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Contributed Paper

A Comparison of Three Extraction Methods for Phenolic Compounds and Antioxidant Activities from *Moringa oleifera* Leaves

Truong Kim Tai* [a], Jittima Thongklay [b], Pisipong Meunprasertdee [c], Papadchaya Kornthattalim [c] and Thammarat Kaaewmanee [a]

[a] Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Amphur Muang, Pattani, 94000, Thailand.

[b] School of Science, Mae Fah Luang University, Amphur Muang, Chiang Rai, 57100, Thailand.

[c] Instrument Center, Faculty of Science and Technology, Prince of Songkla University, Amphur Muang, Pattani, 94000, Thailand.

* Author for correspondence; e-mail: tai.t@camcoll.asia

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ABSTRACT

In our study, the proximate analysis of dried *Moringa oleifera* leaves was firstly evaluated; then three different extracts were obtained by using different extraction techniques including agitation (AG), sonication (SO) and aqueous (AQ) were used to investigate the phytochemical screening (total phenolic content (TPC) and total flavonoid content (TFC)) and antioxidant activities (2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, ferric reducing ability of plasma (FRAP) assay and reducing power (RP) assay) of the Moringa leaves in order to find the best extraction method for further study. Finally, the presence of phenolic compounds was analyzed using high performance liquid chromatography (HPLC) analysis. The results showed Moringa leaves contained a large amount of carbohydrate and protein; while there was only small amount of lipid. The highest extraction yield was obtained aqueous method at 62.22 ± 0.68%. For phytochemical screening, the agitation presented the best results for highest total phenolic content at 74.87 ± 0.19 mg Gallic acid equivalent (GAE)/g dry weight and highest total flavonoid content at 18.46 ± 0.25 mg Catechin equivalent (CE)/g dry weight, respectively. Besides, agitation method also showed significantly different in antioxidant abilities compared to the others. Due to dose-dependent manners of all extracts to scavenging DPPH and ABTS free radicals; it strongly reconfirmed the correlation of TPC to antioxidant abilities. Thus, finding the most effective extraction method for highest yield and phenolic compound content is beneficial for achieving greater antioxidant ability and can be applied in further works in the food and pharmaceutical industry.

Keywords: *Moringa oleifera,* proximate analysis, total phenolic content, total flavonoid content, antioxidant activities, HPLC analysis

1. INTRODUCTION

Moringa is one such genus whose various species have not been fully explored despite the enormous reports concerning the various parts of a few species' potential properties, such as anti-bacterial, anti-diabetic, anti-fungal, anti-inflammatory, antioxidant, anti-spasmodic, anti-tumor, anti-ulcer, cardiac and circulatory stimulants, and cholesterol lowering activities [1]. The *Moringa* species are currently of wide interest because of their outstanding economic potential. Amongst these species, *M. oleifera* is the most prevalent for its nutritious and numerous medicinal uses that have been appreciated for centuries in many parts of its native and introduced ranges [2]. It is a small tree (7-12 m high) with thick grey bark, fragrant white flowers and long green pods, which give the tree its name. The common name is Moringa or drumstick tree and in the Thai language is known as Ma-Rum. In fact, a wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds [3]. All these parts are used in folk medicine; however, the Moringa leaves were mostly used to treat various ailments including anti-bacterial and anti-cancer activity, treatment of inflammation and infectious diseases, along with cardio vascular and gastro intestinal disorders [3].

Many researches have demonstrated the oxidative stress is main factor in the development of chronic degenerative diseases including coronary heart disease, cancer and aging [3, 4]. So, one of key factors to protect the cell component is neutralizing free radicals, -by products of cell metabolism, by using antioxidants [4]. An antioxidant may be defined as any substance that when present at low concentrations, compared with those of the oxidizing substrate, significantly delays or inhibits oxidation of that substrate. Normally, most of antioxidants derived from natural products, mainly plant sources, contributes strong antioxidant ability due to the containing of phenolic compounds including phenolic acids and flavonoids. In order to achieve phenolic compounds, several extraction methods have firstly been used to isolate the phenolic compounds from the plant matrix. This step is very important because these compounds might be chemically altered or degraded during extraction process. Fresh, frozen, or dried plant samples should be extracted in different ways to achieve phenolic compounds with minimum lost. From the ancient times, there are two extraction methods that have been used are the ethanolic extraction and aqueous extraction. Aqueous extraction, also known as hot water extraction, is considered as the easiest and greenest method, which does not use a lot of chemicals (organic solvents) and save a lot of times [5]. On the other hand, ethanolic extraction usually takes longer times in extraction process; however, it is normally considered as a high efficiency method in term of quality. Different varieties of phenolic compounds are usually extracted by ethanolic extraction method, which is broader than in aqueous one due to different polarity [6]. Another reason that ethanol is usually selected as the solvent because it extracts high yield compare to other organic solvents, and it is also less toxic, and easily recovered by reduced pressure distillation. Sonication, another name of ultra-sound assist, is a cell-disruptive process, which might help to release the phenolic compounds from the medicinal plants in a very short period of time [6].

