

Antioxidant Capacity and Phenolic Content of *Mimusops elengi* Fruit Extract

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ABSTRACT

The antioxidant capacities of the phenolic compounds extracted from immature green, mature green and orange ripe fruits of *Mimusops elengi* were investigated. The compounds were first removed as crude extracts from the fruits using methanol-acetone and then further separated into three different fractions designated as free phenolic acids (F1), soluble phenolic esters (F2) and insoluble phenolic acid esters (F3). The antioxidant capacity of each fraction was determined by radical scavenging (DPPH and ABTS) assays. The antioxidant capacity values of these fractions, expressed as gallic acid equivalents (GAE) by ABTS assay for immature and mature fruits were in the range of 13.5 ± 0.1 - 441.7 ± 4.8 mg/g extract with relative capacities being $F2 > F3 > F1$. The GAE values for ripe fruits were in the range of 192.3 ± 1.0 - 212.5 ± 4.6 mg/g extract with relative values of $F2 \approx F3 > F1$. The antioxidant capacity of the crude extract from immature fruit (GAE = 318.5 ± 12.3 mg/g extract) was higher than that of either the mature (GAE = 234.1 ± 9.2 mg/g extract) or the ripe fruit (GAE = 111.9 ± 4.9 mg/g extract) at $p = 0.05$. High performance liquid chromatographic analysis confirmed that all phenolic fractions contained gallic acid as a constituent. The total phenolic contents determined by the Folin-Denis assay were consistent with the antioxidant capacity. From the results, *Mimusops elengi* fruits appeared to be a good source of natural antioxidant.

Key words: antioxidant capacity, phenolic extract, *Mimusops elengi*, pikul fruit, natural antioxidant

INTRODUCTION

Mimusops elengi L. (Sapotaceae), known in Thai as “Pikul”, is an evergreen tree whose distribution extends to India, Burma, Pakistan and Thailand. It is normally cultivated in gardens as a fragrant flowering tree. Various parts of this plant are used in Indian medicines as febrifuges, astringents, purgatives and stimulants (Jahan *et al.*, 1995). Crude extracts of the bark and different fractions of the extracts have been investigated for

their effects on gastric ulcers (Shah *et al.*, 2003). Chemical constituents reported from *Mimusops elengi* extracts are taraxerol, taraxerone, ursolic acid, betulinic acid, α -spinosterol, β -sitosterol glycoside, quercitol (Misra and Mitra, 1967), lupeol (Misra and Mitra, 1968), pentacyclic triterpenes, such as mimusopgenone and mimugenone in the seeds (Sen *et al.*, 1995), triterpenoid saponins, such as mimusopsides A and B, mimusopin, mimusopsin, mimusin, Mi-saponin A and 16 α -hydroxy Mi-saponin A from the seeds

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(Sahu *et al.*, 1995, 1997; Sahu, 1996; Lavaud *et al.*, 1996).

In recent years, there has been much interest in natural antioxidants, especially in plant polyphenols and numerous articles about their beneficial effects on health have been published. Antioxidant nutrients are important for limiting damaging, oxidative reactions in cells which may predispose the organism to the development of major clinical conditions such as heart disease and cancer (Duthie and Crozier, 2000). Synthetic antioxidants can be toxic and expensive. Therefore, there is a need to identify effective, natural and possibly more-economical antioxidants. Ripe *Mimusops elengi* fruits are consumed by some local Thai people. Thus in this research, the antioxidant capacities of *Mimusops elengi* fruits at three stages of development (immature, mature and ripe) were investigated. The crude methanol-acetone extracts of *Mimusops elengi* fruit from each stage of development were resolved into the free phenolic acids (F1), soluble phenolic esters (F2) and insoluble phenolic acid esters (F3) fractions. All phenolic fractions were tested for their antioxidant capacity (DPPH and ABTS assays) and underwent total phenolic analysis (Folin-Denis assay). High-performance liquid chromatography (HPLC) was used to confirm the existence of gallic acid in the F1 and in the after-hydrolysis fractions (F2 and F3). The antioxidant capacities and total phenolics were reported as gallic acid equivalents (GAE).

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS) and sodium tungstate dihydrate were sourced from Fluka, Germany. Potassium persulfate (di-potassium peroxodisulfate) was sourced from Merck, Germany. The standard antioxidant was gallic acid

monohydrate (GA), 98 % sourced from Riedel-de Haen, Germany. Other common reagents were of analytical grade.

