http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.117.126

Research Article
Influence of Extraction Methods on Total Phenolic Content and Antioxidant Properties of Some of the Commonly Used Plants in Thailand

[1]Noppawat Pengkumsri, [2]Kanittha Kaewdoo, [1]Wilaipan Leeprechanon and [3]Bhagavathi Sundaram Sivamaruthi

[1]Faculty of Pharmacy, Huachiew Chalermprakiet University, 10540 Samutprakan, Thailand
[2]Faculty of Nursing, Huachiew Chalermprakiet University, 10540 Samutprakan, Thailand
[3]Innovation Center for Holistic Health, Nutraceuticals and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, 50200 Chiang Mai, Thailand

Abstract

Background and Objective: Several plants have been commonly used in Thailand for health improvement, but the phytochemical content and its bio-activities are not yet elucidated completely. The aim of this research was to study the influence of extraction method on total phenolic content (TPC) and antioxidant activity of representative plants such as *Punica granatum*, *Hibiscus sabdariffa*, *Cleistocalyx operculatus* (Roxb.) Merr., *Clitoria ternatea* Linn., Mulberry and *Oryza sativa* L. *indica*. **Methodology:** The samples were subjected to different extraction procedures. The TPC and phenolic compounds were determined by Folin-Ciocalteu colorimetric assay and HPLC, respectively. The antioxidant capacity of the extracts was measured by 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) assays and ferrous ion (Fe^{2+}) chelating assay. **Results:** The maximum TPC was observed in pomegranate peel (TTP) extract (0.1 M HCl: Ethanol extraction) and low TPC was recorded in aqueous extract of butterfly pea flower (BP) samples. The high content of protocatechuic, p-hydroxybenzoic acid were observed in pomegranate seed and seed coat (TTS). Gallic and syringic acids were found to be rich in pomegranate peel (TTP) and flower of butterfly pea (BP), respectively. Roselle flower samples (KJ) showed high content of chlorogenic, p-coumaric and caffeic acids. **Conclusion:** The maximum antioxidant activity was observed in extracts obtained by 0.1 M HCl: Ethanol extraction methods, especially pomegranate peel exhibited high free radical scavenging activity compared to that of the other samples. The results strongly revealed that the extraction method greatly influences the phytochemical content and bioactivity and strongly recommends that any plant samples, intended to study, must undergo several extraction processes to reveal the actual phytochemical content.

Key words: Phenolic compounds, *Punica granatum*, *Clitoria ternatea* L., *Hibiscus sabdariffa*, *Cleistocalyx operculatus*, *Morus* spp., antioxidant

Citation: Noppawat Pengkumsri, Kanittha Kaewdoo, Wilaipan Leeprechanon and Bhagavathi Sundaram Sivamaruthi, 2019. Influence of extraction methods on total phenolic content and antioxidant properties of some of the commonly used plants in Thailand. Pak. J. Biol. Sci., 22: 117-126.

Corresponding Author: Bhagavathi Sundaram Sivamaruthi, Innovation Center for Holistic Health, Nutraceuticals and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, 50200 Chiang Mai, Thailand Tel: +6653944341 Fax: +6653894163

Competing Interest: The authors have declared that no competing interest exists.

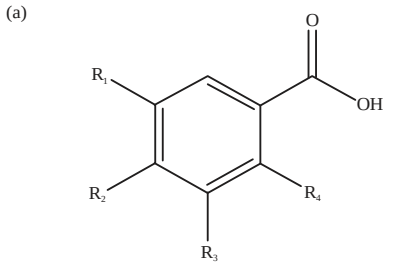
Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The present study focused on determining standard extraction methods to obtain maximum phenolic compounds and bioactivity from the commonly used plants in Thailand. Polyphenols are extensively studied for their possible applications in food and pharmaceutical industries. Polyphenols are derivatives of plant secondary metabolites that are rich in vegetables, fruits, legumes and cereals. The phenolic compounds (PCs) are classified as flavonoids and non-flavonoids[1]. Benzoic and cinnamic acids are the common non-flavonoid compounds. Several hydroxybenzoic and hydroxycinnamic acid derivatives were found to be reported in plants. The derivative compounds are diverse in their R groups. Gallic, protocatechuic, syringic and p-hydroxybenzoic acids are the derivatives of hydroxybenzoic acid. Chlorogenic, caffeic, p-coumaric and ferulic acids are the derivatives of hydroxycinnamic acid (Fig. 1). The PCs are associated with defense mechanism against invading pathogens and radiation and are responsible for flavor, color, odor and acidity of foods[2]. The studies revealed that PCs are protective against the incidence and progress of diabetes, cancers, osteoporosis, cardiovascular and neurodegenerative diseases[3,4].

The reactive oxygen species (ROS) are the by-products of cellular redox process and have both beneficial and destructive role in human health status. A balance in the level of ROS is very crucial. At the optimum level, ROS showed positive effects on immune activity and redox signaling. Whereas, at a high level, ROS cause oxidative stress and leads to cellular damage[5,6]. Oxidative stress is responsible for several neurological, cardiovascular diseases and other disorders. Lifestyle, pollution, smoking, drugs, chemical exposure and stress are major reasons for the increase in oxidative stress. The antioxidants are defensive molecules that acts against the oxidative damages. The consumption of antioxidant-rich foods helps to reduce the incidence of free radical-induced damages and diseases by reducing free radical generation and improve the antioxidant status. Antioxidant-based treatments were used to treat the oxidative stress-related diseases[7,8].