In the southern part of Thailand, especially Pattani province, Moringa, has only been consumed in as a fresh vegetable and has very limitation on research and explore about medicinal applications. Therefore,

the purpose of this study is to investigate the nutritional values of Thai Moringa leaves and the effects of different extraction methods on the comparative analysis of phytochemical contents and concentration- and dose-dependent effects on antioxidant capacities of different ethanolic extracts of Thai *M. oleifera* leaves obtained from three different extraction methods above.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals, reagents, and solvents used in the assay protocols were of analytical grade. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), and Trolox were obtained from Sigma Aldrich, Germany. Other chemicals such as aluminum trichloride, disodium hydrogen orthophosphate dehydrate, iron (III) chloride anhydrous ($FeCl_3$), iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), potassium ferricyanide, potassium persulphate, sodium acetate, sodium carbonate, sodium dihydrogen orthophosphate, and sodium nitrite were purchased from Ajax Finechem NSW, Australia. Hydrochloric acid (HCl), Folin-Ciocalteau, L- Ascorbic Acid, and trichloroacetic acid were bought at Loba Chemie Pvt., India. Dimethyl sulfoxide (DMSO) and sodium hydroxide were ordered at Fisher Chemical, India. The solvents such as ethanol and glacial acetic acid were ordered from Avantor Performance Materials, Malaysia.

2.2 Plant Materials

M. oleifera leaves were collected at a local market in Pattani province, Southern Thailand. The species was identified by comparing with voucher specimen No. 103322 of this plant, which has been deposited in Queen Sirikit Botanic Garden Herbarium, Chiang Mai, Thailand. The samples were washed with clean water and then lyophilized and made into powder (40 mesh sieve).

2.3 Proximate Analysis and Extraction of Moringa Leaves

Proximate analysis of dried Moringa leaves powder was carried out by the methods of the Association of Official Analytical Chemists [7]. The initial temperature was measured at 25°C for all treatments. Five grams of powder was separately extracted using 80% ethanol with ratio 1:50 using 24 hours mechanical agitation or 30 min sonication, repeated three times. Both treatments were operated at 25°C and the sonication technique was reached to 55°C during extraction process [6]. Another 5 g of powder was also autoclaved with 750 mL of distilled water only one time for 20 minutes at 121°C [5]. After filtration through Whatman filter paper No.1 (pore size 11 µm), the ethanolic extracts were then concentrated using rotary evaporator and then dried using centrifuged evaporator at 45°C. On the other hand, the filtered aqueous extract was freeze dried and lyophilized until the powder was obtained. The yields of extraction were then calculated.

2.4 Phytochemical Analysis

2.4.1 Determination of TPC

TPC was determined using the method of Singleton et al. [8] with slight modifications. One milliliter of different concentrations of extracts were added to 5 mL of 10% (1:10 v/v diluted with distilled water) Folin-Ciocalteau reagent and allowed to stand for 5 min at 22°C. After 5 min, 4 mL of 1 M sodium carbonate (Na_2CO_3) was added. These mixtures were incubated for 30 min in the dark, then the development of blue color

was observed and measured at 765 nm using a spectrometer. The phenolic content was finally calculated as mg GAE/g dry weight.

