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FREE RADICAL SCAVENGING ACTIVITY, TOTAL PHENOLIC AND TOTAL ANTHOCYANIN CONTENTS OF EXTRACTS FROM MAMAO (*ANTIDESMA GHAESEMBILLA*) FRUITS

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KEYWORDS: *Antidesma ghaesembilla*, mamao, free radical scavenging activity, total phenolic, total anthocyanin

INTRODUCTION

Antidesma ghaesembilla or mamao is a plant in the Euphorbiaceae family, which has reddish purple fruits suggesting high amount of anthocyanin. The leaves and fruits of this plant have been traditionally used as anti-dysentery agent and blood circulation stimulant while the barks have been used as astringent and tonic. At the present, the ripe fruits of mamao have been developed for many nutritional purposes such as wine, jam, juice and concentrated juice. Therefore, this experiment was set up in order to investigate free radical scavenging activities of extracts from the fruits of mamao prepared by different extraction methods using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Quantitative analysis of total phenolic and total anthocyanin contents of the extracts using spectrophotometric methods was conducted. Finally, phytochemical analysis of active extract by thin layer chromatography was performed.

MATERIALS AND METHODS

Plant materials: Ripe (purple) and nearly ripe (red) fruits of mamao (*A. ghaesembilla*) were collected from Kalasin province, Thailand in January, 2012. The plant materials were identified by Assoc.Prof.Dr. Wandee Gritsanapan, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. Samples were cleaned and air dried, then the seeds were removed and the flesh was cut into small pieces. The flesh of purple and red fruits of mamao was separately extracted using the extraction procedures shown below.

Decoction: The flesh of purple and red fruits of mamao was boiled (80°C) with distilled water (plant/water ratio 1:10 w/v) for 8 h, then filtered. The filtrate was dried using lyophilized method to obtain dried purple and red fruit decoction extracts (PD and RD, respectively).

Maceration: The flesh of purple fruits of mamao was separately macerated with 95% ethanol, methanol and acetone (plant/water ratio 1:10 w/v) for 48 h, then filtered. The filtrate was evaporated under reduced pressure to dryness. These dried ethanol, methanol and acetone extracts of purple fruits were labelled as PME, PMM and PMA, respectively.

Squeezing: The flesh of purple fruits of mamao was minced with distilled water (plant/water ratio 1:10 w/v) using electronic blender, then filtered. The filtrate was dried using lyophilized method to yield squeezed purple fruit extracts (PS).

Determination of free radical scavenging activity using DPPH scavenging assay: The free radical scavenging effect of extracts from mamao fruits, as well of standard trolox, corresponding to the quenching ability on DPPH radical was carried out as described by Yamasaki et al.(1). Each sample was assayed in triplicate and the average of EC₅₀ values was calculated.

Determination of total phenolic content using Folin-Ciocalteu method: Using the method applied from Naithani et al. (2), solutions of plant extracts were oxidized with Folin-Ciocalteu reagent and the reactions were neutralized by sodium carbonate solution. The absorbance of the resulting blue colored solution was measured at 765 nm after 60 min. Each sample was done in duplicate. Total phenolic content was expressed as g gall acid equivalent in 100 g extract (g% GAE).

Determination of total anthocyanin content using pH differential method: Applying the methods from Sutharut and Sudarat (3), plant extract solutions were separately prepared in potassium chloride buffer, pH 1.0 and sodium acetate buffer pH 4.5 and left at room temperature for 15 minutes. Then the absorbance of each solution was measured at 510 and 700 nm. Total anthocyanin content was calculated and expressed as g cyanidin-3-glucoside equivalent in 100 g extract (g% C-3-GE).

Thin layer chromatographic fingerprints: Thin layer chromatography of decoction extract from the red fruits of *A. ghaesembilla* was performed on TLC pre-coated silica gel 60 GF₂₅₄ plate using 2-propanol-acetone-water (7:2:1) as the solvent system. TLC plates were detected under UV 254 and 366 nm and DPPH spray reagent.

Statistical analysis: All data were reported as mean ± standard deviation of triplicate experiment. Student t-test was used to compare mean ($P < 0.05$). All analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., USA).

RESULTS

Free radical scavenging activity: DPPH scavenging assay: Comparing between purple fruit extracts of mamao from various methods of extraction, extract from decoction promoted higher free radical scavenging activity. This method was then selected for the extraction of the red fruits. As shown in Table 1, the red fruit decoction extract (RD) promoted the strongest free radical scavenging activity with an IC_{50} of $72.42 \pm 3.52 \mu\text{g/ml}$.