Punica granatum (Family: Punicaceae), *Hibiscus sabdariffa* (Roselle, Family: Malvaceae), *Cleistocalyx operculatus* (Roxb.) Merr. et L.M. Perry (Family: Myrtaceae), *Clitoria ternatea* Linn. (butterfly pea, Family: Fabaceae), Mulberry and *Oryza sativa* L. *indica* (black rice variety) are the commonly used plants in Thailand. The fruits, seed, peel, leaf, flower, root and bark of *P. granatum* plant was reported for several pharmacological (anti-oxidant, anti-inflammatory, anti-angiogenesis, anti-cancer) and toxicological activities (cytotoxic activity)[9]. The outer ring of the roselle fruit is commonly used in herbal infusions, tea, jellies and jams for the unique color and flavor[10,11]. Roselle has been reported for several pharmacological properties like anti-hypertension, anti-inflammation, anti-cancer and anti-hepatic disorders[12,13]. The *C. operculatus* tree is widely found in Thailand, India, Vietnam, Laos and China. The flower buds of *C. operculatus* is used in traditional medicine (to treat diarrhea) and reported for anti-tumor, antioxidant activity[14-16]. Leaves, roots and seeds of *C. ternatea* plant has been used for the treatment of



Hydroxybenzoic acid derivatives	R_1	R_2	R_3	R_4
Gallic acid	OH	OH	OH	H
Protocatechuic acid	H	OH	OH	H
Syringic acid	OCH_3	OH	OCH_3	H
p-hydroxybenzoic acid	H	OH	H	H

Hydroxybenzoic acid derivatives	R_1	R_2	R_3	R_4
Chlorogenic acid	H	OH	OH	$C_7H_9O_5$
Caffeic acid	H	OH	OH	H
p-coumaric acid	H	OH	H	H
Ferulic acid	OCH_3	OH	H	H

Fig. 1(a-b): Schematic representation of (a) Hydroxybenzoic acid and (b) Hydroxycinnamic acid derivatives

inflammation and dementia and used as a laxative[17]. The mulberry tree is commonly grown in all countries and used for silkworm culturing. The fruit is rich in nutrients and used as raw and processed food[18]. Mulberry fruits and leaves were reported for several pharmacological importance and its phytochemical content[19,20]. The colored rice varieties that are commonly cultured in southeast Asian countries were reported for several pharmacological importance[21]. The phytochemical content of rice bran varies among the cultivars and depends on the extraction methods[22,23].

The present study analyzed the influence of extraction method on PCs, especially the derivatives of hydroxybenzoic and hydroxycinnamic acids of representative plants such as *P. granatum*, *H. sabdariffa*, *C. operculatus*, *C. ternatea* Linn, Mulberry and *Oryza sativa* L. *indica* that are commonly used in Thailand. Correspondingly, the antioxidant property of the extracts has been studied.

MATERIALS AND METHODS

Raw materials: The samples (*Punica granatum* [Tubtim in Thai, Tubtim peel (TTP), Tubtim seed and seed coat (TTS)], *Hibiscus sabdariffa* [Krajeab in Thai, Krajeab flower (KJ)], *Cleistocalyx operculatus* [Ma kiang in Thai, Ma kiang seed coat (MKSc), Ma kiang seed (MKS)], *Clitoria ternatea* L. [Butterfly pea flower (BP)], *Morus* spp. [Mhon in Thai, Mhon fruit (MB)], *Oryza sativa* [Rice berry, Rice berry bran (RB)] were collected at local markets of Samut Prakan province, Thailand and were cleaned and dried. Then subjected to grinding and sieving through mesh-60. The dried samples were defatted by hexane in the ratio of 1:10 (sample: hexane) for 3 times and then they were dried at 50°C under vacuum oven. The defatted samples were stored at -20°C until extraction.

Extraction: The samples were subjected to different extraction processes using four different solvents such as (1) Aqueous extraction, (2) 0.001M HCl-water extraction, (3) 80% ethanol extraction and (4) 0.1 M HCl-Ethanol (15:85) extraction. The different extraction processes were done for 3 times (1 h each). All the extracts were filtered through 0.45 µm filter and dried by either using a vacuum freeze dryer (CHRIST®, UK) or by vacuum oven (Binder, USA). Then the extracts were stored at -20°C until analysis.

Estimation of phenolic content: The total phenolic content (TPC) of the extracted samples were measured by the modified Folin-Ciocalteu colorimetric assay as described earlier[22] and the values were denoted as mg gallic acid equivalent (GAE)/g of extracts. The amount of individual phenolic acids (such as gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, caffeic, syringic, p-coumaric and ferulic acids) were determined by reversed-phase HPLC with gradient elution (YL9100 HPLC system, Korea). The HPLC standards were purchased from Wako (Japan). The mobile phase consists of 100% methanol (A) and 0.1% trifluoroacetic acid with the flow rate of 1.0 mL min^{-1}. The SUPELCO, Ascentis™ C18 column, 5 µm., 250×4.6 mm (Sigma-Aldrich, Germany) were used. Gradient elution was done using solvent A at the concentration of 5, 10, 15 and 15-45% for 0-5, 6-10, 11-15 and 15-50 min, respectively. All samples were measured in triplicate[22].

Determination of antioxidant capacity: The antioxidant capacity of the extracts was measured by 2, 2 -azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) assays as detailed in our previous reports[22,24] and the values were reported as mg trolox equivalent/g extract and mg $FeSO_4$ equivalents/g extracts, respectively. The chelation property of extracts was determined through the ferrous ion (Fe^{2+}) chelating assay[25]. Briefly, 20 µL of extracts, 10 µL of 2 mM ferrous chloride and 25 µL of 5 mM ferrozine were mixed in 96 well plate. The reaction was diluted using 200 µL of deionized water and the plate was incubated at room temperature for 10 min and measured at 562 nm. The values were represented as mg EDTA equivalents/g extracts.

