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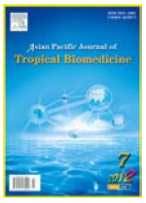

Document heading

Antioxidant potential of phenolic extracts of *Mimusops elengi*

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ABSTRACT

Objective: To evaluate the antioxidant potential of the phenolic extracts of *Mimusops elengi (M. elengi)* L. (Sapotaceae). **Methods:** The extract of stem bark and seeds of *M. elengi* were prepared in methanol and acetone:water (7:3). The acetone: water was further partitioned with ethyl acetate and n-butanol. Antioxidant activity of the extracts and partitioned fractions of *M. elengi* was evaluated in terms of radical scavenging potential (DPPH), inhibition of lipid peroxidation [ferric thiocyanate (FTC)], and total antioxidant activity (phosphomolybdate method). Total phenolics content were calculated using Folin-Ciocalteu reagent. **Results:** The stem bark extract partitioned with ethyl acetate exhibited highest amount of total phenols (98.0 mg GAE/g dry weight), among all other extracts, with 92.0% DPPH radical scavenging activity at concentration of 0.5 mg/mL, while methanol extract (stem bark) had maximum inhibition of lipid peroxidation (62.0%) and total antioxidant activity (771.0 mg/g GAE/g). A positive correlation occurred between total phenols and radical scavenging activity (R^2 = 0.922 9) and total antioxidant activity (R^2 = 0.945 1). **Conclusions:** Our study suggested that antioxidant activity of stembark extract of *M. elengi* is due the presence of phenolic compounds. Furthermore, the bark extract is a valuable source of natural antioxidants.

1. Introduction

Recently much attention has been focused on the use of plant materials such as fruit, vegetables, spices, leaves, roots and bark as natural antioxidants which can effectively replace synthetic antioxidants (tert-butylhydroxytoluene, tert-butylhydroxyanisole, and tert-butylhydroquinone[1]. Plants not only add nutritional value to the food but also contribute in the protection from free radical deterioration by retardation of lipid peroxidation. Free radicals promote oxidative damage which play a role in the pathogenesis of many diseases including cancer, arthritis, antheroclerosis, Alzheimer's disease, and diabetes[2]. The antioxidant activities of plant extracts are usually linked with the presence of anthocyanins, phenolic acids, flavonoids, and tannins. The interest in polyphenolic antioxidants has increased remarkably in the last decade because of their elevated capacity in scavenging free radicals associated with various diseases. This property has been evidenced by a large number of tests measuring the antioxidant activity *in vitro*[3].

The genus Mimusops (Sapotaceae) consists of 30 species of which *Mimusops schimperi* A.Rich., *Mimusops laurifolia* Forssk,, and *Mimusops elengi* (*M. elengi*) L. are found in the tropical and subtropical regions of Asia. *M. elengi* is an ornamental tree with sweet-scented flowers and grows wild in the southern India, Burma, and Pakistan. Various parts of *M. elengi* have been used as a febrifuge, astringent, purgative, and stimulant[4,5]. Furthermore, the studies conducted using bark of the plant has shown dose-dependent inhibition of gastric lesions against ethanol-induced gastric ulcer. The pounded seeds pasted with oil are used for the treatment of obstinate constipation. To date there is no report of extraction of polyphenols and radical scavenging property of this plant. Therefore, in our search of herbal antioxidants from new plant sources, a study was conducted on methanol and acetone-water extracts of stem bark and seed. The acetone-water extracts after evaporation of acetone were extracted successively in ethyl acetate and n-butanol. Total phenols were determined by FC method, while antioxidant potential was assayed by ferric thiocyanate (FTC), DPPH scavenging, and phosphomolybdate methods.

The objective of the present study was to evaluate the stem bark and seed extracts of *M. elengi* as a source of natural antioxidants for food and pharmaceuticals.

