Ghani A: Medicinal Plants of Bangladesh. 2nd Edition (The Asiatic Society of Bangladesh, Dhaka, Bangladesh) 2003: pp. 31, 39-40, 418, 500-504, 589-590.
- Folin O and Ciocalteu V: On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry 1927; 27: 627-650.
- Chang C, Yang M and Wen H: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10: 178-182.
- Prieto P, Pineda M. and Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* 1999; 269: 337- 341.
- Manzocco L, Anese M and Nicoli MC: Antioxidant properties of tea extracts as affected by processing. *Lebens-mittel-Wissenschaft Und-Technologie* 1999; .31: 694-698.
- Govindarajan R, Vijayakumar M, Shirwikar A, Rawat AKS, Mehrotra S and Pushpangadan P: Antioxidant activity of *Desmodium gangeticum* and its phenolics in arthritic rats. *Acta Pharm* 2006; 56: 489-496.
- Oyaizu M: Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 1986; 44: 307-315.
- Resat A, Kubilay G, Mustafa O and Saliha EK: Novel total antioxidant capacity index of dietary polyphenols and vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. *Journal of Agricultural and Food Chemistry* 2004; 52: 7970-7981.
- Fazel S, Hamidreza M, Rouhollah G and Mohammadreza V: Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai Journal of Pharmaceutical Sciences* 2008; 32: 17-20.
- Meyer BN, Ferringni NR, Puam JE, Jacobsen LB, Nicols DE and McLaughlin JL: Brine Shrimp: A convenient general bioassay for active constituents. *Planta Medica* 1982; 45:31-32.
- Ganapathy S and Karpagam S: *In vitro* antibacterial and phytochemical potential of *Aegle marmelos* against multiple drug resistant (MDR) *Escherichia coli*. *Journal of Pharmacognosy and Phytochemmistry* 2016; 5(1): 253-255.
- Prajapat RP, Gupta V, Soni B, Choudhary D, Ram V and Bhandari A: Extraction and isolation of marmelosin from *Aegle marmelos*, synthesis and evaluation of their Derivative as antidiabetic agent. *Der Pharmacia Lettre* 2012; 4 (4):1085-1092.
- Evans CR, Miller N and Paganga G: Antioxidant properties of phenolic compounds. *Trends in Plant Science* 1997; 2(4):152-159.
- Gracia OB, Castilo J, Marin FR, Ortuno A and Rio JAD: Uses and properties of citrus flavonoids. *J. Agric. Food Chem* 1997; 45: 4505-4515.
- Yu-Ling HO, Huang SS, Deng JS, Lin YH, Chang YS and Huang GJ: In vitro antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. *Botanical Studies* 2012; 53: 55-66.

How to cite this article:

Bristy NJ, Hasan AHMN, Alam MN, Wahed TB, Roy P and Alam K MK: Characterization of antioxidant and cytotoxic potential of methanolic extracts of different parts of *Aegle marmelos* (L.). *Int J Pharm Sci Res* 2017; 8(3): 1476-84. doi: 10.13040/IJPSR.0975-8232.8(3).1476-84.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)