Statistical analysis: Independent triplicate samples were used to determine the phenolic acid content and their antioxidant activity to confirm the reproducibility of the results. The values were given as mean±SD. Analysis of variance (ANOVA) was performed to assess the differences in the values. The SPSS software version 17 (Chicago, SPSS Inc, U.S.A) was used for data analysis at 95% confidential level ($p<0.05$).

RESULTS

Polyphenolic content: The samples extracted with 0.1 M HCl-ethanol extraction (acid-ethanol extraction, AEE) showed maximum yield of total phenolic content (150.37±4.17, 36.66±1.04, 61.08±1.04, 117.11±0.35, 46.08±1.39, 48.91±0.52, 44.28±1.04 and 84.30±2.95 mg GAE per g of TTP, TTS, KJ, MKS, MKSc, BP, MB and RB extract, respectively) compared to that of the other extraction methods. The TTS contains less number of phenolic compounds among the studied samples (Fig. 2).

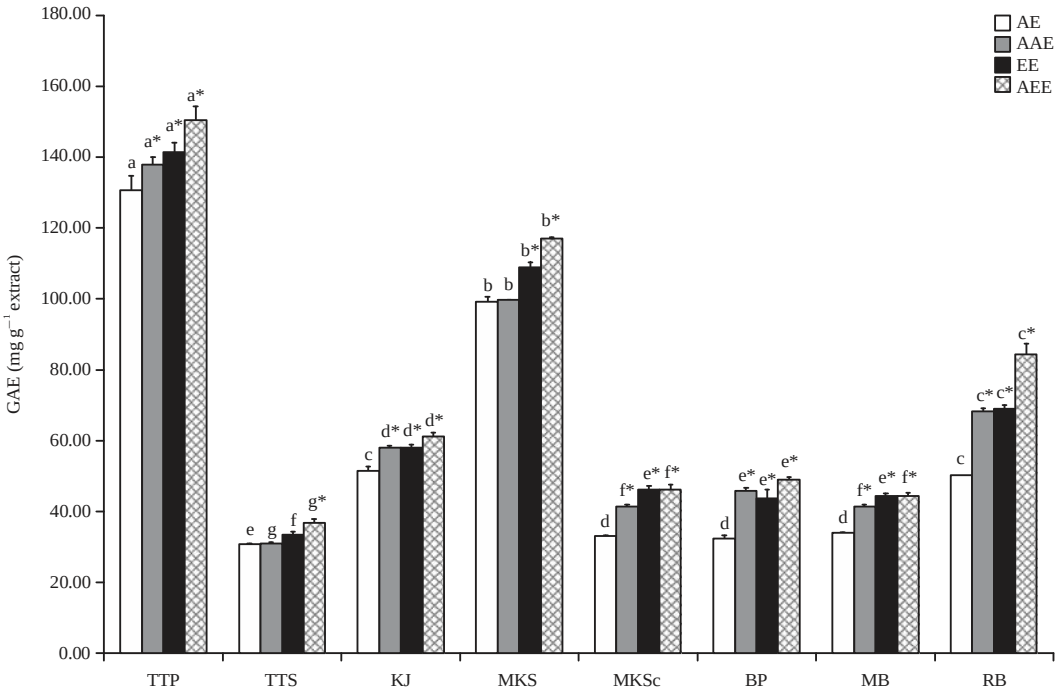


Fig. 2: Total phenolic content of experimental samples. The results were expressed as gallic acid equivalent (GAE). a-f represents the significant changes among the samples and *Indicates the significant changes between different extraction methods
AE: Extract obtained from aqueous extraction, AAE: Extract obtained from 0.001M HCl-water extraction (acid-aqueous extraction, EE: Extract obtained from ethanol extraction, AEE: 0.1 M HCl-ethanol extraction (acid-ethanol extraction)

Hydroxy benzoic acids and hydroxy cinnamic acids content of all extracts were measured separately by HPLC. Among the samples, TTP extracted with aqueous extraction (AE), 0.001M HCl-water extraction (acid in aqueous extraction, AAE), ethanol extraction (EE) and AEE showed maximum gallic acid content (10.18±0.08, 12.18±0.14, 20.22±0.15 and 22.82±0.22 mg per g of extract, respectively) (Fig. 3a). The high content of syringic acid of about 0.75, 0.77±0.01, 1±0.01 and 1.02±0.01 mg per g of BP extract were obtained from the AE, AAE, EE and AEE methods, respectively. The syringic acid was not detected in TTP, TTS and RB samples (Fig. 3b). The TTS extract showed high content of protocatechuic acid (1.80±0.02 and 1.87±0.03 mg per g of extract obtained from EE and AEE methods, respectively) compared to that of the other samples. The protocatechuic acid was not detected in BP (Fig. 3c). Likewise, p-hydroxybenzoic acid was not detected in TTP and RB. Among the samples, TTS extracted with AE, AAE, EE and AEE showed high p-hydroxybenzoic acid content (4.05±0.02, 4.25±0.05, 4.22±0.04 and 5.31±0.04 mg per g of extract, respectively) (Fig. 3d).

The chlorogenic acid was not found in TTP, MKS and MKSc samples. The KJ extract showed high content of chlorogenic acid content (2.44±0.02-4.11±0.05 mg per g extract), p-coumaric acid content (0.74±0.01-1.07±0.02 mg per g extract) and caffeic acid content (0.72-1.46±0.02 mg per g of extract) varied on the extraction methods (Fig. 4). Caffeic acid was not detected in TTS and BP samples (Fig. 4c). The high content of ferulic acid of about 0.78±0.02-1.82±0.04 mg per RB g of extracts were obtained from the extraction (AE, AAE, EE and AEE) methods, respectively. The AE and AAE extract of BP was not detected for ferulic acid (Fig. 4d).

