



## Phenolic Acids Content and Antioxidant Capacity of Fruit Extracts from Thailand

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### ABSTRACT

In this study, soluble and insoluble phenolic acids (SPA and IPA) in selected Thai fruit such as orange, banana, guava and mango using isocratic HPLC-UV method were determined. Results showed that the predominant compounds of all fruit studied were IPA (80.2-99.5%). Gallic and hydroxybenzoic acids were identified as major SPA in guava and orange, respectively. Gallic, hydroxybenzoic, vanillic, caffeic, syringic and ferulic acids were identified as major IPA in all fruit samples. Ferulic acid was the dominant IPA in orange and banana extracts ( $335.8 \pm 13.38$  and  $219.5 \pm 18.47$   $\mu\text{g/g}$  dry weights, respectively). Whilst, gallic acid was the dominant IPA in mango extract ( $542.5 \pm 6.80$   $\mu\text{g/g}$  dry weight) and hydroxybenzoic acid was the dominant IPA in guava extract ( $50.5 \pm 8.12$   $\mu\text{g/g}$  dry weight). The antioxidant capacity of all fruit extracts was also evaluated using a Folin-Ciocalteu's assay and DPPH free radical-scavenging assay. The phenolics in bound form of orange extract contained the highest total phenolic content ( $2.6 \pm 0.02$   $\mu\text{g GAE/ml}$ ). Its antioxidant capacity was 94.9%. Whereas insoluble phenolic content of guava extract had the least antioxidant capacity (22.0%).

**Keywords:** fruit extracts, phenolic acids, soluble phenolic acids, insoluble phenolic acids, antioxidant capacity

### 1. INTRODUCTION

Tropical fruits are well known to be associated with many medicinal properties. Thailand is a major source of fruit varieties, for example, orange, guava, banana and mango. These fruits have been reported to be a potent source of phenolic compounds [1-3]. There have been several studies indicated that these fruits contain the important phenolic acid.

Phenolic acids are simple compounds of non-flavonoid family constituting as a large group of phenolic compounds [4]. They are in the forms of hydroxybenzoic acids (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, sinapic acid) and hydroxycinnamic acids derivatives (gallic acid, vanillic acid, hydroxybenzoic acid, syringic acid) [5-7]. They have excellent antioxidant activities,

which are higher than those of vitamins C and E against reactive oxygen [8]. Thus, they have a wide range of biological activities, such as antioxidant activities, protection against coronary heart diseases, anti-inflammatory, anti-tumour, anti-mutagenic, anti-carcinogenic and anti-microbial activities [9-10].

Phenolic acids can be classified as free (soluble) and bound (insoluble) phenolic acids. The free forms are simply extracted with organic solvent. While, bound phenolic acids are typically involved in cell wall structure and require acid or base hydrolysis to release these bound compounds from the cell matrix. Researcher found that the level of phenolics in plant sources also depends on cultivation techniques, cultivar, growing conditions, extraction methods and extraction temperature, ripening process, as well as processing and storage conditions [2, 5, 21].

Various solvents, such as methanol, ethanol, propanol, acetone, ethyl acetate and their combinations were used in direct solvent extraction of phenolic acids from cereals, fruit and vegetable [11].

However, there have been no reports of the identification and quantification of phenolic acids in fruit of Thailand. Thus, the objective of this study was to analyze the soluble and insoluble phenolic acids composition and antioxidant activities of fruit extracts from Thailand. The solvent extraction method and high performance liquid chromatography (HPLC) technique under isocratic elution method with UV detection were adopted in this study.

## 2. MATERIALS AND METHODS

### 2.1 Samples and Sample Preparation

Orange (*Citrus reticulata*, Tangerine), guava (*Psidium guajava*), banana (*Musa sapientum*, Hom variety) and mango (*Mangifera indica*, Nam-dok-mai variety, ripe) were purchased from the fresh market in Bangkok, Thailand (May

to July, 2011). The fruits sample were immediately prepared by washed, peeled, seed separated, cut in to small pieces and blended in food blender. All prepared fruits were freeze-dried for 24 h. The fruit powders were stored in -20°C prior to extraction.

