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Determination of Total Phenolic Compounds and Flavonoid Contents Related to Antioxidant Activity of "Mao" Fruit Juice from Various Production Sources

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The "Mao" compiled fruits (*Antidesma thwaitesianum* Muell. Arg.) are generally found in tropical forests used as commercial products of juice and wine in Thailand. It is very rich source of phenolic compounds, flavonoids and other antioxidants with their beneficial effects on human health. In the present study, spectrophotometric methods concerning ferric reducing antioxidant power, Folin-Ciocalteu assay and aluminum chloride colorimetric procedure were performed to estimate antioxidant activity and the contents of phenolics and flavonoids in five popular brand-products of 100 % juice sampling from different local sources. From the results, the highest values of antioxidant activity (183.46 ± 1.01 mM Fe^{2+}), total phenolic compounds (643.75 ± 7.33 mg gallic acid equivalents (GAE)/100 mL) and flavonoids (1,989.98 ± 5.32 mg catechin equivalents (CE)/100 mL) were found. While the lowest ones of antioxidant activity (102.42 ± 7.23 mM Fe^{2+}), total phenolics (273.11 ± 14.75 mg GAE/100 mL) and flavonoids (879.10 ± 5.32 mg CE/100 mL) were comparable. The differences in the observed antioxidant activities of these juice samples may relate to their abundant of phenolics and flavonoid contents. Therefore, source of the Mao fruits as raw materials would be a factor providing pronounced antioxidant activity, particularly phenolic compounds and flavonoids in the fruit juices.

Keywords: Antioxidant activity, Phenolic compounds, Flavonoids, Ferric reducing antioxidant power.

INTRODUCTION

Antidesma thwaitesianum Muell. Arg. or "Mao" is classified in the family Euphobiaceae, genus *Antidesma*. Mao is one of many tropical fruits which have been known for long time in Thailand, especially in Phu Phan district, Sakon Nakhon province. The Mao fruit is commonly consumed as commercially available products of concentrate juice and wine, since in particular the fruit juice contains a very rich source of antioxidants. The recent studies have investigated that the health benefits and antioxidant activities of Mao Luang fruits are mainly attributed to almost known phenolic compounds *i.e.*, flavonoids including anthocyanins, proanthocyanidins and tannins, resveratrol and benzoic, caffeic and cinnamic acids[1-4].

Antioxidants are substance which can protect the human body from free radicals and reactive oxygen species induced chronic diseases[5]. It can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction which has very important in the prevention of diseases[6]. Phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals[7]. The part of flavonoids has many beneficial effects on human and animal health such as anti-aging, antioxidant. These are the best known natural antioxidants[8,9]. The capacity of phenolic compounds and flavonoids to act as antioxidants depends upon their position of hydroxyl groups and other features in the chemical structure which are important for their antioxidant and free radical scavenging activities[10,11].

The interests in natural antioxidants have been increased mostly for those containing flavonoids and phenolic compounds which prevent free radical damage. The objective of this study was to measure the antioxidant activity and total phenolics and flavonoid contents in various juice samples. Moreover, the correlation between total phenolics and flavonoid contents and antioxidant activity in the fruit juice from various sources was investigated. The results will provide information of the antioxidants, total phenolics and flavonoid contents in the fruit juices.

EXPERIMENTAL

2,4,6-*tri*(2-Pyridyl)-*s*-triazine (TPTZ) was purchased from Sigma (Switzerland). Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)

was purchased from QRëC® (New Zealand). Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), sodium acetate (CH_3COONa), sodium nitrite ($NaNO_2$) and sodium hydroxide (NaOH) were purchased from Carlo Erba (Italy). Folin-Ciocalteu reagent was purchased from Merck Chemical Supplies (Germany). Sodium carbonate (Na_2CO_3) and Aluminium chloride ($AlCl_3$) were purchased from Analytical Univar Reagent Ajax Finechem (Australia). Catechin was purchased from Aldrich (China). All chemicals and solvents used such as methanol, acetic acid and hydrochloric acid were of analytical grade.

Ultraviolet-visible spectrophotometer (Agilent 8453, USA) was used to determine the antioxidant activity, total phenolics and flavonoid contents.

Sample preparation: Five brands of the Mao juice samples were chosen and analyzed in triplicate. They were purchased from Phu Phan district (Maoluang Phuphan, Chang Palangsong, Maoluang Saitong), Khok Srisuphan district, Sakon Nakhon province and Chaiyaphum province, Thailand. The samples were stored in refrigerator at 4 °C. The 100 % Mao juice samples were extracted using an acidified methanol. Briefly, 5 mL of the Mao juice was added into the mixture of 5 mL 0.1 % (v/v) HCl in methanol (10:90, v/v). After that the mixture was sonicated by ultrasound assisted extraction (Ultrasonic Sonicator, Elma, Transsonic digitals, Germany) for 20 min and kept at room temperature overnight. The mixture was centrifuged at 4000 rpm for 10 min. The supernatant was used for determination of antioxidant activity, total phenolic compounds and flavonoid contents.

