

Evaluation of α -Amylase Inhibitory, Antioxidant and Cytotoxic Activities of *Mimusops elengi* Seeds

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Abstract

Mimusops elengi Linn., locally known as “Bakul, is a commonly used medicinal plant in Bangladesh to treat several maladies. The purpose of this study was to investigate the antioxidant, α -amylase inhibitory and cytotoxic activity of four different solvent fractions such as petroleum ether, chloroform, ethyl acetate, and water of *M. elengi* seed. In this study, the high ferric reducing (1.74 ± 0.005) and DPPH free radical scavenging activity ($IC_{50} 39.49 \pm 0.05 \mu\text{g/ml}$) was observed in the ethyl acetate fraction. The highest amount of flavonoid content ($1059.80 \pm 2.15 \text{ mg of CE/g}$) and total antioxidant capacity (0.17 ± 0.005) was present in chloroform fraction. On the other hand, the ethyl acetate fraction exhibited the highest total phenolic content ($182.90 \pm 0.38 \text{ mg of GAE/g}$). Crude methanolic extract showed highest inhibition of α -amylase with IC_{50} value of $4.09 \pm 0.08 \text{ mg/ml}$. The brine shrimp lethality bioassay of ethyl acetate fraction exhibited maximum toxicity with low LD_{50} value $127.10 \mu\text{g/ml}$. Thus, extracts of *M. elengi* seed could be a source of antioxidant, cytotoxic and α -amylase inhibitory activity containing compounds.

Key words: *Mimusops elengi*, phytochemical screening, antioxidant, α -amylase inhibitory, cytotoxicity.

Introduction

In this era, research on natural products has received a great attention. According to world health organization (WHO), about 70% of the world population is still depending on natural medicines to fulfill their health requirements (Uddin *et al.*, 2012). Now a day, consumption of the natural chemical constitutes enriched foods become interested because of growing awareness of the health benefits. Biologically active chemical constitutes of natural products can be used as a pharmaceutical, nutraceuticals, essential functional foods, and food additives for human health advantage because of their different kinds of structures and functionalities.

According to the International Diabetes Federation (2012), the pervasiveness of diabetes is rising at a worrying order throughout the world and it

is assessed that more than 552 million people will be affected by type 2 diabetes within 2030. Therefore, blood glucose levels should be controlled for delay or prevent type 2 diabetes. The enzyme α -amylase catalyzes the hydrolysis of starch to oligosaccharides and disaccharides in the digestive system. Inhibition of this enzyme suppress blood glucose rising by retard the initial breakdown of starch before liberating complex or simple sugars (Ademiluyi and Oboh, 2013).

At present, biologically active constituent such as phenolics have been reported as a strong antioxidant having inhibition against the action of α -amylase and α -glucosidase enzymes *in vitro* (Kwon *et al.*, 2008; Cheplick *et al.*, 2010; Oboh *et al.*, 2012). These bioactive compounds may bring down the conversion of starch into glucose and hence bring

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down postprandial plasma glucose levels along with other diabetic related complications developing from oxidative stress (Pranprawit *et al.*, 2015). On the other hand, synthetic inhibitors have been found to cause numerous unwanted side effects which led to the search for alternative α -amylase inhibitor from natural products, particularly, a food or medicinal plant origin in recent years to be tested as potentially safer, low cost anti-hyperglycemic agents (Kwon *et al.*, 2008; Oboh *et al.*, 2012).