### 2.2 Extraction of Phenolic Acids in Fruits

Extraction of soluble phenolic acids (SPA) by Soxhlet method (modified by Castro-Vargas et al., 2010) [12], fruit powder (2.5g) was weighed in to a thimble and extracted with 250 ml ethyl acetate for 10 h. After extraction the solvent was evaporated to dryness under vacuum (40°C) by rotary evaporator (BUCHI Rotavapor R-114, Switzerland). Extraction of insoluble phenolic acids (IPA) with base hydrolysis [13], 200 mg of residue from soluble phenolic acids extracted were hydrolyzed with 5 ml of 2N sodium hydroxide to consisted 10 mM EDTA and 1% ascorbic acid, stirred for 30 min at 40-45°C and 1.4 ml of 7.2N hydrochloric acid was added, mixed for 5-10 s. Extracted twice with 6.4 ml of ethyl acetate, vortex for 1 min with vortex shaker and centrifuged at 4000 rpm for 10 min. The combined supernatant was evaporated to dryness under vacuum (40°C) by rotary evaporator. The samples were re-dissolved with mobile phase and filtered using 0.45 µm PTFE syringe filter before analysis by HPLC.

### 2.3 Determination of Antioxidant Capacity

Total phenolic content was determined by the Folin-Ciocalteu assay (modified by Tanawski et al., 2006) [14]. One ml of fruit extract (section 2.2) in test tube was mixed 5 ml of Folin's reagent (diluted with distilled water 1:10) and 4 ml of 7.5% (m/v) sodium carbonate solution. Then, the absorbance was measured at 764 nm after stand for 2 h by spectrophotometer (HACH DR/4000U,

USA) using gallic acid as a standard (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml,  $r^2 = 0.999$ ). Results were expressed as mg of gallic acid equivalent per milliliter (mg GAE/ml).

The antioxidant capacity of the fruit extracts determined by 1 ml of extract (section 2.2) with 3 mL of 0.001M 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (modified by Alothman et al., 2009; Kubola et al., 2008) [2, 15]. Absorbance at 518 nm was read after kept in the dark for 30 min with spectrophotometer (HACH DR/4000U, USA). Percentage of inhibition of the DPPH radical was calculated as:

$$\left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100 \quad (1)$$

(Abs control = Absorbance of DPPH solution without extracts).

#### 2.4 Analysis of Phenolic Acids by HPLC

Reverse phase HPLC analysis was used to identify the phenolic acids of fruit extracts. For quantification analysis, the standard curve of standard phenolic acid was constructed from their concentration (0.0005, 0.001, 0.0025, 0.005, 0.01, 0.02, 0.05, 0.1 mg/ml) and the peak area. HPLC-UV consisted of Waters 510 HPLC pump (Waters, USA), 7125 model Rheodyne injector (Cotati, California, USA) with a 20  $\mu$ L sample loop and UV detector model 2487 (Waters, USA). ACE C18-AR column (5  $\mu$ m, 200  $\times$  4.6 mm) was from Phenomenex (Phenomenex, Inc., Torrance, CA) protected with Mightysil C18, 5  $\mu$ m, 4.6  $\times$  5 mm guard column (Kanto Chemical, Japan). Mobile phase consisted of 2% (v/v) acetic acid in water-methanol 82:18 (v/v), flow rate 1.2 ml/min Phenolic acids were detected at a wavelength of 280-320 nm.

#### 2.5 Statistical Analysis

Analysis was carried out in triplicate and averaged using Microsoft Excel 2010.

### 3. RESULTS AND DISCUSSION

Table 1 showed the retention time ( $t_R$ ), linear equation between concentration and peak area, regression coefficient ( $R^2$ ) and maximum absorption wave length. All of phenolic acids could be based line separated on ACE C18-AR column. Although, vanillic and caffeic acid were eluted at the same time however, their maximum absorption wavelengths were different.

Solvents, such as methanol ethanol propanol, acetone, ethyl acetate, dimethylformamide and their combinations have also been used for the extraction of phenolics, often with different proportions of water [11, 22].

Table 2 and 3 showed that the amount of SPA and IPA in orange, guava, banana, and mango. All fruit extracts contained different phenolic acids contents and phenolic acids composition, with some earlier observations on similar fruit obtained from different geographical origins [2, 5].

SPA, gallic acid was mostly found in guava and mango extracts ( $11.6 \pm 0.30$  and  $3.0 \pm 0.68$   $\mu$ g/g dry weight, respectively), while hydroxybenzoic acid ( $10.4 \pm 2.14$   $\mu$ g/g dry weight) was mostly found in orange extract and vanillic acid ( $6.5 \pm 0.50$   $\mu$ g/g dry weight) was mostly found in banana extract. Chlorogenic acid was not detected in all fruit extracts. IPA, orange and banana extracts were mostly found ferulic acid ( $335.8 \pm 13.38$  and  $219.5 \pm 18.47$   $\mu$ g/g dry weight, respectively), which consistent with earlier research found mostly ferulic acid in orange and banana [5]. Guava and mango