Plant materials

Fresh *Mimusops elengi* fruits were collected during April-July 2006 from Pikul trees cultivated on the Sanarnchan Palace campus, at Silpakorn University, in Nakhon Pathom province. The plant materials were identified by Associate Professor Aree Thongpukdee in the Biology Department, Faculty of Science, Silpakorn University. The fruits were separated into three stages based on their development: immature green, mature green and orange ripe fruits. The exo- and meso-carp of *Mimusops elengi* fruits were freeze-dried in sealed packs and kept at -4°C until use.

Preparation of the extracts

Each freeze-dried sample was ground with a pestle and mortar and the phenolic extraction procedure of Krygier *et al.* (1982) was followed (Figure 1). Ten grams of freeze-dried material was extracted five times at room temperature with 50 mL of 70% methanol in water and 70% acetone in water (1:1). After centrifugation at 700 rpm for 5 min, the combined supernatants, the so-called crude extract, was further separated into three fractions. The free phenolic acids fraction (F1) was extracted from the supernatant of the crude extract using diethyl ether (DE)-ethyl acetate (EA) (1:1) as a solvent-to-water phase in the ratio of 1:1. To the resulting water phase from F1, 20 mL of 4 M NaOH was added and the mixture was incubated for 4 h at room temperature to hydrolyze the phenolic esters. The resulting solution was acidified to pH 2 with 6 M HCl before removing fatty acid substances by extraction with hexane. The liberated phenolic acids were then extracted with diethyl ether-ethyl acetate, as described above, yielding the soluble phenolic acid esters fraction (F2). The residues

from the methanol-acetone extractions were hydrolyzed directly with 4 M NaOH under the same conditions as the esters. After acidification and centrifugation, the clear supernatants were extracted with hexane and then with diethyl ether-ethyl acetate, using the same procedure described for F2, to yield the insoluble-bound phenolic acids fraction (F3).

HPLC analysis

The HPLC apparatus used was an HP1100 system (Agilent Technologies, U.S.A) with isocratic delivery and a UV-Vis detector monitored at 270 nm. The separation was carried out at room temperature on a Zorbax Eclipse XDB-C18 column (15 cm × 3 mm i.d., 5 µm particle) with a guard column C 18. The mobile phase