In Bangladesh, all part of *M. elengi* plant is consumed traditionally by large number of people for many illnesses. It was observed that different parts of this plant exhibited various biological activities such as leaves possess antioxidant, anti-inflammatory (Ganesh *et al.*, 2013), antibacterial (Padhi and Mahapatra, 2013), and cytotoxic activities (Karmakar *et al.*, 2011). Bark shows wound healing (Gupta and Jain, 2011), diuretic activities (Koti and Ashok, 2010). In addition, flowers show hypoglycemic effect (Zahid *et al.*, 2012). Only antibacterial activity of *M. elengi* seed was examined. But seed extracts did not show any effect against gram positive and gram negative strains (Shahwar, D. and Raza, M.A., 2009). No researches have been studied on the α -amylase inhibition and antioxidant activity of *M. Elengi* seed. Thus, the present study aims to investigate the highest inhibitory potential of α -amylase and antioxidant activity of *Mimusops elengi* Linn. seed by using both polar and non-polar solvent.

Materials and Methods

Chemicals and reagents: In this study, all the chemicals used were of analytical grade. Petroleum ether, chloroform, ethyl acetate, methanol, sodium carbonate, ascorbic acid, potassium buffer, trichloroacetic acid and sodium hydroxide were purchased from Merck, Germany. Aluminium chloride, sulphuric acid, dimethyl sulfoxide and 3,5-dinitrosalicylic acid were obtained from Merck, Mumbai, India. Folin-ciocalteau, gallic acid, DPPH and sodium phosphate were bought from Sigma Chemical Company, USA. Catechin (Wako Pure Chemicals Ltd., Japan), sodium nitrite (Thomas

Baker, Mumbai), ammonium molybdate (The British Drug house Ltd, England), potassium ferricyanide (Matheson Coleman and Bell, America), ferric chloride (Loba Chemic Pvt Ltd, Mumbai) and α -amylase enzyme (Sigma-Aldrich, Germany) were procured from the mentioned sources.

Collection and extraction of plant material: The fruits of *M. elengi* were collected from the campus of University of Rajshahi, Rajshahi, Bangladesh during April to May. The plant species were authenticated (voucher specimen no. 125) at the Department of Botany, University of Rajshahi. Then the seeds were taken out from the fruits at that time. The collected seeds were washed with clean water, dried in air and ground to a coarse powder with an electric grinder (PHILIPS, HL7750/00). The pulverized seeds were stored in an airtight container until used. About 1618 gm of powdered seed were macerated with methanol in a 2.5 litre glass container at room temperature and kept for 48 hours with shaking occasionally. The extract was filtered by using Whatman no. 1 filter paper, and the solvent was evaporated by using the Buchi Rota-vapor (R-200; DS, MODEL: WB-1000E) under reduced pressure at 45°C. Finally, the extract was dried by using nitrogen gas. The crude methanolic extract was dissolved in 10% aqueous methanol and then fractionated with petroleum ether, chloroform, and ethyl acetate to provide pet ether, chloroform, ethyl acetate and water soluble fractions. Then all fractions were subjected to bioactivity test.

Phytochemical screening: Qualitative phytochemical screening of some major secondary metabolites such as saponins, tannins, glycosides, steroids, alkaloids, phenol and flavonoids in *M. elengi* seed was carried as per the published methods (Khandelwal *et al.*, 2008; Harborne *et al.*, 1998; Wadood *et al.*, 2013) with some modifications. The presence of major secondary metabolites was generally confirmed by visual inspection of color and precipitate formation after addition of specific test reagents.

Estimation of total phenolic and flavonoid content (TPC and TFC): Total phenolic content was determined by using the Folin-Ciocalteu reagent

(Slinkard and Singleton, 1977; McDonald *et al.*, 2001). About 0.30 ml of each sample (1000 µg/ml by methanol) was transferred in triplicate to the vial.

A gallic acid standard curve with varying concentrations (5, 10, 20, 40, 80, 100 µg/ml) was constructed by plotting gallic acid concentration on the abscissa and absorbance on ordinates for quantification of total phenolic value.

Total flavonoid content assay was performed using aluminum chloride colorimetric technique by following the slightly modified method described by Olajire and Azeez (2011).

Determination of total antioxidant capacity: The total antioxidant capacity of the methanolic extract and its four fractions were assessed spectrophotometrically by the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999) with minor modifications and ascorbic acid used as the standard.