**Table 1.** Validation method of HPLC.

Standards	Retention time, $t_R$ (min)	Linear equation	$R^2$	$\lambda_{max}$ (nm)
Gallic acid	5.58	$y = (5.86 \times 10^4)x$	0.9940	280
Hydroxybenzoic acid	8.95	$y = (3.55 \times 10^4)x$	0.9994	280
Chlorogenic acid	9.79	$y = (6.47 \times 10^4)x$	0.9979	320
Vanillic acid	11.68	$y = (3.64 \times 10^4)x$	0.9965	280
Caffeic acid	11.68	$y = (10.9 \times 10^4)x$	0.9996	320
Syringic acid	14.22	$y = (6.50 \times 10^4)x$	0.9955	280
<i>p</i> -Coumaric acid	21.49	$y = (18.2 \times 10^4)x$	0.9972	320
Ferulic acid	29.56	$y = (12.1 \times 10^4)x$	0.9990	320
Sinapic acid	32.51	$y = (9.79 \times 10^4)x$	0.9971	320

**Table 2.** The amount of soluble phenolic acids (SPA) in fruit extracts.

Phenolic acids	Banana	Guava	Mango	Orange
Gallic acid	$1.0 \pm 0.7$	$11.6 \pm 0.3$	$3.0 \pm 0.7$	N.D.
Hydroxybenzoic acid	$3.2 \pm 1.1$	$1.7 \pm 0.3$	$0.4 \pm 0.2$	$10.4 \pm 2.1$
Chlorogenic acid	N.D.	N.D.	N.D.	N.D.
Vanillic acid	$4.4 \pm 0.2$	$2.4 \pm 0.7$	N.D.	$2.9 \pm 0.4$
Caffeic acid	$1.0 \pm 0.0$	$0.2 \pm 0.1$	N.D.	$1.2 \pm 0.8$
Syringic acid	$1.4 \pm 0.3$	$4.5 \pm 0.8$	$0.3 \pm 0.2$	$2.9 \pm 0.3$
<i>p</i> -Coumaric acid	$0.2 \pm 0.0$	$0.5 \pm 0.0$	N.D.	N.D.
Ferulic acid	$0.4 \pm 0.0$	$0.5 \pm 0.0$	N.D.	$3.3 \pm 0.3$
Sinapic acid	N.D.	$0.5 \pm 0.0$	N.D.	$3.4 \pm 0.3$
Total phenolic acids	$11.6 \pm 2.3$	$21.9 \pm 2.1$	$3.7 \pm 1.0$	$24.0 \pm 4.2$

Values are specified on a dry weight basis in  $\mu\text{g/g}$  and are given as mean  $\pm$  standard deviations ( $n = 3$ ). Not detected = N.D.

**Table 3.** The amount of insoluble phenolic acids (IPA) in fruit extracts.

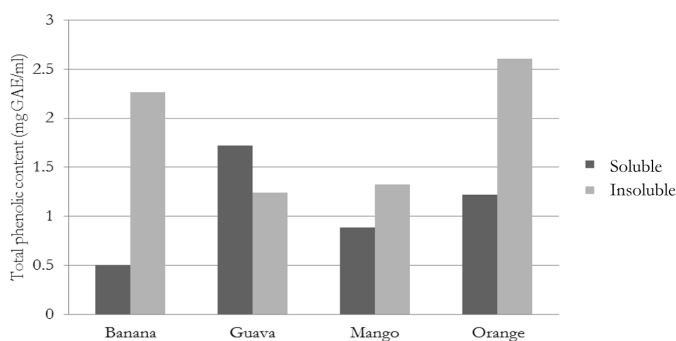
Phenolic acids	Banana	Guava	Mango	Orange
Gallic acid	$21.5 \pm 1.1$	$17.2 \pm 2.4$	$542.5 \pm 6.8$	$14.5 \pm 1.5$
Hydroxybenzoic acid	$15.0 \pm 0.5$	$50.5 \pm 8.1$	$118.2 \pm 28.2$	$25.5 \pm 1.6$
Chlorogenic acid	N.D.	N.D.	N.D.	$2.7 \pm 0.8$
Vanillic acid	$8.2 \pm 2.2$	$17.9 \pm 1.4$	$1.3 \pm 0.1$	$24.1 \pm 8.5$
Caffeic acid	$4.9 \pm 0.4$	$0.5 \pm 0.0$	$0.2 \pm 0.0$	$41.0 \pm 2.2$
Syringic acid	$6.2 \pm 1.5$	$3.3 \pm 0.8$	$0.6 \pm 0.2$	$8.0 \pm 1.7$
<i>p</i> -Coumaric acid	$9.5 \pm 1.3$	N.D.	$0.8 \pm 0.3$	$10.0 \pm 0.3$
Ferulic acid	$219.5 \pm 18.5$	$1.0 \pm 0.5$	$1.0 \pm 0.0$	$335.8 \pm 13.4$
Sinapic acid	$1.3 \pm 0.3$	N.D.	N.D.	$39.1 \pm 0.6$
Total phenolic acids	$286.1 \pm 25.7$	$90.5 \pm 13.3$	$664.6 \pm 35.6$	$500.7 \pm 30.6$

Values are specified on a dry weight basis in  $\mu\text{g/g}$  and are given as mean  $\pm$  standard deviations ( $n = 3$ ). Not detected = N.D.