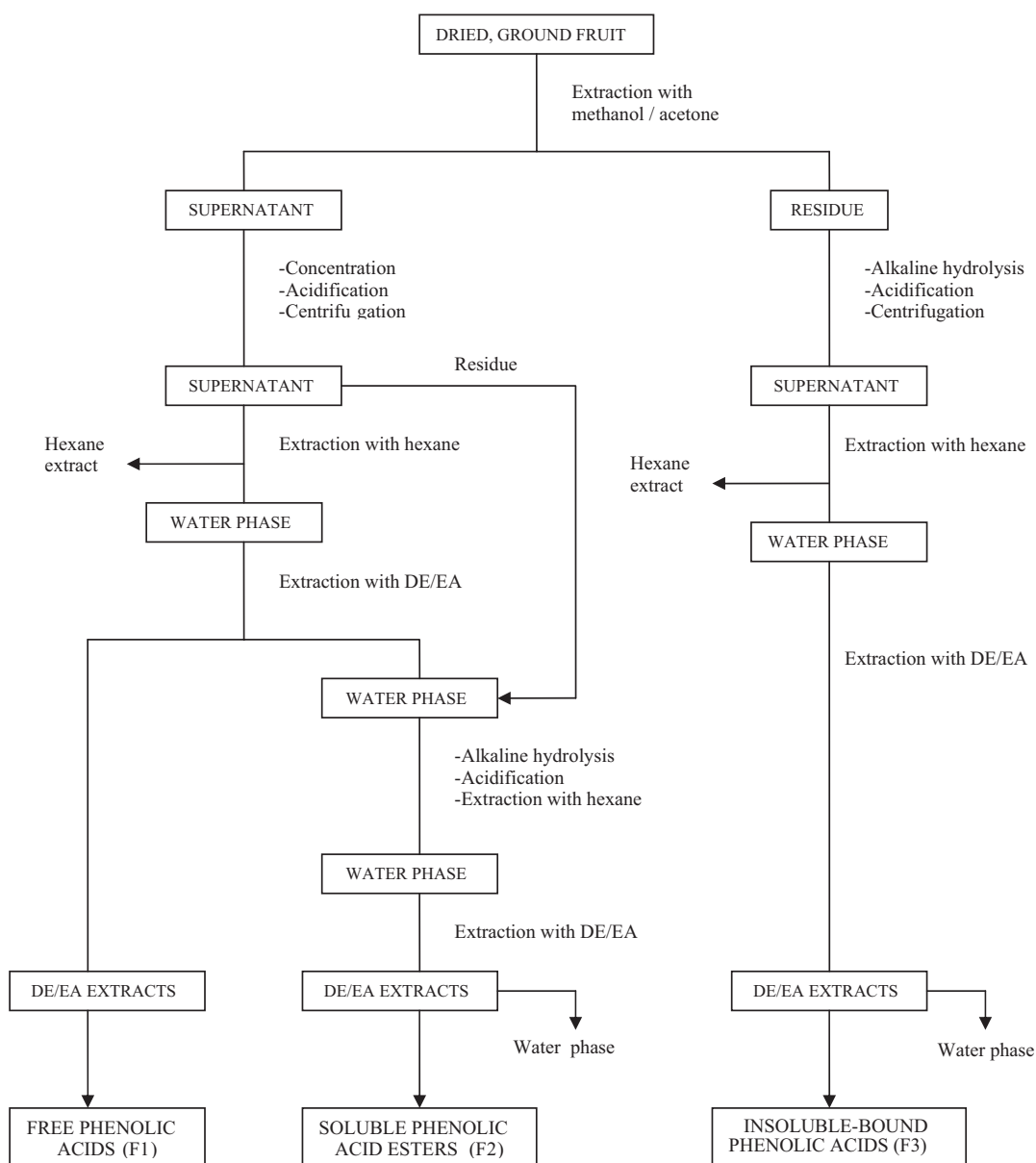


Figure 1 Procedure for extraction and separation of phenolics from *Mimosa elengi* fruit.

contained 0.1% phosphoric acid in methanol and 0.1% phosphoric acid in water at the ratio of 10:90 (v/v). The injection volume of the extract was 20 μ L.

Antioxidant capacity tests

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

This method used was a modification of Atoui *et al.* (2005). The commercially available free radical DPPH• was dissolved in methanol to a concentration of 6×10^{-5} M. Different aliquots of the sample or standard solution in microliters were mixed with 4.50 mL DPPH and the final volume was adjusted to 5.0 mL with water. After 10 min incubation, the absorbance at 517 nm was measured in a 1 cm cell using a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). The percentage of radical remaining was calculated from the equation (1):

$$\% \text{ radical remaining} = (\text{Abs sample} / \text{Abs control}) \times 100 \quad (1)$$

where Abs is the absorbance at 517 nm.

The absorbance corresponded directly to the radical concentration, the control being a mixture of 4.50 mL DPPH reagent and 0.50 mL deionized water. For each sample, the % radical remaining was plotted against the concentration of the sample and the concentration that caused a 50% decrease of Abs relative to that of the control was determined as the effective concentration, EC₅₀. A calibration curve of % radical remaining against concentration of standard gallic acid in the concentration range 0.4-2.5 ppm was prepared. The EC₅₀ of each sample was determined and calibrated with the EC₅₀ of standard gallic acid to give the antioxidant capacity of the sample in GAE units (mg/g extract).

2,2'-azinobis (3-ethylbenzothiazoline-sulphonic acid) (ABTS) assay

The ABTS assay followed the method described by Lo and Cheung (2005) with some modification. The radical ABTS•⁺ was generated

by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration), following 12-16 h incubation in the dark at room temperature. The resulting solution was diluted with water to give an absorbance of 0.70 ($\pm$ 0.05) at 734 nm before use. A 2.90 mL aliquot of this solution was mixed with several volumes of each sample and the total volume was adjusted to 3.00 mL with water. The absorbance at 734 nm was measured by a Lambda 35 UV/VIS spectrophotometer after standing for 10 min. The "percentage of radical remaining" was calculated and then plotted against the concentration of the sample. The antioxidant capacity of the sample in GAE units (mg/g extract) was determined by calibration of the EC₅₀ as in the DPPH assay, but with the calibration curve of standard gallic acid in the concentration range 0.2-3.8 ppm.