2. Materials and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, gallic acid and α-tocopherol

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were purchased from Sigma-Aldrich (USA). Solvents and butylhydroxytoluene (BHT) of analytical grade were purchased from Panerac (Spain). All other chemicals and reagents of analytical grade were from Merck (Germany).

2.2. Plant material

Stem bark and seed of *M. elengi* were collected in June 2006, from Government College University campus Lahore, Pakistan. The plant was identified at Department of Botany, GCU Lahore where a voucher specimen was deposited (036-GCU-BOT-06).

2.3. Extraction

The plant material (stem bark and seed) was shade dried, powdered, and extracted by percolation method for seven days at room temperature (100 g each) with 1.0 L each of methanol (100%) and acetone:water (70:30%). The crude extracts were filtered through Whatman No 40 filte paper and concentrated using rotary evaporator. The acetone:water extracts were defatted with diethyl ether and partitioned with ethyl acetate and n-butanol. The percentage yields of bark and seed in methanol (20.6%, 15.4%), aetone:water (21.2%, 17.9%), ethyl acetate (9.3%, 9.6%), and 1-butanol (13.9%, 14.6%), respectively.

2.4. Determination of total phenols

The total phenols in extracts and fractions of *M. elengi* were determined using Folin-Ciocalteu reagent[6]. The extract (20 mg) was dissolved in (10 mL) methanol. 40 μL of each sample was mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.8 mL of 10% sodium carbonate solution. The mixture was allowed to stand for 30 min and the absorption was measured at 765 nm against a blank which contained 40 μL of methanol in place of sample. The total phenolics content were expressed as gallic acid equivalents in mg/g of dry weight (Table 1). Correlation studies between total phenolic content and antioxidant activities in DPPH, lipid peroxidation, and phosphomolybdate assays were also performed.

2.5. Ferric-thiocyanate assay (FTC)

Inhibition of lipid peroxidation in linoleic acid system was determined using thiocyanate method[7,8]. Extract/fraction (2 mg) was added to a mixture of linoleic acid (2 mL of 2% v/v), absolute ethanol (10 mL), and 10 mL of 0.2 M sodium phosphate buffer (pH 7). The solution was incubated at 40 ℃ and the degree of oxidation was measured. BHT, α-tocopherol, and gallic acid were used as positive control.

$$\text{Inhibition percentage} = \frac{\triangle_{\text{Abs. of blank}} - \triangle_{\text{Abs. of sample}}}{\triangle_{\text{Abs. of blank}}} \times 100$$

2.6. DPPH radical scavenging assay

The radical scavenging ability of different extract/fraction of *M. elengi* was measured using stable free radical of DPPH[9]. Methanol solution (1.0 mL) of all the extract/fraction at various concentrations (0.01-1.5 mg/mL) was added to 1.0 mL (0.2 mg/mL) methanol solution of DPPH and kept in dark. The decrease in absorbance at 517 nm was noted after 30 min.

The % scavenging of radical was determined by the following formula.

$$\text{Percentage scavenging of DPPH} = \frac{\triangle_{\text{Abs. of blank}} - \triangle_{\text{Abs. of sample}}}{\triangle_{\text{Abs. of blank}}} \times 100$$

Concentration of sample where absorbance of DPPH decreases 50% (SC_{50} values) were also determined. Gallic acid, BHT, and α-tocopherol were used as standard reference. The amount of the sample (μg/mL) was also determined by measuring decrease in absorbance at 517 nm to half of the initial value as SC_{50}.

2.7. DPPH TLC autographic assay

Minimum amount of the active extract/fraction required to scavenge the DPPH radical was determined by applying different concentrations (0.01-1.5 mg/mL) on TLC plates. The plates were sprayed with 0.2% (2 mg/mL) DPPH solution and examined after half an hour. Light yellow color on purple background indicated the positive result.