2.4.2 Determination of TFC

TFC of various samples were determined using the aluminum chloride assay by Zhishen et al. [9] with some modifications. An aliquot (1.0 mL) of extracts was taken in different test tubes containing 4 mL of deionized water (DI) water, then 0.3 mL of sodium nitrite (5% $NaNO_2$, w/v) was added and allowed to stand for 5 min. Later, 0.3 mL of aluminum trichloride (10% $AlCl_3.6H_20$) was added and incubated for 5 min, followed by the addition of 2 mL of 1 M sodium hydroxide (NaOH), and the total volume was made up to 10 mL with DI water. After 15 min of dark incubation, the mixture turned to pink whose absorbance was measured at 510 nm using a spectrometer. The flavonoid content was calculated as mg CE/g dry weight.

2.5 Anti-oxidative Activity

2.5.1 DPPH radicals scavenging assay

DPPH scavenging potential was measured based on the scavenging ability of stable DPPH radicals of fractions by Payet et al. [10]. About 0.2 mL of extracts in various concentrations was added to 1.8 mL DPPH working solution (A_{517} = 1.0 ± 0.02). After vortexing, the mixture was incubated for one hour in dark place at room temperature. The decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was measured at 517 nm. Results were expressed in µg Trolox equivalents (TE)/g dry weight. 50% inhibition concentration (IC_{50}) was also calculated.

2.5.2 ABTS scavenging assay

For ABTS scavenging assay, the method of Re et al. [11] was followed with some modifications. The stock solution of the ABTS radical was prepared by dissolving 7.4 mM of ABTS in 2.45 mM of sodium persulphate solution, and the mixture was stored in dark for 12-16 hours. The freshly-prepared working solution was then obtained by diluting 1 mL $ABTS^{\bullet+}$ stock solution with 60 mL water to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Eighty microliters of extracts were allowed to react with 1920 µL of the $ABTS^{\bullet+}$ solution for 30 min in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed in µg TE/g dry weight. IC_{50} was also calculated.

2.5.3 FRAP assay

The FRAP assay was followed by the method of Kulkarni et al. [12] with slight modifications. A working solution was prepared freshly by mixing 200 mL of 300 mM acetate buffer (pH 3.6), 20 mL of 10 mM TPTZ solution, and 20 mL 20 mM of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min and referred as FRAP solution. 200 µL of ethanolic extract of *M. oleifera* in different concentrations was mixed with 1800 µl of FRAP solution and kept for 45 minutes at 37°C in a water bath. Then the blue color from ferrous tripyridyltriazine complex was measured by reading the absorbance at 595 nm. The activity was expressed as µmol TE/g dry weight.

2.5.4 Reducing power assay

The reducing power of ethanolic extract of *M. oleifera* was determined by the method of Jayanthi and Lalitha [13] with slight modification. Briefly, 2.5 mL of different extracts were mixed with 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide (K_4FeCN_6), and the

mixture was incubated for 20 min at 50°C. After cooling the solution, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The supernatant (2.5 mL) was mixed with 2 mL distilled water and 0.5 mL of 0.10% freshly prepared $FeCl_3$ solution. The ferric reducing capacities of the extracts were checked by measuring the absorbance at 700 nm against a reagent blank after 30 minutes incubation. The activity was expressed as mg Ascorbic acid equivalents (AAE)/g dry weight.

2.6 HPLC Analysis

The condition for HPLC analysis was used as described by Siddhuraju and Becker [14]. Phenolic compounds were determined by HPLC using a C18 column (250 mm × 4.6 mm, 5 µm). Gradient elution was performed using two solvents; 50 mM H_3PO_4, pH 2.5 (solution A), and acetonitrile (solution B) for determining phenolic compounds. Ten microliters of the 10 mg of different extracts was dissolved in 1 mL of diH2O and injected into the column with a flow rate 0.7 ml/min and detect the phenolic compounds with different wavelengths including 254 nm for Gallic acid and Chlorogenic acid, 270 nm for Ellagic acid, 280 nm for (+)-Catechin and (-)-Epicatechin, 340 nm for Ferulic acid, and 370 nm for Myricetin, Quercetin, and Kaempferol. Peak area and retention time of the extract sample were compared with standard curves of various concentrations of standards. Results (µg/g dry weight) were obtained by comparison of peak areas (specific wavelength) of samples with that of standards.

2.7 Statistical Analysis

All values were given as mean ± standard derivation (X ± SD) from triplicate samples of three independent experiments. Statistical analysis was performed by were analyzed by one-way ANOVA and Tukey's multiple range test at $p \leq 0.05$, 0.01, or 0.001, using the statistical package SPSS, Version 17 (USA).