Antioxidant capacity: The ABTS assay was performed to measure the total antioxidant capacity of the samples. The TTP showed high trolox equivalent of antioxidant capacity (TEAC) (1311.55±25.53, 1405.47±35.14, 1550.66±30.05, 1755.5±33.89 mg TEAC per g of TTP samples extracted from AE, AAE, EE, AEE methods, respectively) compared to the TEAC of other extract samples (Fig. 5a). The ferric-reducing antioxidant power (FRAP) of extracts was measured. Similarly, TTP showed high FRAP value in a range of 6551.25±125.27-8893.93±145.67 mg $FeSO_4$ per g of extract, respectively (Fig. 5b). The ion chelating capacity of the TTP and RB samples were over served to be higher in a range of 166.40±2.33-215.50±4.30 and 27.75±0.56-144.60±2.89 mg EDTA equivalent per g of TTP and RB extract, respectively (Fig. 5c).

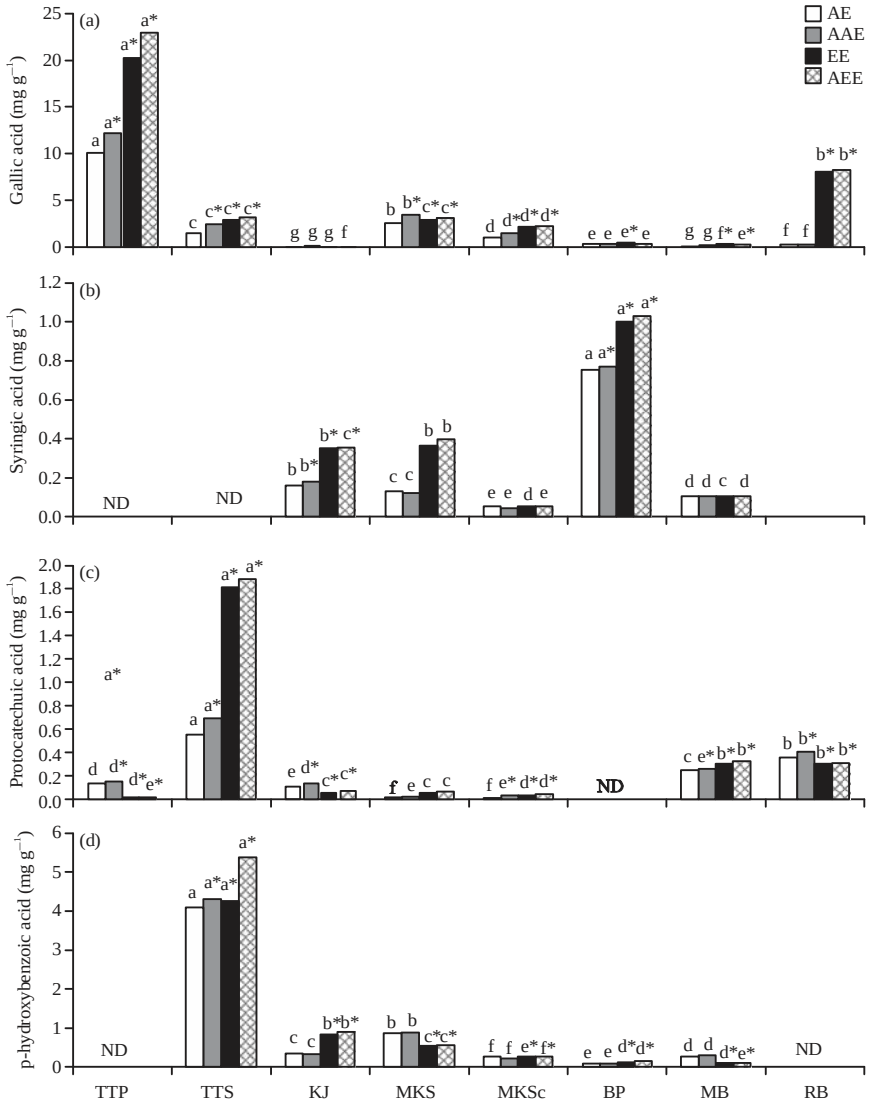


Fig. 3(a-d): Concentration of (a) Gallic acid, (b) Syringic acid, (c) Protocatechuic acid and (d) P-hydroxybenzoic acid in different samples measured by HPLC. A-f represents the significant changes among the samples and *Indicates the significant changes between different extraction methods

AE: Extract obtained from aqueous extraction, AAE: Extract obtained from 0.001M HCl-water extraction (acid-aqueous extraction, EE: Extract obtained from ethanol extraction, AEE: 0.1 M HCl-ethanol extraction (acid-ethanol extraction)

