Ultrasound – Assisted Extraction of Phenolic Compounds from Indigenous Vegetables In Southern Thailand

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ABSTRACT

Ultrasound-assisted extraction has indicated numerous advantages over traditional extraction methods for extracting compounds based on plant materials. An objective of the investigation was to determine optimal conditions in an ultrasound-assisted extraction process of indigenous vegetables in the south of Thailand. Dried ground of indigenous vegetables (*Ficus racemosa* L. (Look ching), *Etlingera elatior* (Dok dala), *Curcuma longa* L. (Kamint) and *Glochidion perakense hook.f*. (Mun pu) in the south of Thailand (5 g, all edible part of indigenous vegetables) were subjected to ultrasound-assisted extraction at 35-75°C, ratio of raw material with ethanol (60% v v^{-1}) (1:15-1:30 mg mL^{-1}) and extraction time between 5-60 minutes. Extracts were filtered through filter paper (whatman no.1) and extracts obtained were used for determination of total phenolic content using Folin – Ciocalteu reagent and antioxidant activities using 2,2–diphenyl–1–picrylhydrazyl (DPPH). Results showed that total phenolic contents and antioxidant activities of *Glochidion perakense hook.f* was found to yield the highest total phenolic content and antioxidant activity (47.24±0.04 mg GAE g^{-1} DW and 52.74±0.68%, respectively). Optimum extraction conditions in ultrasound-assisted extraction from *Glochidion perakense hook.f* was as follows: solid to liquid ratio of 1:20 mg mL^{-1}, extraction temperature of 65°C and extraction time of 30 minutes. Under above-mentioned conditions, experimental total phenolic content and antioxidant activity was 61.50±0.25 mg GAE g^{-1} DW and 74.05±0.65%, respectively. *Glochidion perakense hook.f* was considered as a good source of natural antioxidants

Key Words: Ultrasound – assisted extraction, Phenolic compounds, Indigenous vegetables, Antioxidant activity

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INTRODUCTION

There is a growing interest concerning lipid oxidation because of its significance for food deterioration. Lipid oxidation is a complicated free radical chain process involving a variety of radicals formation. Oxidation is influenced by various parameters e.g. temperature, light, air, physical and chemical properties of the substrates, and presence of oxidation catalysis or initiators. Use of antioxidants in lipid-containing foods is one potential method to minimize rancidity, retard formation of toxic oxidation products, maintain nutritional quality and increase shelf-life of food products (Maisuthisakul *et al*. 2007). Since consumers are usually concerned about use of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroxyquinone (TBHQ) and propyl, octyl, and dodecyl gallates in lipid-containing foods, synthetic antioxidants have been applied for decreasing lipid oxidation during storage of processed food products tend to cause possible worry among consumers. In addition, use of chemical additives has raised questions regarding food safety and toxicity (Chang *et al*. 1977). There is also an interest in developing natural antioxidants from plants.

Various parts of many Thai indigenous plants are used as food, beverage, medicine, or for chewing and these are potential sources of new natural antioxidants (Maisuthisakul *et al*. 2007). Thai native vegetables have been reported to be good sources of antioxidants, and many also consist of with anti-bacterial, anti-inflammatory, anti-mutagenic, and anti-carnogenic related compounds (Kongkachuichai *et al*., 2015). Extraction of antioxidant compounds from plants can be carried out in a variety of ways, for examples, traditional extractions (soxhlet, infusion and maceration), and supercritical fluid extraction. However, these methodologies have been employed for decades, it is important to mention that some of them could be a cause of degradation of targeted bioactive compounds due to high temperature and long extraction time used. There are also some health risk for analysts because of relatively large number of toxic solvents.

The conventional extraction methods of polyphenols from plants have some limitations regarding the high solvent consumption, the long extraction time requirement, the improvement of yield and quality of the extracts. Actually, ultrasound-assisted extraction (UAE) is widely used for extracting plant compounds in order to overcome these drawbacks (Porto *et al*., 2013). Ultrasonic-assisted extraction (UAE) has been proven to be one of the most promising techniques for extracting bioactive compounds from plants, and it is relatively adaptable on a small or large scale, in addition it is a promising technology due to cheaper and fewer instrumental requirements. Use of ultrasound can enhance extraction process efficacy by increasing mass transfer between solvents and plant materials. Collapse of cavitation bubbles leads to better cell disruption through formation of microjets due to asymmetrical bubble collapse near a solid surface. This permits for improved solvent

penetration into the plant body itself and can also break down cell walls (Toma *et al*. 2001). Application of UAE offers many advantages including: less amount of solvent, lower temperatures and shorter extraction time which is very useful for extraction of thermo-labile and unstable compounds and also increased extraction yield (Muniz-Marquez *et al*. 2013). Therefore, objectives of this work were to investigate influences of solid/liquid ratio, extraction time and extraction temperature by using UAE on total phenolic contents and antioxidant activities of the indigeneous vegeatable in the South of Thailand.