3. RESULTS AND DISCUSSIONS

3.1 Proximate Analysis

Proximate analysis, the most common analysis done for nutritional testing, were divided the food into five categories: moisture, ash, crude protein, lipid, carbohydrate. The proximate analysis of dried *M. oleifera* leaves is presented in Table 1. The chemical compositions of protein, lipid, ash, fiber, and carbohydrate were 30.46 ± 0.08 %, 2.42 ± 0.10 %, 9.00 ± 0.01 %, 10.02 ± 0.08 % and 58.12 ± 0.09 %, respectively. The Moringa leaves powder was not only an excellent source of protein and carbohydrates, but also a very low source of lipids. The high contain of soluble fiber can used for many benefits including: support the growth of good bacteria in gut, reduce absorption of LDL cholesterol, and reduce the absorption of glucose into bloodstream for diabetes people. Thus, the leaves are one of the best plant foods that have potentially to be produce as nutritional and pharmaceutical products.

Table 1. Nutritional value (%) of dried *M. oleifera* leaves.

Proximate Analysis	Dried Leaves
Protein (%db)	30.46 ± 0.08
Lipid (%db)	2.42 ± 0.10
Ash (%db)	9.00 ± 0.01
Fiber (%db)	10.02 ± 0.08
Carbohydrate (%db)	58.12 ± 0.09

db, dry basis

There are some studies that reported the information of proximate analysis in Moringa leaves, such as Makkar et al. [15]

showed that the chemical compositions of Nicaraguan *M. oleifera* leaves (db) were 25.1 % protein, 5.4 % lipid, 11.5 % ash and 58.0 % carbohydrate or Lalas et al. [16] showed the chemical compositions (db) of Greek Moringa leaves were 26.3 % protein, 5.6 % lipid, 12 % ash and 56.1 % carbohydrate, which were slightly different from our results. These variations might be due to differences in agro-climatic conditions by the origin of samples, or due to a different age of trees. Different origin of Moringa would produce different strains which contain different amounts of chemical compositions. However, it might not be due to different stages of maturity of the leaves samples, since fresh tender green leaves have been used in all studies.

3.2 Yield of Extraction and Phytochemical Analysis

The extraction methods had been strongly emphasized from the beginning. A great extraction method should not only be easy but also have the high extraction efficiency. Ethanol had been used as extraction solvent in both agitation and sonication extractions. Normally, ethanol would be mixed herbs or raw materials for several months to infuse and dissolve phenolic compounds based on concept of "like dissolve like". With the aid of sonication, the ultrasound strongly vibrated cell membranes and forced phenolic compounds easily release. However, ultrasonic process also created heat during their work which may degrade phenolic compounds. Due to the broad range of polarity, ethanol extracted both high polarity or low polarity compounds which represented in final product as dark pink sticky substance.

On the other hand, aqueous required a shorter time by allowing samples could be boiled for several hours in order to break the cell membranes and then the phenolic compounds were released to the water. To mimic the instruction for the use in daily life, the aqueous extract has been prepared by autoclaved herb with water at 121°C for 20 min which save a lot of time. The solution will be then lyophilized in order to get rid of the water. The final product was in lightly pink powder form, which had a strong water absorption property.

The yields of extraction, total phenolic content, and total flavonoid content in the different extracts of plant material were also summarized in Table 2. The extraction yields by agitation, sonication, and aqueous were 45.12 ± 0.80 %, 56.44 ± 0.82 % and 62.22 ± 0.68 %, respectively. The aqueous extraction method gave the highest extraction yield and significantly different from sonication, and agitation methods. It was due to the hot temperature had been used during extraction; cell membranes were easily lysed and untargeted substances such as carbohydrate also released into solution which was able to increase the yield of aqueous extract. However, agitation method extracted significantly more phytochemicals than the other ones. For the total phenolic content test, extracts of *M. oleifera* by agitation, sonication, and aqueous were 74.87 ± 0.19, 59.91 ± 0.20 and 56.00 ± 0.21 mg GAE/g dry weight, respectively. In the total flavonoid contents from extracts of *M. oleifera* by agitation, sonication, and aqueous were 18.46 ± 0.25, 13.50 ± 0.22 and 12.55 ± 0.22 mg CE/g dry weight, respectively. The TPC and TFC of aqueous extract were significantly lower than the others, which also reconfirmed by HPLC analysis.