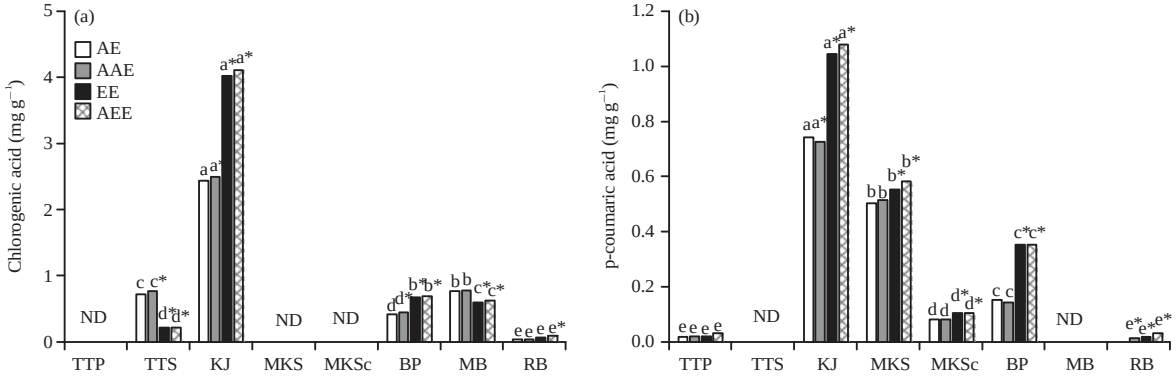


Fig. 4(a-d): Continue

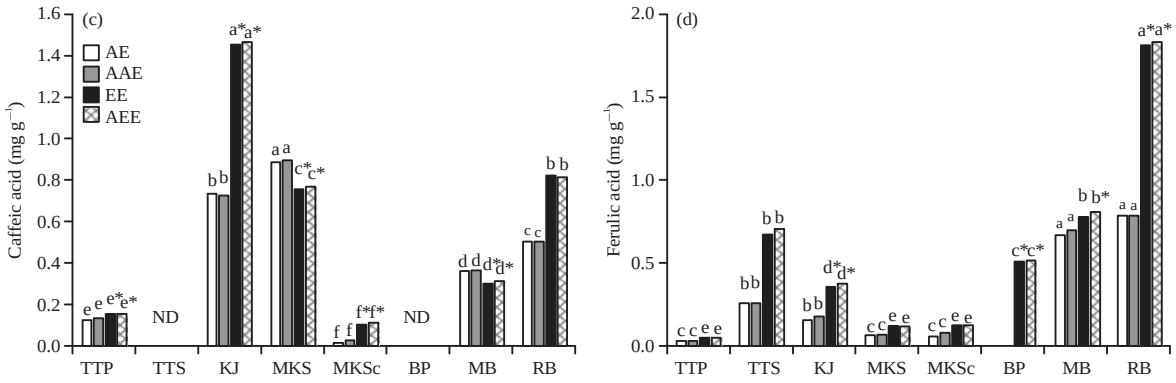


Fig. 4(a-d): Concentration of (a) Chlorogenic acid, (b) P-coumaric acid, (c) Caffeic acid and (d) Ferulic acid in different samples measured by HPLC. A-f represents the significant changes among the samples, and * indicates the significant changes between different extraction methods

AE: Extract obtained from aqueous extraction, AAE: Extract obtained from 0.001M HCl-water extraction (acid-aqueous extraction, EE: Extract obtained from ethanol extraction, AEE: 0.1 M HCl-ethanol extraction (acid-ethanol extraction)

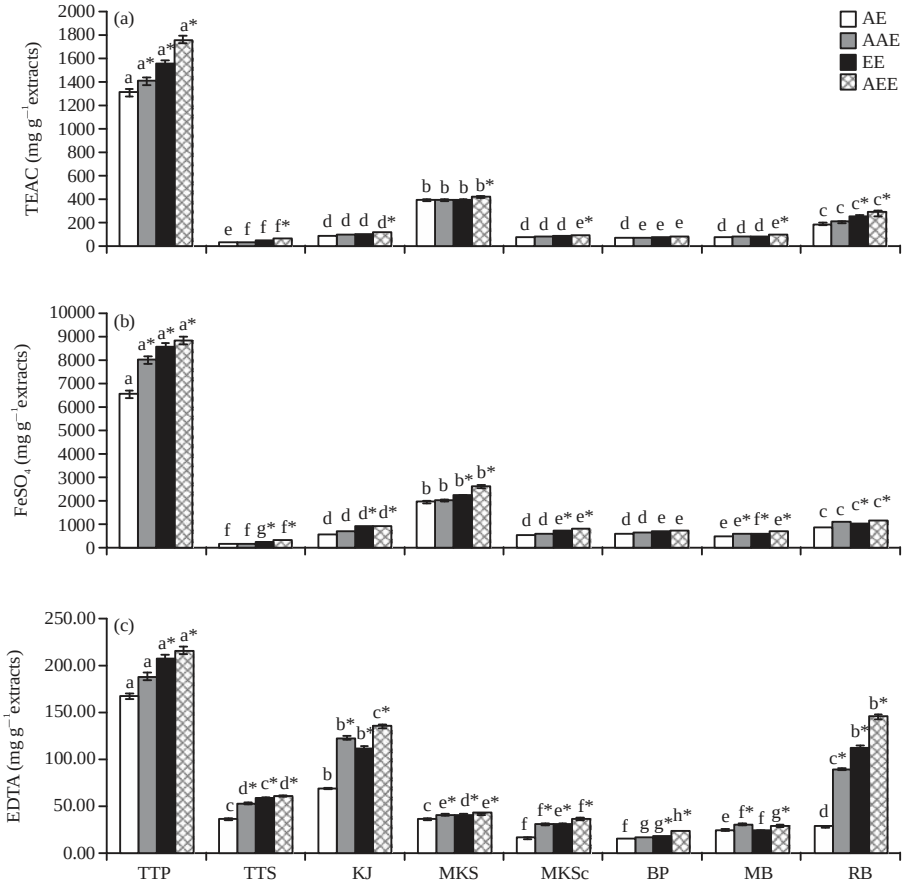


Fig. 5(a-c): Trolox equivalent of (A) Anti-oxidant capacity, (b) Ferric reducing antioxidant power and (c) Ferrous ion chelating capacity (C) of experimental samples. A-f represents the significant changes among the samples and *Indicates the significant changes between different extraction methods