2.8. Evaluation of total antioxidant capacity by phosphomolybdate method

The total antioxidant capacity of the plant extract/fraction was evaluated by measuring the absorbance of Mo(V) at 695 nm[8]. An aliquot of 0.2 mL (0.5 mg/mL) of the sample solution was mixed with 2.0 mL of the reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 ℃ for 60 minutes and absorbance was measured at 695 nm against a blank contained 2 mL of reagent solution. Total antioxidant activity was expressed equivalent to gallic acid.

2.9. Statistical analysis

The statistically analysis was carried out by the computer program ANOVA. The replicate readings taken with each bioassay were used. The concentration of extract has been mentioned in the experimental section. The Duncan's multiple range tests were used to determine differences at each point which were considered significant at $P< 0.05$.

3. Results

Total phenolic compounds of different extracts/fractions were determined according to Folin-Ciocalteu method with gallic acid as standard (R^2= 0.996, y=0.0035+0.0295). Total phenolics ranged from 70-980 GAE mg/g of dry weight (Table 1). Antioxidant activity of extracts/fractions was determined by inhibition of peroxidation in linoleic acid system using FTC[10]. The effect of all bark and seed extracts/fractions of *M. elengi* on the rate of peroxidation was compared with α-tocopherol, BHT, and gallic acid. Methanol extract of stem bark (MBM) of *M. elengi* exhibited higher inhibition of peroxidation (62.0%) compared to that displayed by gallic acid (51.5%). Extract in acetone:water (MBA) and ethyl acetate fraction (MBE) of bark were also found to have antioxidant activity with 44.7% and 47.7%, respectively, while *n*-butanol fraction (MBB) showed very low response to this assay. All seed extracts/fractions except ethyl acetate fraction (MSE,

Table 1
Total phenolics content and antioxidant activity of different extracts/fractions of *M. elengi*.

Sample/ Standard	Total phenolics (mg/g of dry weight)	Antioxidant activities				
		Percentage of DPPH neutralization	DPPH-SC_{50} (μg/mL)	Total antioxidant activity (mg/g)	FTC (% inhibition)	TLC (μg)
MBM[a]	98.0 ± 1.2	91.0 ± 0.5	2.0 ± 0.1	771.0 ± 12.0	62.0 ± 0.2	1.00 ± 0.00
MBA[b]	91.0 ± 0.8	89.0 ± 0.3	4.0 ± 0.3	565.0 ± 14.0	44.7 ± 0.6	1.00 ± 0.00
MBE[c]	78.0 ± 1.6	92.0 ± 0.5	10.0 ± 0.1	603.0 ± 21.0	47.7 ± 0.4	1.00 ± 0.00
MBB[d]	65.0 ± 1.5	72.0 ± 0.7	35.0 ± 0.4	494.0 ± 13.0	21.7 ± 0.7	4.00 ± 0.10
MSM[e]	21.0 ± 0.5	12.2 ± 0.3	–	94.0 ± 5.0	19.0 ± 0.2	18.00 ± 0.40
MSA[f]	7.0 ± 0.7	9.5 ± 0.7	–	102.0 ± 9.0	8.0 ± 0.5	25.00 ± 0.30
MSE[g]	65.0 ± 1.2	88.0 ± 1.1	28.0 ± 0.4	396.0 ± 14.0	48.4 ± 0.7	2.50 ± 0.10
MSB[h]	20.0 ± 0.7	20.4 ± 0.5	–	113.0 ± 3.0	29.4 ± 0.5	12.00 ± 0.20
Gallic acid	–	93.0 ± 0.7	4.0 ± 0.2	–	51.5 ± 0.7	0.05 ± 0.00
BHT	–	77.5 ± 0.4	12.0 ± 0.3	–	65.2 ± 0.3	1.00 ± 0.00
α-tocopherol	–	65.0 ± 0.7	68.0 ± 0.4	–	74.5 ± 0.4	5.00 ± 0.40

[a] Methanol bark extract; [b] Acetone-water (7:3) bark extract; [c] Ethyl acetate fraction of acetone-water (7:3) bark extract; [d] *n*-butanol fraction of acetone-water (7:3) bark extract; [e] Methanol seed extract; [f] Acetone-water (7:3) seed extract; [g] Ethyl acetate fraction of acetone-water (7:3) bark extract; [h] *n*-butanol fraction of acetone-water (7:3) bark extract.