Table 2. Extraction yield (% dried mass), total phenolic content (mg/g GAE dry weight), and total flavonoid content (mg/g CE dry extract) of different extracts of *M. oleifera* leaves.

	% Yield	TPC	TFC
Agitation	45.12 ± 0.80^{c}	74.87 ± 0.19^{a}	18.46 ± 0.25^{b}
Sonication	56.44 ± 0.82^{b}	59.91 ± 0.20^{b}	13.50 ± 0.22^{c}
Aqueous	62.22 ± 0.68^{a}	56.00 ± 0.21^{c}	12.55 ± 0.22^{a}

*, mg GAE/g dry weight; **, mg CE/g dry weight; Values are mean ± SD for n = 3; $^{a-c}$ different small case superscript letters within the same column for a significant difference between two different extraction methods ($p < 0.001$).

3.3 Anti-oxidative Activity

3.3.1 Scavenging ability and reducing ability

The majority functions of antioxidant were usually separated into two types: scavenging ability (DPPH assay, ABTS assay) and reducing ability (FRAP assay, RP assay). In scavenging ability, with the presence of antioxidant compounds, $DPPH^{\bullet}$ could accept only protons from the antioxidant scavenger molecule to be converted to the reduced form of DPPH which is pale yellow [10]; while in the ABTS assay, the $ABTS^{+}$ could only receive elections in order to convert to ABTS which is transparent [11]. The DPPH radicals scavenging assay is suitable for samples containing the low polarity compounds, which can be dissolved in alcoholic solvents; while ABTS radicals scavenging assay is suitable for samples containing high polarity compounds, especially antioxidants dissolve in water. In reducing ability, FRAP assay directly measured the ability of antioxidants to reduce a ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to the ferrous complex (Fe^{2+}-TPTZ), blue color, at low pH condition (pH = 3.6) [12], while in reducing power assay, antioxidants also reacted with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) at neutral pH condition (pH = 6.6) [13]. Many previous researches pointed out that the antioxidant activity of plant extract is corresponding to phenolic capacities.

The results of antioxidant activities (Table 3) strongly confirmed the correlation to the results of phenolic content in the previous part. Phenolics are able to act as antioxidants in many different ways including in acting as good hydrogen donors, generating free radicals by hydroxyl groups to stabilize other free radicals, or acting as a metal chelator [17]. The phenolic compounds of extract are, the better the antioxidant it will be. In DPPH, ABTS, and FRAP assays, antioxidant ability results of the extract from agitation were 18.62 ± 0.05, 35.29 ± 0.37 and 34.64 ± 0.07 mg TE/g dry weight, respectively, which was significantly higher than other methods. Moreover, in reducing power assay, the results from extracts of *M. oleifera* by agitation, sonication, and aqueous were 21.86 ± 0.08, 17.22 ± 0.03 and 14.73 ± 0.04 mg AAE/g dry weight, respectively, which strongly reconfirmed the correlation of phenolic content to antioxidant ability. Using the Trolox equivalence as a standard unit in different antioxidant capacity tests (DPPH, ABTS, and FRAP assays), we recognized that the antioxidant abilities of extracts did not equally exhibit in different systems. All extracts exhibited their highest antioxidant abilities in the FRAP assay among three assays following the trend ATBS>DPPH assay.

Table 3. DPPH, ABTS, FRAP, and Reducing power (RP) assays of different extracts from *M. oleifera* leaves.

	DPPH (*)	ABTS (*)	RP (**)
Agitation	18.62 ± 0.05[a]	35.29 ± 0.37[a]	21.86 ± 0.08[a]
Sonication	15.26 ± 0.11[b]	32.45 ± 0.32[b]	17.22 ± 0.03[b]
Aqueous	13.23 ± 0.08[c]	26.89 ± 0.48[c]	14.73 ± 0.04[c]

*, mg TE/g DW; **, mg AAE/g DW; Values are mean ± SD for n = 3;[a-c] different small case superscript letters within the same column for a significant difference between two different extraction methods ($p < 0.001$).