Determination of antioxidant activity: Antioxidant activity was determined using ferric reducing antioxidant power (FRAP) assay which was done according to Ali *et al.*[12] with some modifications. The method is based on the reduction of a ferric-2,4,6-tripyridyl-*s*-triazine complex (Fe^{3+}-TPTZ) to its ferrous (Fe^{2+}-TPTZ), an intensive blue colored form in the presence of antioxidant. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM solution of 2,4,6-tripyridyl-*s*-triazine in the mixture of 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL solution of 2,4,6-tripyridyl-*s*-triazine and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C. One-mL of the supernatant (diluted 1:200 fold) was allowed to react with 3 mL of the FRAP solution. The absorbance of the mixture was measured at 593 nm after incubation at 37 °C for 10 min. Standard solutions of $FeSO_4 \cdot 7H_2O$ (50-300 mg/L) were used for the calibration curve. The results were expressed as mM Fe^{2+} per 100 mL sample.

Determination of total phenolic compounds: The total phenolic compounds were determined as described by Škerget *et al.*[13] with slightly modified. Briefly, 0.5 mL of the supernatant and 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent (FCR) were mixed, followed by the addition of 2 mL of 7.5 % (w/v) Na_2CO_3, then mixed well on a vortex vibrator for 5 min and incubated in the dark at ambient temperature (28 °C) for 1 h before the absorbance was measured at 765 nm. Gallic acid was used as a calibration curve and the results were expressed as mg of gallic acid equivalents per 100 mL sample.

Determination of flavonoid contents: The flavonoid was determined by aluminum chloride colorimetric method[14]. One mL of the supernatant or standard solution of catechin (100-400 mg/L) was added into a 10 mL volumetric flask, then 4 mL of deionized water was added and kept still for 5 min. After that 0.3 mL of 5 % (w/v) $NaNO_2$ and 1.5 mL of 2 % (w/v) $AlCl_3$ were added. The mixture was shaken for 5 min and then 2 mL of 1 M NaOH was added. The absorbance was measured at 510 nm. The flavonoid content was expressed as mg of catechin equivalents per 100 mL sample.

RESULTS AND DISCUSSION

Antioxidant activity: The antioxidant activity of the Mao juice was determined by FRAP assay, which measures the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ. The results are presented in Fig. 1.

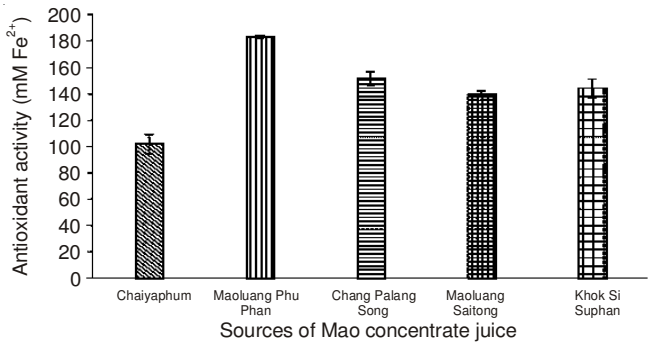

Fig. 1. Antioxidant activities of the Mao juice from five production sources

The results show that the highest antioxidant activity was observed in Maoluang Phuphan (183.46 ± 1.01 mM Fe^{2+}) followed by Chang Palangsong (152.08 ± 4.84 mM Fe^{2+}), Khok Sri Suphan (144.49 ± 7.16 mM Fe^{2+}), Maoluang Saitong (140.18 ± 2.25) and Chaiyaphum (102.42 ± 7.23 mM Fe^{2+}). These results gave 144.53 mM Fe^{2+} in averaged significant ferric reducing power, indicating the hydrogen donating ability of the existing phytochemicals. Therefore, almost of the juice samples possesses in high antioxidant activity.

Total phenolic compounds: The total phenolic compounds (TPCs) of various sources of the Mao juice were determined using the FCR method. This method is an electron transfer reaction which the phenolic compounds react with FCR and measures the reducing ability of the sample. The contents of total phenolic compounds in juice sample are shown in Fig. 2.