Total phenolics analysis

The Folin-Denis assay was used for total phenolics determination. Folin-Denis reagent was prepared as in the work of Erdemoglu *et al.* (2000). A mixture of 10.0 mg of sodium tungstate, 2.0 g of phosphomolybdic acid, 5 mL of phosphoric acid and 75 mL of de-ionized water was refluxed for 2 h before making the volume up to 100 mL with de-ionized water. To 0.10-1.0 mL of standard gallic acid or sample solution, 2.0 mL of the Folin-Denis reagent and 2.0 mL of saturated sodium carbonate solution were added and the total volume was made up to 25 mL with de-ionized water. After 10 min incubation, the absorbance at 700 nm was measured. A calibration curve for gallic acid in the concentration range 1.0-10.0 ppm was used for determining total phenolics in the sample fractions and the findings were expressed as mg of gallic acid per gram of the extracted fraction.

RESULTS AND DISCUSSION

The percentage yield of all phenolic fractions from the three ripening stages of

Mimusops elengi fruits are shown in Table 1. The yields implied that the phenolic constituents of the fruit changed during the ripening process. They showed decreasing amounts of free phenolic acids and soluble phenolic esters, but increasing amounts of insoluble-bound phenolic acids as the fruit proceeded through the ripening stages. From the HPLC analysis (Figure 2), the phenolic fractions contained gallic acid, as their chromatograms show a dominant chromatographic peak at 2.9 min which is the retention time of the standard for gallic acid. Hence, gallic acid was chosen as the standard for expression of antioxidant capacity and total phenolics, expressed as gallic acid equivalents (GAE) in this work.

The antioxidant capacities of all the *Mimusops elengi* fruit extracts determined by both DPPH and ABTS assays are shown in Table 2.

Table 1 Percentage yield (w/w of freeze dried) *Mimusops elengi* fruit extracts from three stages of the ripening process.

Extract	% Yield
Immature fruit	
F1	0.36
F2	3.53
F3	0.59
Mature fruit	
F1	0.12
F2	2.73
F3	0.80
Ripe fruit	
F1	0.10
F2	1.61
F3	8.29

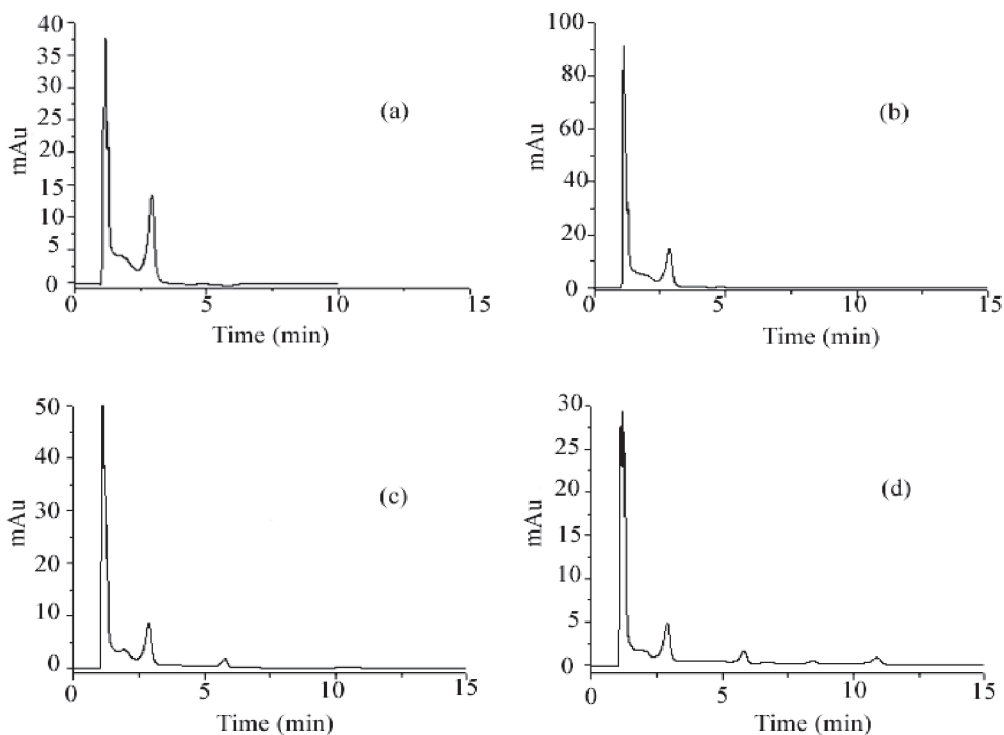


Figure 2 HPLC chromatograms indicated the presence of gallic acid: (a) standard gallic acid 3 ppm, (b) F1, (c) F2 and (d) F3 of the immature fruit extracts. Chromatographic conditions are detailed in the text .