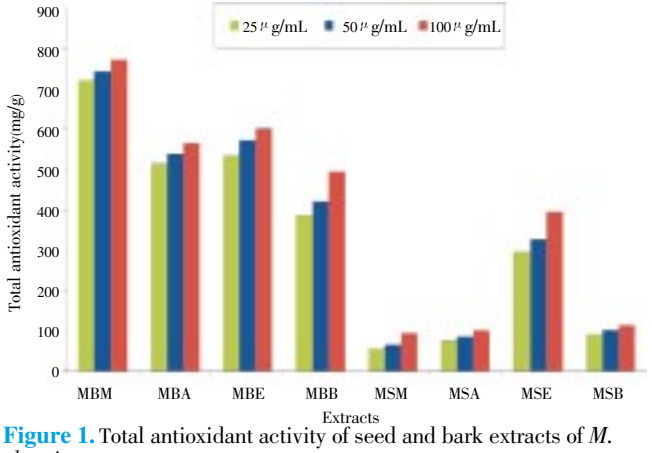

Figure 1. Total antioxidant activity of seed and bark extracts of *M. elengi*.

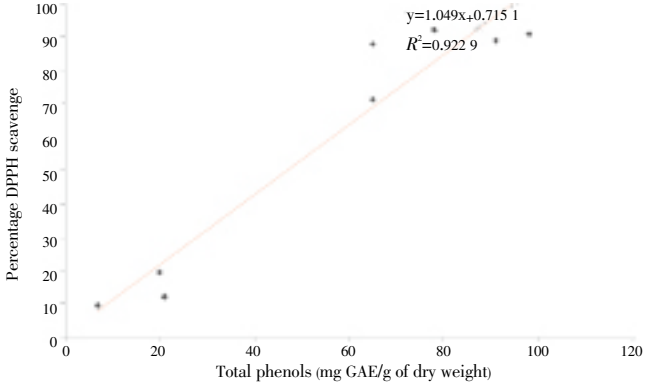

Figure 2. Correlation between total phenols and percentage DPPH radical scavenge activity of *M. elengi*.

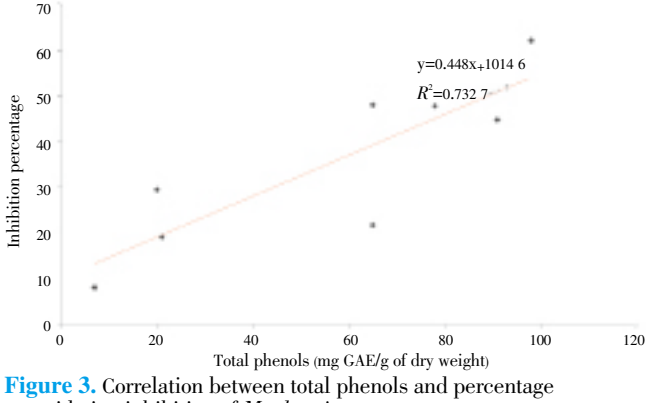

Figure 3. Correlation between total phenols and percentage peroxidation inhibition of *M. elengi*.

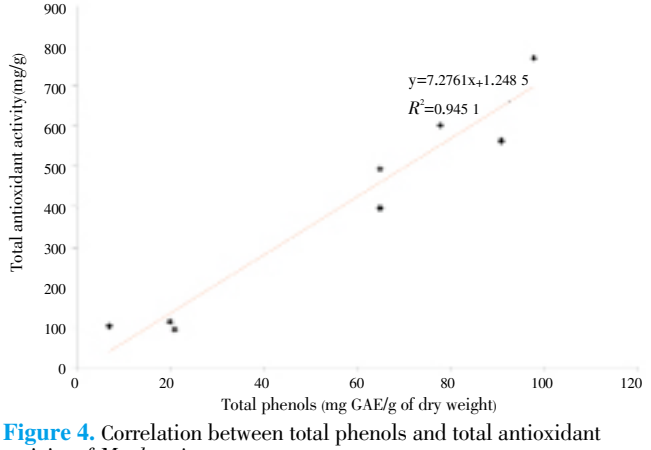

Figure 4. Correlation between total phenols and total antioxidant activity of *M. elengi*.