Determination of ferric reducing power: The ferric reducing power of *M. elengi* seed extracts was investigated by using the potassium ferricyanide-ferric chloride according to the procedure described by Oyaizu *et al.* (1986) with some modifications by using ascorbic acid used as a standard.

Determination of DPPH free radical scavenging activity: The DPPH method was used to evaluate the free radical scavenging activity of the extracts of *M. elengi* seed according to Choi *et al.* (2000) with few modifications.

The percentage of DPPH radical scavenging activity was calculated by using the following formula.

$$\% \text{ DPPH radical scavenging activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The % of scavenging was plotted against concentration and from the graph the IC₅₀ value was calculated. Ascorbic acid was used as standard for this study.

Determination of in vitro α-amylase inhibitory activity: Inhibition of α-amylase enzyme was determined according to the procedure described by Jabir *et al.* (2014) with some modifications.

The IC₅₀ values were determined from the plots of % of inhibition vs inhibitor concentration. Acarbose was used as a standard.

Determination of cytotoxic activity: Brine shrimp (*Artemia salina*) lethality bioassay was used for evaluation of cytotoxic activity of *M. elengi* seed extracts according to the method described by Meyer *et al.* (1982) and Parvin *et al.* (2009) with some modifications. Brine shrimp eggs were collected from Natore Fishing Agriculture and Research Center, Rajshahi, Bangladesh. Eggs were placed in a 1000 ml beaker containing artificial sea water (3.8% of sodium chloride, pH 8 to 9) for 48 hrs with constant oxygen supply at 37°C. After 24 hrs of hatching period, the nauplii were ready for the experiment. Solution of the seed extracts and standard with concentrations 600, 300, 150, 75 and 37 ppm were prepared by using dimethyl sulfoxide (DMSO). The prepared solutions were taken in six test tubes and twenty brine shrimp nauplii were then placed in each test tube. Volume of liquid was adjusted by brine water and the controls were also arranged in the same fashion in DMSO without the extracts. After 24 hours of incubation, the test tubes were observed at 6th, 12th, 18th and 24th hrs using a magnifying glass and the number of survivors in each tube was counted. Vincristine sulfate and DMSO was used as the positive control and negative control, respectively.

Statistical analysis

All experiments were performed in triplicate. The TFC, TPC and DPPH assays results have been expressed as means ± SD using Microsoft Excel. The enzyme analysis was assessed by one-way analysis of variance (ANOVA) and Tukey's comparison test. A p < 0.05 value was regarded as significant. Probit analysis was used to determine the LD₅₀ values, 95% confidence limits and regression equations for each extract and standard from the mortality data. The cytotoxicity of the extracts was compared with the standard gallic acid. Probit software was used to calculate the LD₅₀ value from the mortality data.

Results and Discussion

The present study was carried out to evaluate the antioxidant, α -amylase inhibitory activity and cytotoxicity of *M. elengi* seed extracts and the results have been summarized in different tables and figures. The results of phytochemical screenings of the crude methanolic extract and four different fractions of *M. elengi* seed are depicted in Table 1.

The crude extract and all fractions were found to contain glycosides. On the other hand, alkaloids

could not be detected in any of the test samples. Saponins, tannins, steroids and flavonoids were also present in the crude extract and some of its partitionates. The result of this study was found to be in agreement with the previously available reports on the same plant (Gami and Parabia, 2010).

Total phenolic and flavonoid contents of the different fractions and crude extract of *M. elengi* seed are shown in figure 1 and figure 2, respectively.

Table 1. Phytochemical screening of the crude methanol extract of *M. elengi* seeds and its four fractions.