Table 2 Antioxidant capacities by DPPH and ABTS assays of *Mimusops elengi* fruit extracts.

Extract	GAE (mg/g extract) $\pm$ SD*	
	DPPH assay	ABTS assay
Crude extract		
Immature	183.2 $\pm$ 3.6	318.5 $\pm$ 12.3
Mature	140.1 $\pm$ 2.8	234.1 $\pm$ 9.2
Ripe	58.4 $\pm$ 0.9	111.9 $\pm$ 4.9
Immature fruit		
F1	10.0 $\pm$ 0.1	13.5 $\pm$ 0.2
F2	311.0 $\pm$ 9.4	292.0 $\pm$ 10.0
F3	146.8 $\pm$ 5.8	109.7 $\pm$ 0.8
Mature fruit		
F1	13.1 $\pm$ 0.3	17.2 $\pm$ 0.5
F2	402.8 $\pm$ 12.5	441.7 $\pm$ 4.8
F3	231.1 $\pm$ 8.4	110.8 $\pm$ 2.4
Ripe fruit		
F1	184.4 $\pm$ 9.7	192.3 $\pm$ 1.0
F2	186.4 $\pm$ 6.6	212.5 $\pm$ 4.6
F3	209.0 $\pm$ 13.7	209.2 $\pm$ 5.9

* based on five samples.

The antioxidant capacities of the crude extracts at different ripening stages changed according to the following sequence: immature > mature > ripe. The paired-t test of the GAE values of the crude extracts at each stage from the DPPH and the ABTS assays revealed a significant difference (one-tailed, $P = 0.05$) between the two methods. Arnao (2000) reported the possibility that the DPPH assay was prone to sample matrix interferences more than the ABTS assay, due to the lower wavelength monitoring, so the results from the ABTS assay should be more reliable. However, after further separation of the crude extracts at different stages, so that there were three different forms of phenolics, statistical tests between the results from the two methods indicated no significant difference (paired t-test, one-tailed, $P = 0.05$). The relative antioxidant capacities of the three phenolic fractions of the immature and mature stages were $F2 > F3 > F1$, while for the ripe stage, it was $F2 \approx F3 > F1$, except for the DPPH assay ($F3 > F2 \approx F1$). The total phenolics analysis by Folin-Denis assay (Table 3)

showed the highest amount of phenolics in the form of soluble phenolic acid esters (F2) at the mature stage, as well as the highest antioxidant capacity of this fraction from both DPPH and ABTS assays. The correlations between results of

Table 3 Total phenolics analysis by Folin-Denis assay.

Extract	Folin-Denis GAE (mg/g) $\pm$ SD*
Immature fruit	
F1	20.1 $\pm$ 0.8
F2	361.9 $\pm$ 8.5
F3	168.8 $\pm$ 2.4
Mature fruit	
F1	31.7 $\pm$ 0.6
F2	516.9 $\pm$ 14.7
F3	310.9 $\pm$ 5.1
Ripe fruit	
F1	221.6 $\pm$ 13.5
F2	284.2 $\pm$ 18.6
F3	298.5 $\pm$ 7.9

* Based on five samples.

the antioxidant capacity from both the DPPH and ABTS assays relative to the results of the total phenolics from the Folin-Denis assay were highly significant ($P < 0.01$) with correlation coefficients of 0.9856 and 0.9348, respectively. Hence, the phenolic compounds could have been the main cause of antioxidant capacity. Further studies to elucidate the chemical structures of the phenolic compounds of different *Mimusops elengi* fractions would clarify this issue.

CONCLUSION

Phenolic extracts of *Mimusops elengi* fruit at different ripening stages were analyzed for their antioxidant capacities and total phenolic contents. The good yield of the fruits from this species and its high antioxidant capacity strongly suggested that this tropical, edible fruit could be a potential, rich source of natural antioxidant. Further studies on the isolation and structural characterization of the active components in Pikul are underway. Information about the active components could lead to promising alternative food and cosmetic ingredients with antioxidant capacity.

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