AE: Extract obtained from aqueous extraction, AAE: Extract obtained from 0.001M HCl-water extraction (acid-aqueous extraction, EE: Extract obtained from ethanol extraction, AEE: 0.1 M HCl-ethanol extraction (acid-ethanol extraction)

DISCUSSION

A detailed study on phenolic compounds of Italian pomegranate revealed that pomegranate peel has a high content of phenolic compounds compared to juice and pulp[26]. In the present study, Thai pomegranate peel (Thai Tubtim peel, TTP) extracts showed high TPC compared to the Thai Tubtim seed and seed coat (TTS) extracts. The maximum TPC (150.37±4.17 mg GAE/g of TTP extract) was observed in TTP sample obtained from AEE method and low TPC (32.36±0.87 mg GAE/g of BP extract) was noted in BP sample obtained from AE method. The TTP extracts obtained from AE, AAE, EE and AEE was highly rich in TPC compared to other tested samples (Fig. 2). A study suggested that methanolic extract of Ma Kiang leaves exhibited high TPC (511.44±18.23 mg GAE/mg) and anthocyanin (262.96±2.98 mg Quercetin equivalent/mg) and exhibited anti-aging properties[27]. In the current study, the extracts of Ma kiang seed (MKS) showed high TPC than that of the extracts of Ma kiang seed coat (MKSc) (Fig. 2). The aqueous extracts of leaves and flowers of Malaysian *C. ternatea* L. was ~20.7 and 18.5 mg GAE/g extract, respectively while methanolic extract exhibited high TPC content of ~61.7 and 64.8 mg GAE/g extract, respectively[28]. The present study results proved that AEE yielded high TPC from *C. ternatea* L. flower (BP). High yield of 84.30±2.95 mg GAE/g extracts was observed in the RB extract obtained from AEE method. Turkey mulberries were reported with ~18-19 µg GAE/mg of sample[29] of TPC. The ethanolic extract of Thai white mulberry fruits was reported with 104.78-213.53 mg GAE per 100 g dry weight of TPC and 69.58-211.01 mg catechin equivalent per 100 g dry weight of flavonoid content[30]. The TPC of 33.71-44.28 mg g^{-1} of MB extract was observed in the present study. The yield and bioactivity in the current study was higher than previous reports on Thai mulberry fruits[30]. The results suggested that AEE method yielded high TPC from the tested samples. The statistical analysis proved that the extraction methods significantly influence the polyphenolic content of the extracts (Fig. 2).

The study proved that Spanish mulberry leaves contain high caffeoylquinic acids and flavonols[20]. In the present study, the samples KJ, MB and BP contains a relatively reduced amount of gallic acid. The RB extract showed very low gallic acid level in AE and AAE extraction methods while EE and AEE methods yielded RB extract that are relatively high content of gallic acid (Fig. 3a). The results showed that the TTP, TTS and RB samples were not comprising a detectable level of syringic acid. Likewise, BP samples lack a detectable amount of protocatechuic acid and TTP and RB samples were not containing a detectable amount of p-hydroxybenzoic acid (Fig. 3b-d). The TTP and BP samples were recorded for high content of gallic acid and syringic acid, respectively. The TTS samples were recorded with a high content protocatechuic and p-hydroxybenzoic acids (Fig. 3). Sentandreu *et al.*[31] reported several phenolic, anthocyanins and some of the new non-anthocyanin phenolic compounds in pomegranate juice. Turkey pomegranate juice was reported for containing gallic, ferulic, protocatechuic, caffeic, chlorogenic acids and several organic acids[32]. In our study, chlorogenic acid content was not detectable in the extracts of TTP, MKS and MKSc samples. Likewise, p-coumaric acid content was not detectable in the extracts of TTS and MB and caffeic acid content was not detectable in the TTS and BP extracts. Each extracts of the tested samples contained ferulic acid (Fig. 4). The KJ extracts were found to contain high content of chlorogenic, p-coumaric and caffeic acid compared to other samples. The RB extracts contained high content of ferulic acid. P-coumaric acid was not detectable in the AE of RB, but RB extract obtained from other extraction methods displayed a detectable level of p-coumaric acid (Fig. 4). These results and statistical analysis suggested that extraction methods play a crucial role in phenolic compound extraction. The results also suggested that the plant samples need several extraction steps to acquire the potential phytochemicals.

The TTP extracts exhibited high antioxidant property compared to that of the other samples and activity was attributed to the rich gallic acid content of TTB (Fig. 5, 3a). Next to TTP extracts, RB extract showed high ion chelating property, which is possibly due to the presence of ferulic acid in AEE extract of RB (Fig. 5, 4d). About 18-42 mM TEAC of antioxidant capacity was reported in pomegranate juice of Iranian cultivars[33]. Arils, rinds and juice of Italian pomegranate were documented for high antioxidant activity[34]. Likewise, Thai pomegranate peel (TTP) showed a maximum of 1311.55±25.23 to 1755.5±33.89 mg TEAC per g of extracts, whereas Thai pomegranate seed and seed coat (TTS) showed only 29.22±1.05 to 61.03±1.53 mg TEAC per g of extracts (Fig. 5a). Next to TTP, MKS extract showed high antioxidant property (417.81±9.95 mg TEAC/g of MKS extract obtained from AEE method) (Fig. 5a).