48.5% inhibition) did not show significant activity in FTC assay. Phosphomolybdate assay is based on the reduction of Mo (VI) to Mo (V) by the sample with the formation of a green phosphate/Mo (V) complex at acidic pH. MBM showed maximum activity (771.0 mg/g equivalent to gallic acid), while seed extract in methanol give very low response in this assay (Figure 1). The correlation between the radical scavenging activity and phenolics content of *M. elengi* extract/fraction was studied using a linear regression analysis. The high correlations between DPPH scavenging activity (R^2 = 0.922 9) and total antioxidant activity (R^2 = 0.945 1) (Figure 2 & 4, respectively), and weak correlation between inhibition of peroxidation (R^2 = 0.732 7) (Figure 3), and total phenolics content of different extracts/fractions of *M. elengi* were detected.

4. Discussion

Phenolic compounds are commonly found in both edible

and non-edible plants and exhibit multiple biological effects, including antioxidant activity[8]. All bark extracts/fractions were found to have higher amount of phenolic components than extracts/fractions of seed. DPPH radical is a stable free radical which upon receiving proton from hydrogen donor loses its chromophore and become yellow. As the concentration of phenolic compounds or number of OH group on aromatic ring increases, the ability of phenols to scavenge the DPPH radical also increases[11,12]. All the samples of bark extracts/fractions exhibited appreciable scavenging activity ranging from 72.0% to 92.0%, while all the seed samples, except ethyl acetate fraction (88.0%), have no ability to scavenge the DPPH radical. The antioxidant potential of extracts/fractions measured by the DPPH assay was also expressed as SC_{50} values. The values were calculated in μg/mL of extract/fraction, required to lower the initial absorbance of DPPH solution by 50%. MBM fraction exhibited the lowest SC_{50} value among all the others extracts/fractions. To determine the lowest concentration (μg) which has ability to neutralize the DPPH free radical DPPH. TLC autographic assay was applied on the active extracts/fractions. The lowest inhibitory concentration calculated was 1 μg each for MBM, acetone:water extract (MBA) and ethyl acetate fraction (MBE) of bark.

The principle of the antioxidant activity is the ability of a sample to neutralize or capture the free radicals. Different parts of *M. elengi* were used to evaluate their antioxidant activities using DPPH scavenging, FTC, and phosphomolybdate assays. Total phenolic content were estimated by FC method which showed highest amount in the bark extracts/fractions. Several authors have reported that the concentration of phenolic compounds is generally higher in the young tissues which later on condensed to form complex phenolic compounds[13, 14]. Higher amount of phenolic compounds in bark can be attributed towards the accumulation of condensed phenols with the maturity of plant.

The results revealed that the scavenging ability of the extracts/fractions of *M. elengi* was dependent on the concentration of phenols. Similarly the results of total antioxidant activity were also correlated with the phenolic contents. Our results are consistent with the findings of many research groups[15-17] who reported positive correlation between total phenolic content and antioxidant activity. According to Ronald[18] in DPPH and phosphomolybdenum assay, antioxidant activity can either proceed through hydrogen atom transfer (HAT) or reduction (SET), while the peroxyl radicals generated in lipid peroxidation are quenched by HAT mechanism. Antioxidant activity of phenolic compounds is often associated with their redox properties[19], therefore it can be suggested that the phenolic extracts/fractions of *M. elengi* show antioxidant activity through single electron transfer mechanism (SET). The low inhibition of lipid peroxidation by the extracts/fractions of *M. elengi* was also consistent with the lack of HAT mechanism in the antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Esam HM, Ali HK. Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chem* 2000; **69**: 135-141.