MATERIALS AND METHODS

Materials

Plant materials

Thai indigenous vegetables in Souhern Thailand were collected from Nakhon Sri Thamarat Province, Thailand and as authenticated by the corresponding author. Materials were washed thoroughly in potable water and dried in a hot air oven at 50°C, 10 hr, then ground to pass through a 0.25 mm sieve. Power was kept in sealed metalize bags and stored at room temperature until the analyses.

Chemicals and reagents

Ethanol and Folin-Ciocalteu's phenol regents were obtained from Merk, Bangkok, Thailand. Gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Fluka Analyticals, Bangkok, Thailand. All chemicals and reagents were of analytical reagent grade.

Methods

Ultrasound-assisted extraction

Ultrasound-assisted extraction of phenolic compounds from Thai indigenous vegetables in the south of Thailand was performed in an ultrasonic bath (120 W, 45 kHz) by employing some extraction variables including: solid/liquid ratio 1:15, 1:20, 1:25 and 1:30 g mL^{-1}, extraction temperature 35, 45, 55, 65 and 75°C and time of sonication 5, 15, 30 and 60 minutes consecutively.

Samples (5 g, all edible part of indigenous vegetables) were placed in 100 mL Erlenmeyer flasks and 100 mL of 60% volume per volume (v v^{-1}) ethanol was added. Sample was submitted to ultrasonic irradiation at different extraction conditions. Extracts obtained were filtered by vacuum filtration. Total phenolic contents of Thai indigenous vegetables extracts were determined using Folin-Ciocalteu method (Yingngam *et al*., 2014). All calculations about the quantitative analysis were compared with the calibration curve of gallic acid and the results were expressed as gallic acid equivalents (mg GAE g^{-1} dried sample) and antioxidant activities were calculated as follow,

inhibition (%) = $[A_0-(A_1-A_2)/A_0]$x100 where A_0, A_1, A_2 are the absorbance value of the DPPH solution without sample solution, test sample, and sample without DPPH solution, respectively (Yingngam *et al*., 2014).

Statistical analysis

All experiments were repeated 3 times; average and standard deviations were calculated. Statistical analysis was carried out and the comparisons of averages of each treatment were based on the analysis of variance (ANOVA) at significance level 5%. Values followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic contents and antioxidant activities in Thai indigenous vegetables

Phenolic compounds are very important vegetable constituents because they exhibit antioxidant activities by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic contents but it is known that different phenolic compounds have different response to Folin-Ciocalteu method. Therefore, in this work, we calculated total phenolic contents in units of mg gallic acid equivalents of phenolic compounds as shown in Table 1. It was found that *Glochidion Perakense Hook.f* (Mun pu) obtain the highest total phenolic content and antioxidant activity (47.24±0.04 mg GAE g^{-1} DW and 52.74±0.68%, respectively). Kongkachuichai *et al*. (2015) and Tangkanakul *et al*. (2005) found that the total phenolic content of *Glochidion Perakense Hook.f* (Mun pu) was of 47.63 mg GAE g^{-1} DW and 49.06 mg GAE g^{-1} FW, respectively. The wide variation in total phenolic contents in vegetables might arise from the variety of the plant, plant portions, stage of ripening, place of cultivation, climate condition, fertilization, sample collection, transportation, sample preparation, and methods of analysis (Rop *et al*., 2011).

Table 1 Total phenolic content and antioxidant activity of indigenous vegetables in the southern Thailand.

Scientific name	Local name	Phenolic content (mg GAE g^{-1} DW)	Antioxidant activity (%)
Ficus racemasa L.	Look ching	18.61 ± 0.26[a]	36.42 ± 0.69[a]
Etlingera elatior	Dok dala	20.93 ± 0.34[a]	38.41 ± 0.69[b]
Curcuma longa L.	Kamint	34.11 ± 0.08[b]	43.04 ± 1.05[c]
Glochidion Perakense Hook.f.	Mun pu	47.24 ± 0.04[c]	52.74 ± 0.68[d]

[a-d] Means within the same column followed by different letters were significantly different ($p \leq 0.05$).

Effects of extraction temperature on total phenolic content of *Glochidion Perakense Hook.f.*

Effects of extraction temperature on phenolic contents and antioxidant activities were probed. Results demonstrated that, when temperature increased from 35 to 65°C, phenolic contents and antioxidant activities of extracts increased (Table 2), which is possibly owing to high number of cavitational nuclei formed during higher extraction temperature as a consequence of high cavitation threshold which is accountable for acoustic cavitation. A relative greater force ruptured cavitational nuclei and disrupted cell tissues during extraction, which will enhance mass transfer (Toma *et al*. 2001). Moreover, increasing of temperature enhanced solubilities of phenolic compounds and diffusion coefficients of extraction solvent (Al-Farsi and Lee, 2008). When extraction temperature was above 65°C, phenolic contents and antioxidant activities decreased. It is true that higher temperature (40°C) may increase solubilities of phenolics (Japon-Lujan *et al*. 2006) but too high (60°C) may cause their degradation (Rostagno *et al*. 2007). Moreover, the temperature at 75°C, the efficiency decreased. It may from the effect of solvent evaporation to reduce the ratio between solvent to material. Mild heating might soften plant tissue, weaken cell wall integrity, hydrolyze bonds of bound phenolic compounds (phenol-protein or phenol-polysaccharide) as well as enhance phenolics solubility, thus more phenolics would distribute to solvent system (Spingno *et al*. 2007).