Phytochemical constituents	Name of test	Crude extract	Petroleum ether fraction	Chloroform extract	Ethyl acetate fraction	Water Fraction
Saponins	Frothing test	+++	-	+	++	++
Tannins	Lead acetate test	+++	-	-	+	-
Glycosides	Bal Jets test	+++	++	+++	++	++
Steroids	Libermann-Burchard's test	+++	++	+	-	-
	Salkowski test	+++	+++	++	-	-
Alkaloids	Mayer's test	-	-	-	-	-
Phenol	Ferric chloride test	++	-	+	++	+
Flavonoids	Shinoda test	++	-	+	++	-

“+” = small amount, “++” = moderate amount, “+++” = large amount, “-” = not present.

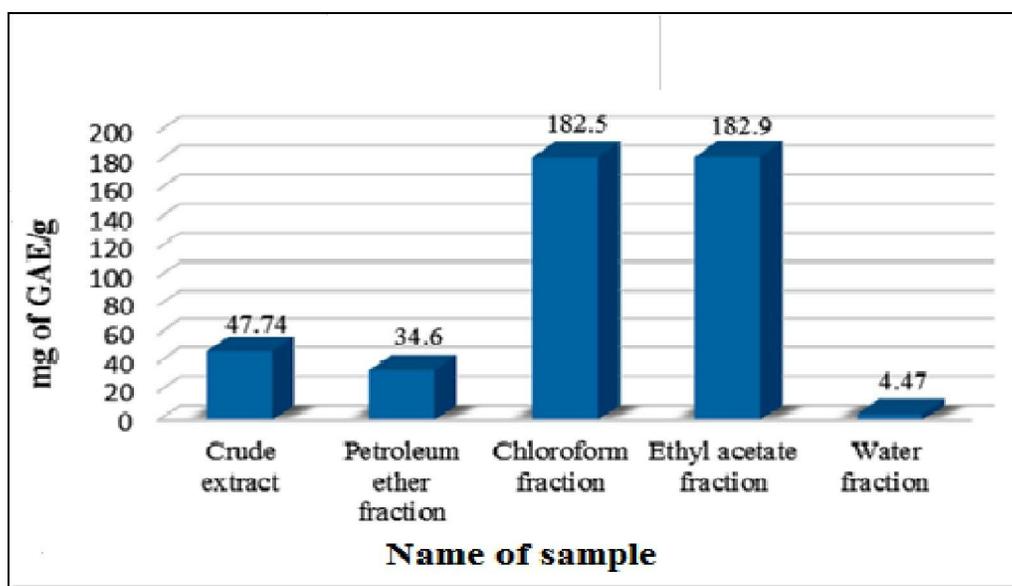


Figure 1. Total phenolic contents of methanolic crude extract of *M. elengi* seed and its different fractions.