[2] Rimm EB, Ascherio A, Grovannucci E, Spielgelman D, Stampfer MJ, Willet WC. Vegetable, fruits, and cereal fiber intake and risk of coronary heart disease among men. *J Am Med Assoc* 1996; **275**: 447-451.

[3] Huang D, Ou B, Hampsch-Woodill MF, Judith A, Prior RL. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agr Food Chem* 2002; **50**: 4437-4444.

[4] Payal JS, Mitesh SG, Mamta BS, Sunita SG, Devdas S. Study of *Mimusops elengi* bark in experimental gastric ulcers. *J Ethnopharmacol* 2003; **89**: 305-311.

[5] Shahwar D, Raza MA. *In vitro* antibacterial activity of extracts of *Mimusops elengi* against gram positive and gram negative bacteria. *Afr J Microb Res* 2009; **3**(8): 458-462.

[6] Shahwar D, Raza MA, Mirza ASM, Abbasi MA, Ahmad VU. Comparative study of antioxidant and antimicrobial activities of stem-bark extracts of *Litchi chinensis* and its organic fractions. *J Chem Soc Pak* 2010; **32**(3): 357-362.

[7] Osawa T, Namiki M. Novel type of antioxidant isolated from leaf wax of *Eucalyptus leaves*. *Agric Biol Chem* 1981; **45**: 735-739.

[8] Huda-Faujan N, Noriham A, Norrakiah AS. Babji AS. Antioxidant activity of plants methanolic extracts containing phenolic compounds. *Afr J Biotechnol* 2009; **8** (3); 484-489.

[9] Shahwar D, Shafiq UR, Naeem A, Ullah S, Raza MA. Antioxidant activities of the selected plants from the family *Euphorbiaceae, Lauraceae*, *Malvaceae* and *Balsaminaceae*. *Afr J Biotechnol* 2010; **9**(7): 1086-1096.

[10] Abbasi MA, Aslam A, Riaz T, Aziz UR, Shahzadi T, Siddiqui SZ, et al. Investigation on the comparative antioxidant potential of various fractions of *Vitex negundo* of Pakistani origin. *Biosci Res* 2011; **8**(1): 1-7.

[11] Miguel MG. Antioxidant activity of medicinal and aromatic plants. *Flav & Fragr J* 2010; **25**(5): 291-312.

[12] Shahwar D, Raza MA, Toqir A, Viqar, UA. Microbial transformation of vanillin isolated from *Melia azedarach* to vanillyl alcohol followed by protease inhibition and antioxidant Activity. *J Chem Soc Pak* 2011; **33**(5): 715-719.

[13] Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flower and fruit pulp. *Food Chem* 2002; **79**: 61-67.

[14] Wang SY, Lin HS. Antioxidant activity of fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stages. *J Agr Food Chem* 2000; **48**: 140-146.

[15] Muhammad ZQ, Faraz AR, Rukhsana K, Durre S, Raza MA. *In-vitro* antioxidant potential of aqueous and organic extracts of *Clematis connata*. *Asian J Chem* 2011; **23**(9): 4017-4020.

[16] Shahwar D, Raza MA, Saeed A, Riasat M, Chatta FI, Javaid M, et al. Antioxidant potential of the extracts of *Putranjiva roxburghii*, *Conyza bonariensis*, *Woodfordia fruiticosa* and *Senecio chrysanthemoids*. *Afr J Biotechnol* 2012; **11**(18): 4288-4295.

[17] Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agr Food Chem* 2011; **49**: 5165-5170.

[18] Ronald LP, Xianli W, Karen S. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agr Food Chem* 2005; **3**: 4290-4302.

[19] Yıldırım A, Mavi A, Oktay M, Kara AA, Algur F, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* D.), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis*) extracts. *J Agr Food Chem* 2000; **48**: 5030-5034.