3.3.2 IC_{50} values

The IC_{50} values for antioxidant capacity tests were also presented in Table 4. Lower IC_{50} value means higher antioxidant capacity. The agitation extract was found most powerful for both DPPH scavenger and ABTS scavenger as evidenced by lowest IC_{50} value (139.60 ± 0.25 µg/mL in DPPH assay and 57.07 ± 0.53 µg/mL in ABTS assay) compared to other extracts. Therefore, agitation should be a potential method for the purpose of extracting phenolic compounds.

Charoensin [18] also reported the IC_{50} of methanol and dichloromethane extracts of Moringa leaves. The IC_{50} values of methanol and dichloromethane extracts in DPPH assay using agitation method were 160 µg/mL and 321 µg/mL, respectively. IC_{50} value of Trolox standard was 6.72 µg/mL in DPPH assay, which was a similar value to Trolox standard in this study. Compared to the study of Charoensin, the ethanolic extract in our study achieved stronger antioxidant ability achieved due to the lower value of IC_{50}. The reason reconfirmed that ethanol solvent was not only safe in term of using, but it also had stronger extraction ability compared to the methanol one due to the broader polarity of ethanol.

Table 4. IC_{50} in DPPH and ABTS assays of different extracts from *M. oleifera* leaves.

Treatment	50% Inhibition concentration	
	DPPH IC_{50} (*)	ABTS IC_{50} (*)
Agitation	139.60 ± 0.25[a]	57.07 ± 0.53[a]
Sonication	159.49 ± 0.35[b]	80.70 ± 0.55[b]
Aqueous	250.66 ± 0.33[c]	100.51 ± 0.53[c]
Trolox (µg/mL)	6.05 ± 0.02	4.40 ± 0.04

Values are mean ± SD for n = 3;[a-c] different small case superscript letters within same column for significant difference between three different extraction methods ($p < 0.001$).

3.3.3 Dose dependence abilities

The agitation (red) shows the highest coefficient value in both DPPH and ABTS assays according to the slope in the graphs shown in Figure 1. This again shows that agitation exhibited the strongest antioxidant activity. The more phenolic active compounds in the extract were, the higher radical

scavenging of the treatment would be. Hence, agitation obtained the highest dose dependence ability in both the assays. On the other hand, the coefficient values of sonication (yellow) and aqueous (green) extractions are lower than that of the agitation in both DPPH and ABTS assays. This showed the lower dose dependence ability. It might have caused by the unsuitable extraction methods due to the degradation of phenolic compounds by high temperature in aqueous extraction or the changing of the structure of phenolic compounds in the sonication extraction. With the linear correlation between concentration and radical scavenging ability, the antioxidant activity of all Moringa leave extracts were reported in term of concentration and dose-dependent.

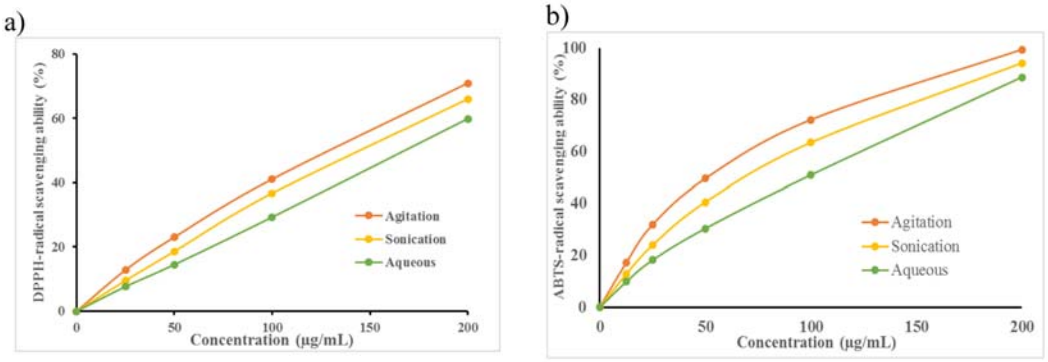

Figure 1. Antioxidant activity determined by (a) DPPH-radical scavenging assay, (b) ABTS-radical scavenging assay.