The AEE method was employed to reveal the phytochemical content (anthocyanins and phenolic compounds), antimicrobial and antioxidant properties of various Mexican *H. sabdariffa* (Roselle) varieties[35]. The phytochemical content of roselle was found to be varied based on the cultivation conditions[36]. The antioxidant evaluation of all parts of roselle suggested that roselle seeds exhibited high antioxidant activity and that could be a potent food additive[37,38]. Recently, phytochemical content,

antioxidant and anti-inflammatory properties of roselle leaves of different origin were reported. The study reported that Thai variety consists of 22.4±3.1 mg g^{-1} extract of TPC and 126.7±5.9 mg g^{-1} extract of total antioxidant capacity[11]. In the present study, about 61.08±1.04 mg g^{-1} extract of TPC and 115.86±2.9 mg g^{-1} extract of TEAC were observed in roselle flower (Fig. 2, 5a). The flowers of *C. ternatea* L. have been reported for antioxidant activities[17,39]. In the present study, BP also exhibited antioxidant activity (Fig. 5a).

The phytochemical content, antioxidant activity and neuroprotective property of Taiwan mulberries at different ripening stages were reported. The study concluded that above bioactive properties were found to be varied based on the types of phenolic compounds present in every ripening stage[40]. The free-radical scavenging property (70.25-95.18 mg TEAC/g extracts) were observed in the present study. The influence of extraction method on antioxidant property of rice bran was reported previously[23]. The results of the current study also proved that the extraction method influences the yield and quality of rice bran extract.

Collectively, AEE extracts of all the samples showed maximum activity in all studied free-radical scavenging models. The results suggested that AE was less effective, in terms of phenolic content and antioxidant capacity, compared to other studied extraction methods. Addition of acid in water and ethanol extraction significantly improved the TPC and antioxidant activity. The results of the current study strongly revealed that the extraction method greatly influences the phytochemical content of extracts that is responsible for bio-activity. The study strongly recommends that a study material (plant) should undergo several extraction processes to reveal the actual phytochemical content.

CONCLUSION

High amount of phenolic compositions was extracted with AEE methods than that of AE, AAE and EE methods, respectively. Rationally, bound form of phenolics including phenolic acids, flavonoids and anthocyanin pigments are eluted easily from phytosomal vesicle. In this study, pomegranate or Tubtim peel extracts was denoted as TTP, which contained high total phenolic content represented as gallic acid equivalent. Besides, highest subtype of each phenolic acid compositions were analyzed including gallic acid of TTP, protocatechuic acid and p-hydroxybenzoic acid of TTS, chlorogenic acid, caffeic acid and p-coumaric acid of KJ, syringic acid of BP and ferulic acid of RB. Therefore, TTP extracts obtained from AEE exhibited high biological anti-oxidant activity (the electron transferring, reducing power and chelation of iron). Further investigation is required to determine the better extraction method to yield more bio-active compounds rich extracts to develop cosmetics or nutraceuticals.

ACKNOWLEDGMENT

We gratefully acknowledge to the Faculty of Pharmacy and Huachiew Chalermprakiet University, Samutprakan, Thailand. The BSS also wish to acknowledge the CMU Post-Doctoral Fellowship, Chiang Mai University, Chiang Mai, Thailand.

REFERENCES

1. Bravo, L., 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev., 56: 317-333.
2. Beckman, C.H., 2000. Phenolic-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? Physiol. Mol. Plant Pathol., 57: 101-110.
3. Arts, I.C.W. and P.C.H. Hollman, 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr., 81: 317S-325S.
4. Graf, B.A., P.E. Milbury and J.B. Blumberg, 2005. Flavonols, flavones, flavanones and human health: Epidemiological evidence. J. Med. Food, 8: 281-290.
5. Pham-Huy, L.A., H. He and C. Pham-Huy, 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 4: 89-96.
6. Sen, S., R. Chakraborty, C. Sridhar, Y.S.R. Reddy and B. De, 2010. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. Int. J. Pharm. Sci. Rev. Res., 3: 91-100.
7. Gupta, V.K. and S.K. Sharma, 2006. Plants as natural antioxidants. Nat. Prod. Radiance, 5: 326-334.
8. Rathore, G.S., M. Suthar, A. Pareek and R.N. Gupta, 2011. Nutritional antioxidants: A battle for better health. J. Nat. Pharm., 2: 2-14.
9. Rahimi, H.R., M. Arastoo and S.N. Ostad, 2012. A comprehensive review of *Punica granatum* (Pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. Iran. J. Pharm. Res., 11: 385-400.
10. Mahadevan, N., Shivali and P. Kamboj, 2009. *Hibiscus sabdariffa* Linn: An overview. Natl. Prod. Radiance, 8: 77-83.
11. Zhen, J., T.S. Villani, Y. Guo, Y. Qi and K. Chin *et al*., 2016. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. Food Chem., 190: 673-680.