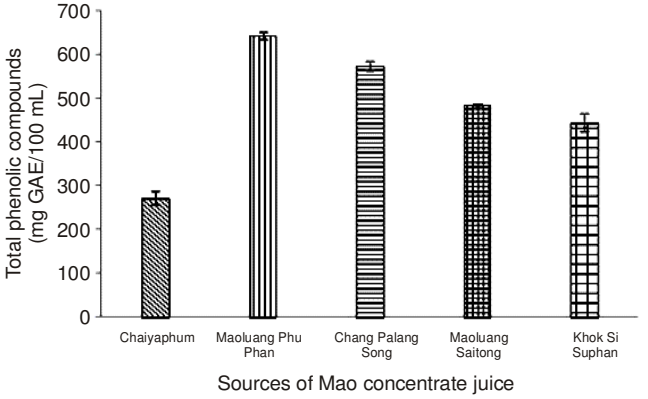

Fig. 2. Total phenolic compounds of the Mao juice from five production sources

From Fig. 2, total phenolic compounds (TPCs) found in five juice samples are ranged from 273.11 ± 14.75 to 643.75 ± 7.33 mg GAE/100 mL juice sample, with average of 484.09 mg GAE/100 mL. Maoluang Phu Phan still shows the highest value of total phenolic compounds (643.75 ± 7.33 mg GAE/100 mL) followed by Chang Palang Song (573.75 ± 10.8 mg GAE/100 mL), Maoluang Saitong (484.96 ± 4.01 mg GAE/100 mL), Khok Si Suphan (444.89 ± 20.35 mg GAE/100 mL), while Mao juice from Chaiyaphum gives the lowest one (273.11 ± 14.75 mg GAE/100 mL). Similar trend in total phenolic compounds is found comparing with those of their antioxidant activities (Fig. 1). Comparing these results with other fruit juices, total phenolic compounds of the Mao juice are higher than ready-to-drink orange juices (18.7-54.2 mg GAE/100 mL)[15], apple juices (5.2-14 mg GAE/100 mL)[16] and pomegranate juices (204.6-290.7 mg GAE/100 mL)[17].

Determination of flavonoid contents: The flavonoids in the Mao juice was determined by reacting with sodium nitrite, resulted in the colored flavonoid-aluminium complex formation, which can be detected at maximum wavelength[18] of 510 nm. The flavonoid contents found in these juice samples are shown in Fig. 3.

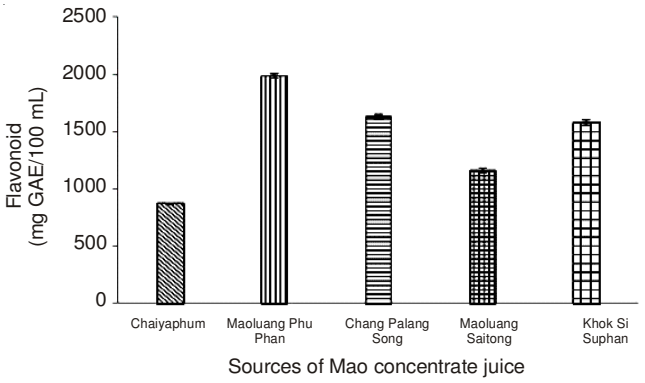

Fig. 3. Flavonoid contents of the Mao juice from five production sources

The flavonoid contents were calculated from the linear equation based on the calibration curve (y = 0.0023x + 0.0058) and expressed as mg of catechin equivalents per 100 mL sample. The results demonstrated that the juice from Maoluang Phu Phan has the highest flavonoid contents (1989.98 ± 5.32 mg CE/100 mL) followed by Chang Palang Song (1632.22 ± 22.50 mg CE/100 mL), Khok Si Suphan (1584.82 ± 27.42 mg CE/100 mL), Maoluang Saitong (1167.86 ± 19.62 mg CE/100 mL). While that of Chaiyaphum gives the lowest flavonoid contents (879.10 ± 5.32 mg CE/100 mL). Similar trend in the flavonoids of these juices is also found as the same as total phenolic compounds and antioxidant activity as mentioned above. The average amount of the total flavonoids is 1450.80 mg CE/100 mL. Therefore, it is evident that the Mao juices are highly potential source of flavonoids.

Conclusion

The antioxidant activities and both total phenolic compounds and flavonoid contents of five production sources of the Mao juices were investigated. The results show that Maoluang Phu Phan was found to possess the highest antioxidant activity, total phenolic compounds and flavonoid contents while the Mao juice obtained from Chaiyaphum gave the lowest data. The differences in the observed antioxidant activities from various juice samples may relate to their abundant of phenolic and flavonoid contents. The results suggest that their antioxidant activity would be corresponding with the contents of both phenolic compounds and flavonoids.

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