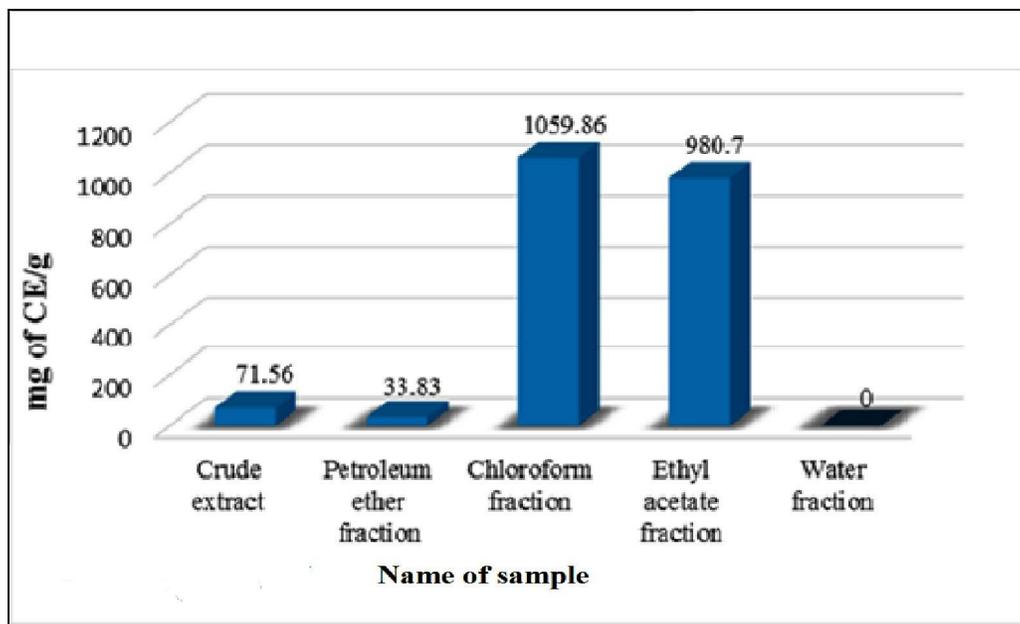


Figure 2. Total flavonoid contents of methanolic crude extract of *M. elengi* seed and its different fractions.

The result showed that ethyl acetate fraction has high phenolic content with the value of 182.90 ± 0.38 mg of GAE/g of dried extract. The water fraction showed lower phenol content with the value 4.47 ± 0.19 mg of GAE/g of dried extract. This essential changeability pointed out the influence of solvents on the extractability of phenolic compounds. By several studies, it was well-known that phenols are moderately polar compounds, thus they tend to gather in the fraction of medium polarity solvent such as ethyl acetate. The similar results were observed when using this solvent for the extraction of different medicinal plant (Wei *et al.*, 2010; Mariem *et al.*, 2014; Mariod *et al.*, 2009; Mannan *et al.*, 2017). Ethyl acetate is used as solvent for the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols (Dauer *et al.*, 2003).

In the present study, high flavonoid content was observed in chloroform fraction with the value 1059.80 ± 2.15 mg of CE/g of dried extract and it was absent in the aqueous fraction.

Figure 3 shows the total antioxidant capacities of the four different fractions and crude extract of *M. elengi* seed as well as the standard ascorbic acid.

The chloroform fraction of *M. elengi* seed extract exhibited highest antioxidant capacity (0.17 ± 0.005) compared with standard (0.35 ± 0.005). It can be deduced that maximum total flavonoid content in chloroform fraction is responsible for the highest total antioxidant capacity (Prasain *et al.*, 2010). The result is agreeable with that of reported by Mannan *et al.* (2017) which showed the highest antioxidant capacity of the ethyl acetate fraction obtained from the leaves of *M. elengi*. Thus, the result of total antioxidant capacity of *M. elengi* seeds extract followed the order of chloroform>ethyl acetate>methanol extract (crude)>petroleum ether>water.

The ferric reducing power was analyzed to determine the reducing power of methanol extract and the fractions of *M. elengi* seeds. Ferric reducing power of the four different fractions and crude extract of *M. elengi* seeds in addition to the standard ascorbic acid are depicted in figure 4.