12. Lin, H.H., J.H. Chen, W.H. Kuo and C.J. Wang, 2007. Chemopreventive properties of *Hibiscus sabdariffa* L. on human gastric carcinoma cells through apoptosis induction and JNK/p38 MAPK signaling activation. Chemico-Biol. Interact., 165: 59-75.
13. Chang, H.C., C.H. Peng, D.M. Yeh, E.S. Kao and C.J. Wang, 2014. *Hibiscus sabdariffa* extract inhibits obesity and fat accumulation and improves liver steatosis in humans. Food Funct., 5: 734-739.
14. Ye, C.L., J.W. Liu, D.Z. Wei, Y.H. Lu and F. Qian, 2005. *In vivo* antitumor activity by 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone in a solid human carcinoma xenograft model. Cancer Chemother. Pharmacol., 56: 70-74.
15. Ye, C.L., Y. Liu and D.Z. Wei, 2007. Antioxidant and anticancer activity of 3'-formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone. J. Pharm. Pharmacol., 59: 553-559.
16. Mai, T.T., N. Fumie and N.V. Chuyen, 2009. Antioxidant activities and hypolipidemic effects of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr. and Perry. J. Food Biochem., 33: 790-807.
17. Kamkaen, N. and J.M. Wilkinson, 2009. The antioxidant activity of *Clitoria ternatea* flower petal extracts and eye gel. Phytother. Res., 23: 1624-1625.
18. Gundogdu, M., F. Muradoglu, R.G. Sensoy and H. Yilmaz, 2011. Determination of fruit chemical properties of *Morus nigra* L., *Morus alba* L. and *Morus rubra* L. by HPLC. Scient. Hortic., 132: 37-41.
19. Wang, Y., L. Xiang, C. Wang, C. Tang and X. He, 2013. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. PLoS One, Vol. 8, No. 7. 10.1371/journal.pone.0071144.
20. Sanchez-Salcedo, E.M., P. Mena, C. Garcia-Viguera, F. Hernandez and J.J. Martinez, 2015. (Poly) phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves: Their potential for new products rich in phytochemicals. J. Funct. Foods, 18: 1039-1046.
21. Sivamaruthi, B.S., P. Kesika and C. Chaiyasut, 2018. A comprehensive review on anti-diabetic property of rice bran. Asian Pac. J. Trop. Biomed., 8: 79-84.
22. Pengkumsri, N., C. Chaiyavat, S. Chalermpong, S. Sasithorn and S. Prasit *et al*., 2015. Physicochemical and antioxidative properties of black, brown and red rice varieties of Northern Thailand. Food Sci. Technol., 35: 331-338.
23. Pengkumsri, N., C. Chaiyavat, S.S. Bhagavathi, S. Chalermpong and S. Prasit *et al*., 2015. The influence of extraction methods on composition and antioxidant properties of rice bran oil. Food Sci. Technol., 35: 493-501.
24. Chaiyasut, C., N. Pengkumsri, S. Sirilun, S. Peerajan, S. Khongtan and B.S. Sivamaruthi, 2017. Assessment of changes in the content of anthocyanins, phenolic acids and antioxidant property of *Saccharomyces cerevisiae* mediated fermented black rice bran. AMB Express, Vol. 7. 10.1186/s 13568-017-0411-4.
25. Ebrahimzadeh, M.A., F. Pourmorad and A.R. Bekhradnia, 2008. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol., 7: 3188-3192.
26. Russo, M., C. Fanali, G. Tripodo, P. Dugo and R. Muleo *et al*., 2018. Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC-PDA-ESI/MS and evaluation of their antioxidant activity: Application to different Italian varieties. Anal. Bioanal. Chem., 410: 3507-3520.
27. Manosroi, J., C. Chankhampan, K. Kumguan, W. Manosroi and A. Manosroi, 2015. *In vitro* anti-aging activities of extracts from leaves of Ma Kiang (*Cleistocalyx nervosum* var. paniala). Pharm. Biol., 53: 862-869.
28. Rabeta, M.S. and Z.A. Nabil, 2013. Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* Linn. Int. Food Res. J., 20: 495-500.
29. Gungor, N. and M. Sengul, 2008. Antioxidant activity, total phenolic content and selected physicochemical properties of white mulberry (*Morus alba* L.) fruits. Int. J. Food Propert., 11: 44-52.
30. Butkhup, L., W. Samappito and S. Samappito, 2013. Phenolic composition and antioxidant activity of white mulberry (*Morus alba* L.) fruits. Int. J. Food Sci. Technol., 48: 934-940.
31. Sentandreu, E., M. Cerdan-Calero and J.M. Sendra, 2013. Phenolic profile characterization of pomegranate (*Punica granatum*) juice by high-performance liquid chromatography with diode array detection coupled to an electrospray ion trap mass analyzer. J. Food Comp. Anal., 30: 32-40.
32. Poyrazoglu, E., V. Gokmen and N. Artik, 2002. Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. J. Food Compo. Anal., 15: 567-575.
33. Mousavinejad, G., Z. Emam-Diomeh, K. Rezaei and M.H.H. Khodaparast, 2009. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem., 115: 1274-1278.
34. Ricci, D., L. Giamperi, A. Bucchini and D. Fraternale, 2006. Antioxidant activity of *Punica granatum* fruits. Fitoterapia, 77: 310-312.

35. Borras-Linares, I., S. Fernandez-Arroyo, D. Arraez-Roman, P.A. Palmeros-Suarez and R. Del Val-Diaz *et al*., 2015. Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). Ind. Crops Prod., 69: 385-394.
36. Formagio, A.S.N., D.D. Ramos, M.C. Vieira, S.R. Ramalho and M.M. Silva *et al*., 2015. Phenolic compounds of *Hibiscus sabdariffa* and influence of organic residues on its antioxidant and antitumoral properties. Braz. J. Biol., 75: 69-76.
37. Mohd-Esa, N., F.S. Hern, A. Ismail and C.L. Yee, 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chem., 122: 1055-1060.
38. Wang, J., X. Cao, H. Jiang, Y. Qi, K.L. Chin and Y. Yue, 2014. Antioxidant activity of leaf extracts from different *Hibiscus sabdariffa* accessions and simultaneous determination five major antioxidant compounds by LC-Q-TOF-MS. Molecules, 19: 21226-21238.
39. Iamsaard, S., J. Burawat, P. Kanla, S. Arun and W. Sukhorum *et al*., 2014. Antioxidant activity and protective effect of *Clitoria ternatea* flower extract on testicular damage induced by ketoconazole in rats. J. Zhejiang Univ.-Sci. B, 15: 548-555.
40. Yang, J., X. Liu, X. Zhang, Q. Jin and J. Li, 2016. Phenolic profiles, antioxidant activities and neuroprotective properties of mulberry (*Morus atropurpurea* Roxb.) fruit extracts from different ripening stages. J. Food Sci., 81: C2439-C2446.