Determination of total phenolic content: Folin-Ciocalteu method: As shown in Table 1, RD exhibited the highest total phenolic content of $2.81 \pm 0.88 \text{ g\% GAE}$. Total phenolic content of extracts from the purple fruits of mamao prepared from different extraction methods ranged from 0.87 ± 1.00 to $2.63 \pm 1.02 \text{ g\% GAE}$.

Determination of total anthocyanin content: pH differential method: Squeezing extract from the purple fruits of mamao gave the highest amount of total anthocyanin content of $61.51 \pm 0.95 \text{ g\% C-3-GE}$. Maceration with organic solvents yielded the extracts with lower anthocyanin contents (Table 1).

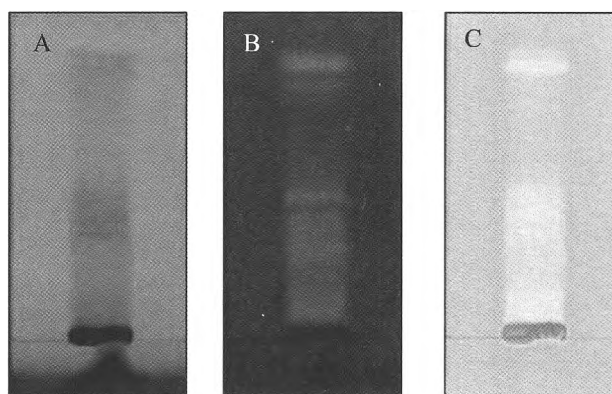
Thin layer chromatographic fingerprints: Decoction extract from the red fruits of mamao produced the strongest free radical scavenging activity with high total phenolic and total anthocyanin contents. Therefore, this extract was phytochemically analyzed using TLC. Thin layer chromatograms of RD showed specific fingerprint with some major compounds positive to DPPH spray reagent suggesting antioxidant compounds (Figure 1).

Table 1 Free radical scavenging activity, total phenolic and total anthocyanin contents of extracts from the mamao fruits

Sample	Yield (%w/w)	DPPH assay (IC_{50} , $\mu\text{g/ml}$)	Total phenolic (g% GAE)	Total anthocyanin (g% C-3-GE)
PD	9.32	91.62 ± 4.74^a	1.69 ± 1.11^a	8.67 ± 0.56^a
RD	3.38	72.42 ± 3.52^b	2.81 ± 0.88^b	15.79 ± 2.09^b
PME	15.76	575.18 ± 17.14^c	0.87 ± 1.00^c	6.21 ± 0.57^c
PMM	10.95	3552.23 ± 393.03^d	1.72 ± 1.56^a	6.55 ± 1.04^c
PMA	6.91	541.70 ± 51.79^c	1.49 ± 0.86^a	6.56 ± 0.84^c
SPS	7.06	120.73 ± 5.75^c	2.63 ± 1.02^d	61.51 ± 0.95^d
Trolox	-	29.98 ± 0.63^1	-	-

Values in each column with different superscripted letters are significantly different ($P < 0.05$)

Figure 1 TLC fingerprints of decoction extract from the red fruits of mamao
Stationary phase: silica gel GF₂₄₅
Solvent system: propanol-acetone-water (7:2:1)
Detection: A= UV 254 nm,
B= UV 366 nm, C= DPPH spray reagent



DISCUSSION

The fruits of mamao which have red to purple color when they are ripening have popularly been developed as various nutritional foods and drinks such as wine, jam, juice and concentrated juice. The red to purple color of mamao fruits suggest their abundance in anthocyanins which were previously reported to exhibit antioxidant activity (4). From the results of extracts from purple fruits, decoction method gave the extract with the strongest free radical scavenging activity, while squeezing gave the extract with the highest total phenolic and total anthocyanin contents. These results could be the guidelines for the preparation of plant extract in manufacturing level. Moreover, decoction extract from the red fruits exhibited the highest free radical scavenging activity with high total phenolic content. The results suggested that the nearly ripe fruits of mamao (red fruits) are also good raw material that promoted the

extract with strong free radical scavenging and high phenolic content. Thin layer chromatogram of red fruit decoction extract showed some major antioxidative compounds.

CONCLUSION

Squeezing extract from the ripe fruits of mamao contained the highest total anthocyanin, while decoction of the nearly ripe fruits yielded the extract with the strongest free radical scavenging activity and the highest total phenolic compounds.

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