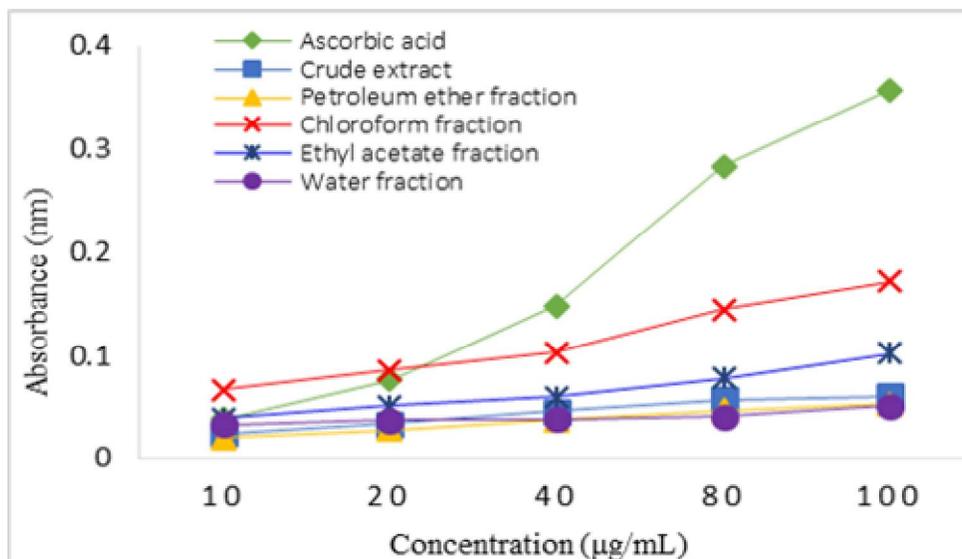


Figure 3. Total antioxidant capacities of crude extract of seeds of *M. elengi* and its four different fractions.

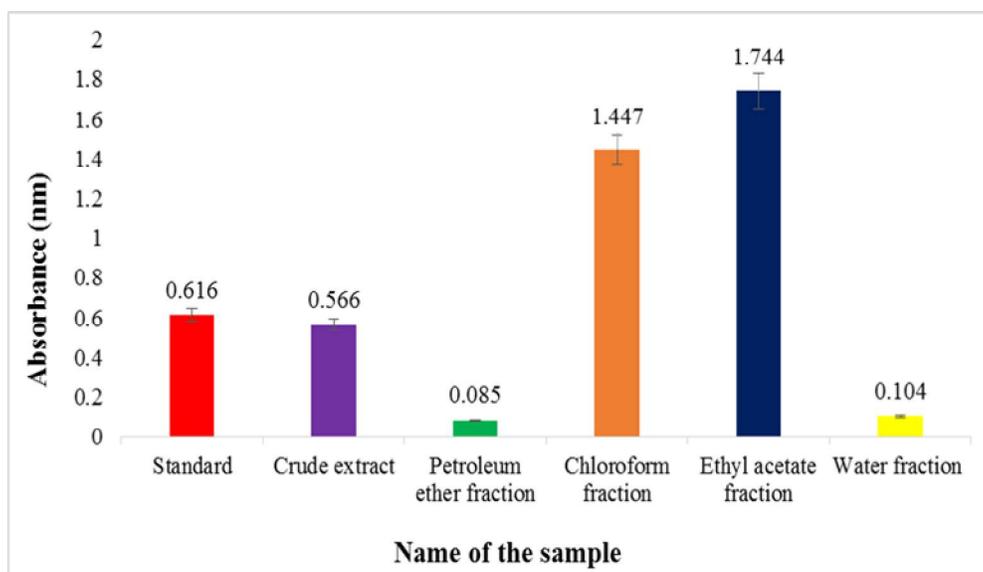


Figure 4. Ferric reducing power of crude extract of *M. elengi* seed and its different fractions.

In this method, the ethyl acetate fraction showed higher ferric reducing power (1.74 ± 0.005) compared with the standard ascorbic acid (0.61 ± 0.003). The water fraction showed lower activity. This result corroborates with other studies which showed that the ethyl acetate fraction had better ferric reducing power than the other fractions (Liu *et al.*, 2011; Trabelsi *et al.*, 2012). Thus, it can be seen that all the extracts of *M. elengi* seed possess ferric

reducing power and the order of ferric reducing power was ethyl acetate > chloroform > methanol extract (crude) > water > petroleum ether. By these observations, it can be suggested that both solvent polarity and plant phenolic natures influence the antioxidant activity.

The DPPH free radical scavenging activity of crude extract, other test samples and standard ascorbic acid are shown in figure 5.