3.4 HPLC Analysis

In the HPLC analysis, all fractions were examined for their specific polyphenolic composition using HPLC. There were nine phenolic compounds, Gallic, Chlorogenic, Ellagic and Ferulic acids as phenolic acids and Catechin, Epicatechin, Myricetin, Quercetin and Kaempferol as flavonoids which were shown in Figure 2. The result revealed that the number of phenolics compounds in agitation fraction were four compounds including Gallic acid (119.2 µg/g), Catechin (594 µg/g), Epicatechin (321.2 µg/g), and Ferulic acid (62.7 µg/g). However, there were only three phenolic compounds presented in the sonication one including Catechin (743.51 µg/g), Epicatechin (322.43 µg/g), and Ferulic acid (73.96 µg/g). On the other hand, there were also only three phenolic compounds presented in the aqueous one including Epicatechin (700.5 µg/g), Ferulic acid (65.4 µg/g), and Myricetin (705.4 µg/g).

Previously, Gupta et al. [19] have noticeably reported that M. oleifera leaves possess major phenolic compounds of phenolics, such as quercetin and kaempferol, which are responsible for antioxidant activity. However, Epicatechin and Ferulic acid are two main components that contributed to the antioxidant abilities in Moringa leaves [20]. Different amounts of Epicatechin in different extracts showed that high temperature helped to break the cell and extracted the Epicatechin easily. Catechin is very sensitive to heat and pressure, which is easily degraded by high heat, over 100°C [20]. On the other hand, the Gallic acid was disappeared in the sonication fraction might be due to high attitude level (frequency = 50 kHz) of extraction and the long time applying (30 minutes) [21]. Myricetin from Moringa leaves was deeply packed to many

complexes inside the cells; thus, the aqueous was preferably used to extract bioactive compounds by breaking down the cells [17]. The amount of extracted phenolic compounds of different techniques was not the same due to different physical conditions such as time, temperatures, pressure applying to the samples.

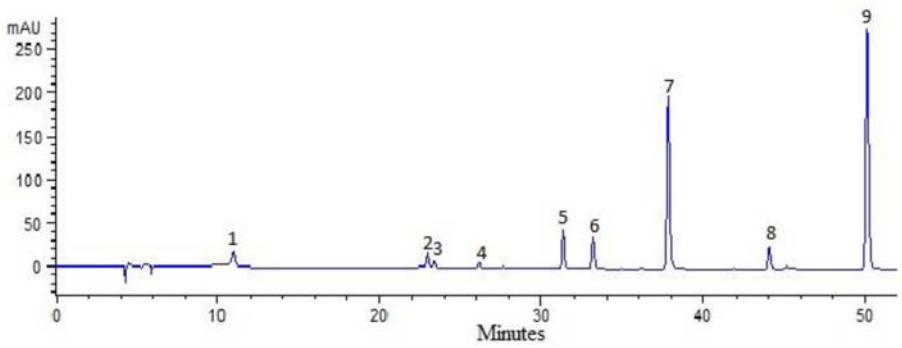

Figure 2. Chromatogram of 9 standard mixture used in HPLC analysis recorded at 280 nm. Gallic acid (1), Chlorogenic acid (2), Catechin (3), Epicatechin (4), Ellagic acid (5), Ferulic acid (6), Myricetin (7), Quercetin (8), Kaempferol (9).

4. CONCLUSIONS

In summary, we have demonstrated the nutritional screening, phenolic screening, and antioxidation activity of different extracts of Thai Morning leaves. The Moringa leaves powder was not only an excellent source of protein and carbohydrates, but also can be a good source for nutraceutical products due to high content of dietary fiber. Although the agitation method showed lower extraction yield and it consumes lots of energy during the extraction, it was the best method for extracting phytochemicals and antioxidants in *M. oleifera* leaves due to the highest contents of phenolic compounds (74.87 ± 0.19 mg GAE/g dry weight) in the extract. The agitation extract was also found most powerful for both DPPH scavenger and ABTS scavenger as evidenced by lowest IC_{50} value (139.60 ± 0.25 µg/mL in DPPH assay and 57.07 ± 0.53 µg/mL in ABTS assay) and highest coefficient values in dose-dependence abilities compared to other extracts. In conclusion, although the aqueous showed highest extraction yield and it is environmentally-friendly and economical, the agitation was the best method for extracting the phytochemicals and antioxidants in *M. oleifera* leaves.

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