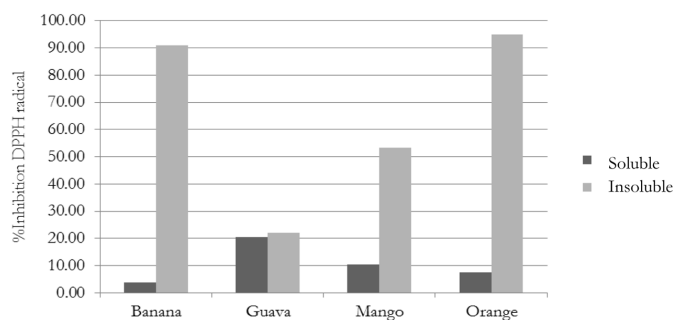
extracts were mostly found hydroxybenzoic acid ( $50.5 \pm 8.12 \mu\text{g/g}$  dry weight) and gallic acid ( $542.5 \pm 6.80 \mu\text{g/g}$  dry weight), respectively.

IPA are distributed in the cell walls, while SPA are compartmentalised within the plant cell vacuoles [7, 16]. They have a wide range of biological activities. IPA was more likely to be bio-available for microbial metabolism and uptake in the colon, while SPA was absorbed in the small intestine, either for rapid metabolism and/or excretion [5]. Figure 1 showed the overall total phenolic content of selected Thai fruit extracts. The insoluble phenolic content of orange, banana, and mango extracts ( $2.6 \pm 0.02$ ,  $2.3 \pm 0.05$ , and  $1.3 \pm 0.36 \text{ mg GAE/ml}$ , respectively) had higher than soluble phenolic content ( $1.2 \pm 0.24$ ,  $0.5 \pm 0.07$ , and  $0.9 \pm 0.30 \text{ mg GAE/ml}$ , respectively). On the other hand, soluble phenolic content of guava extract ( $1.7 \pm 0.34 \text{ mg GAE/ml}$ ) was higher than insoluble

phenolic content ( $1.2 \pm 0.30 \text{ mg GAE/ml}$ ). It agreed well with the study of Sun et al. [20] reported that the phenolic in soluble free from were higher than that of bound from in common fruits. These results indicated that different fruits had the different amounts of soluble and insoluble phenolic contents. The antioxidant capacity of the fruit extracts, percentage inhibition of the DPPH radical of insoluble phenolic content higher than soluble phenolic content (orange > mango > banana > guava). Insoluble phenolic content of orange extract contained the highest total phenolic content ( $2.6 \pm 0.02 \text{ mg GAE/ml}$ ) and was consistent with high antioxidant capacity (94.9%), (Figure 2). Whereas insoluble phenolic content of guava extract had the least antioxidant capacity (22.0%). These results demonstrate that high total phenolic content was consistent with high antioxidant capacity from banana, mango and orange extracts.



**Figure 1.** The phenolic content in soluble and insoluble form of fruit extracts by Folin-Ciocalteu's assay.



**Figure 2.** Antioxidant capacity of fruit extracts by DPPH free radical-scavenging assay.

There was a direct relationship between total phenolic content and total antioxidant activity in phytochemical extracts of different fruits. The higher total phenolic content in fruits resulted in higher total antioxidant activity, indicating phenolics may be the major contributor to the total antioxidant activities of fruits. While, guava extract in the part of insoluble phenolic content, its relatively high total phenolic content has low antioxidant capacity. The possible reason may be due to the phenols in guava extract have a higher redox potential than that of other fruit extracts [18]. Another possible reason may be due to the slow rate of reaction between DPPH and the phenol molecules of guava extract [19].

#### 4. CONCLUSION

There were large amount of phenolic acids are found in fruit extracts from Thailand and the dominant was the insoluble form. Ferulic acid was the dominant IPA in orange and banana extracts; while gallic acid was the dominant IPA in mango extract and hydroxybenzoic acid was the dominant IPA in guava extract. IPA of orange extract contained the highest total phenolic content. It was also consistent with high antioxidant capacity.

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