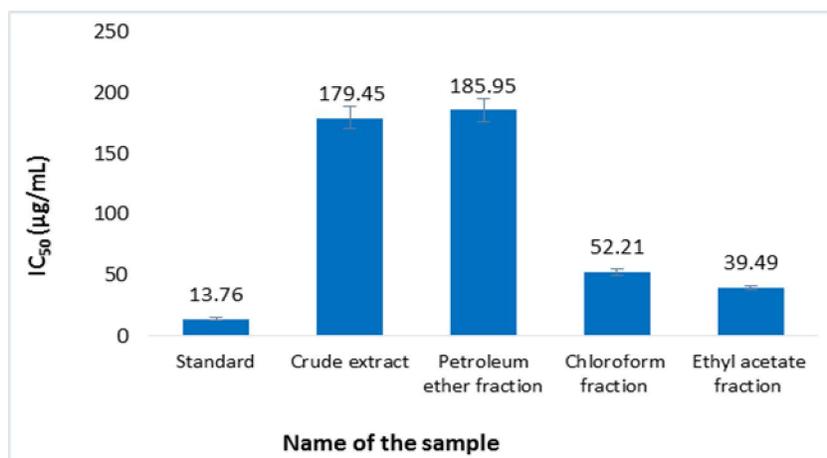


Figure 5. DPPH free radical scavenging activity of crude extract of *M. elengi* seed and its fractions.

Table 2. α -Amylase inhibitory activity of crude extract of *M. elengi* seed, its fractionates and acarbose.

Name of sample	Conc. (µg/ml)	% Of inhibition \pm SD	IC ₅₀ (mg/ml) \pm SD	Name of sample	Conc. (µg/ml)	% of inhibition \pm SD	IC ₅₀ (mg/ml) \pm SD
Acarbose (standard)	50	2.56 \pm 0.14	0.62 \pm 0.003	Chloroform fraction	50	0.56 \pm 0.06	5.01 \pm 0.05
	100	4.27 \pm 0.21			100	1.19 \pm 0.02	
	200	8.64 \pm 0.14			200	3.06 \pm 0.22	
	500	48.82 \pm 0.54			500	5.13 \pm 0.11	
Crude extract	50	1.52 \pm 0.30	4.09 \pm 0.08	Ethyl acetate fraction	50	2.11 \pm 0.10	7.31 \pm 0.05
	100	2.13 \pm 0.11			100	3.42 \pm 0.20	
	200	3.40 \pm 0.20			200	4.46 \pm 0.22	
	500	6.93 \pm 0.31			500	5.49 \pm 0.11	
Petroleum ether fraction	50	2.20 \pm 0.21	5.08 \pm 0.07	Water fraction	50	2.2 \pm 0.01	9.13 \pm 0.07
	100	2.56 \pm 0.14			100	3.34 \pm 0.10	
	200	4.67 \pm 0.69			200	4.08 \pm 0.03	
	500	6.49 \pm 0.15			500	4.94 \pm 0.12	

Free radical scavenging activity is mostly assigned to the phytochemicals present in the natural products derived food and is related with the total phenolic content or total flavonoid content of the food (Ehlenfeldt and Prior, 2001; Liu *et al.*, 2008; Borneo *et al.*, 2009). The ethyl acetate fraction of *M. elengi* seeds showed maximum DPPH free radical scavenging activity with the lowest IC₅₀ value (39.49 \pm 0.05 µg/ml). The aqueous fraction did not show any scavenging activity. Many studies investigated the impact of the extracting solvents on antioxidant

activity and found that antioxidant values obtained from organic solvent extraction were generally higher than those obtained with water (Cheplick *et al.*, 2010; Sulaiman *et al.*, 2011; Wang *et al.*, 2012). This result is in agreement with other studies where the ethyl acetate fraction had better DPPH free radical scavenging activity than the other fractions (Desire *et al.*, 2010; Liu *et al.*, 2011). The ethyl acetate fraction probably due to its polarity allows the accumulation of a variety of antioxidant compounds such as polyphenols, flavonoids and other bioactive

molecules that exhibit the strongest radical-scavenging activity. Thus, the order of DPPH free radical scavenging activity as ascorbic acid > ethyl acetate > chloroform > methanol extract (crude)> petroleum ether.

Overall the ethyl acetate fraction and crude methanolic extract of analyzed medicinal plant exhibited the highest total phenolic content, iron reducing power and DPPH radical scavenging activity whereas the chloroform fraction showed the highest total flavonoid content and total antioxidant activity.

The % inhibition of α -amylase at 50, 100, 200, 500 μ g doses the crude extract and four fractionates of *M. elengi* and standard acarbose has been shown in table 2.

Displayed highest percentage of inhibitory effects ($6.93 \pm 0.31\%$) on the α -amylase enzyme at 500 μ g/ml with an IC_{50} value of 4.09 ± 0.08 mg/ml. On the other hand, the water fraction showed lowest activity with highest IC_{50} value 9.13 ± 0.07 mg/ml. The standard exhibited the IC_{50} value of 0.62 ± 0.003 mg/ml at the same concentration. The order of α -amylase inhibitory activity was methanol > chloroform > petroleum ether > ethyl acetate > water. The phytochemical assay of this study showed large amount of tannins, phenols and flavonoids present in methanol extract which may be the cause of the highest inhibitory activity (McDougall *et al.*, 2005).

The result of brine shrimp lethality bioassay has been summarized in table 3.

Table 3. Effect of different extracts of *M. elengi* seed and vincristine sulfate on *Artemia salina*.

Name of sample	LD ₅₀ (μ g/ml)	95% Confidence limits		Regression equation	χ^2 value
		Lower	Upper		
Crude extract	369.3	178.01	766.14	$Y = 2.258 + 1.068X$	0.26009
Petroleum ether	6912.5	16.372	2918.56	$Y = 3.419 + 0.4117X$	0.52541
Chloroform	915.7	156.73	5350.18	$Y = 2.937 + 0.6966X$	0.48172
Ethyl acetate	127.1	73.958	218.41	$Y = 2.687 + 1.099X$	0.51401
Aqueous	374.0	188.82	740.80	$Y = 2.045 + 1.149X$	0.66255
Vincristine sulfate (Standard)	81.48	45.113	147.19	$Y = 2.239 + 1.44X$	0.12610

χ^2 (chi-square) statistic is a single number that tells how much difference exists between the observed counts and the counts expected if there were no relationship at all in the population. A low value for chi-square means there is a high correlation between two sets of data.

The LD₅₀ values of the crude extract, petroleum ether, chloroform, ethyl acetate and water fractions were found to be 369.3, 6912.5, 915.7, 127.1 and 374.0 μ g/ml, respectively. The standard vincristine sulfate had an LD₅₀ value of 81.48 μ g/ml. The lowest LD₅₀ value of 127.1 μ g/ml was found in the case of ethyl acetate fraction, indicating its more potent cytotoxicity compared with other fractions in this study. The ethyl acetate fraction has been reported previously for its potent cytotoxic properties compared with other fractions (Mannan *et al.*, 2017).

Conclusions

In present study, the antioxidant, α -amylase inhibitory and cytotoxic activities of crude extract of seeds and its different fractions of a well-known medicinal plant, *M. elengi* were evaluated. The results of this study revealed that all extracts except the aqueous fraction of this medicinal plant contain polyphenols and flavonoids in good order. The chloroform and ethyl acetate fractions exhibited high antioxidant activity, iron reducing power and free radical scavenging activity; on the other hand least activity was exposed by petroleum ether and aqueous fractions. All samples except the aqueous fractions

showed α -amylase inhibitory activity. Compared to the standard, over all α -amylase inhibitory activity of this plant was satisfactory. However, all test samples (other than the petroleum ether fraction) possess slight toxicity towards brine shrimp nauplii bioassay. Thus, the results recommend that the plant is a possible source of antioxidant and antidiabetic. However, further extensive studies are necessary to isolate and characterize the compounds responsible for the bioactivities.

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Conflict of interest

No conflict of interest.

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