Table 2 Effect of extraction temperature on total phenolic content and antioxidant activity.

Extraction temperature (°C)	Phenolic contents (mg GAE g^{-1} DW)	Antioxidant activities (%)
35	47.23 ± 0.35^{a}	52.74 ± 0.69^{a}
45	49.21 ± 0.44^{a}	54.83 ± 1.35^{a}
55	58.72 ± 0.71^{b}	66.62 ± 0.80^{b}
65	61.20 ± 0.17^{b}	73.97 ± 2.75^{c}
75	42.32 ± 0.13^{a}	39.35 ± 2.34^{d}

$^{a-d}$ Means within the same column followed by different letters were significantly different ($p \leq 0.05$).

Effects of solid/liquid ratios on total phenolic contents of *Glochidion Perakense Hook.f.*

In this study, ethanol was used as the extraction solvent because ethanol is safe and classified in class 3 pharmaceutical level according to the United Stage of Food and Drug Administration (US-FDA) (McConville, 2002) and can be easily recovered by reduced pressure distillation. In addition, according to Wang *et al*. (2008) found that ethanol extracts contained the highest content of total phenolics, followed by methanol extracts and acetone extracts. Table 3

showed effect of solid/liquid ratio extraction phenolic compounds. Results indicated that effects of solid/liquid ratio on the phenolic contents and antioxidant activities of extracts were variables that were considerably affected during extraction of phenolic compounds. It can be observed that, when a liquid/solid ratio increases from 15 to 20, phenolic content and antioxidant activity would increase. One of probable explanations for this phenomenon is that usually usage of larger volume of solvent could obtain larger amount of bioactive compounds (Xia *et al*. 2011), as it could accelerate diffusion of compounds, which could be favorable for increase of phenolic contents (Lui *et al*. 2010). Moreover, concentration differences between interior plant cells and exterior solvent were caused by higher solid-liquid ratios, which could dissolve more constituents (phenolic compounds) more effectively and lead to an enhanced mass transfer rate and increase the extraction yield (Toma *et al*. 2001). By further increase in solvent-to-material ratio, a decline in phenolic contents and antioxidant activities was above 20 mL g^{-1}. This indicated that a larger volume of solvent did not improve the driving force. Similar results were also reported by other researchers (Gan *et al*., 2011; Wang *et al*., 2013; Yingngam *et al*., 2014).

Table 3 Effects of solid/liquid ratio on total phenolic content and antioxidant activity.

Solid/liquid ratios (mg mL^{-1})	Phenolic contents (mg GAE g^{-1} DW)	Antioxidant activities (%)
1:15	52.91 ± 0.14[a]	58.45 ± 1.74[a]
1:20	61.20 ± 0.16[b]	73.97 ± 2.75[b]
1:25	48.32 ± 0.32[c]	45.94 ± 0.63[c]
1:30	45.00 ± 0.93[c]	41.65 ± 1.48[d]

[a-d] Means within the same column followed by different letters were significantly different ($p \leq 0.05$).

Effects of extraction time on total phenolic content of ***Glochidion Perakense Hook.f.***

Contents of the phenolic compounds and antioxidant activities extracted at different times of sonication were presented in Table 4. A substantial increase of phenolic contents and antioxidant activities were observed over the specified extraction period (5-60 minutes), and phenolic compounds and antioxidant activities reached a maximum of approximately 61.00±0.25 mg GAE g^{-1} DW and 73.85±0.52%, respectively. Mass transfer controls a solvent extraction of any component from a plant matrix; when solvent saturates, extracted compounds, concentration gradient becomes null and an ultrasound extraction of phenolic compound from *Glochidion Perakense Hook.f,* ceases and finally mass transfer stopped after 30 minutes and the process can then be interrupted.

Table 4 Effects of extraction time on total phenolic contents and antioxidant activity.

Extraction time (minutes)	Phenolic contents (mg GAE g^{-1} DW)	Antioxidant activities (%)
5	15.22±0.32[a]	37.42±0.51[a]
15	34.83±0.16[b]	45.67±0.83[b]
30	61.00±0.25[c]	73.85±0.52[c]
60	61.20±0.10[c]	73.84±0.64[c]

[a-c] Means within the same column followed by different letters were significantly different ($p \leq 0.05$).

Results obtained from these experiments showed that the optimum extraction conditions for extracting the phenolic content from *Glochidion Perakense Hook.f* extracts were obtained by ultrasound-assisted extraction at liquid to solid ratio 20, extraction temperature 65°C, extraction time 30 minutes which produced maximal phenolic content of 61.50±0.25 mg GAE g^{-1} DW and antioxidant activities of 74.05±0.65%.

CONCLUSION

Glochidion perakense hook.f can be regarded as a good source of natural antioxidants. Moreover, The best condition based on four types of Thai indigenous vegetables in Southern Thailand with ultrasound-assisted extraction was solid/liquid ratio 1:20 (g L^{-1}), extraction temperature 65°C, and extraction time 30 minutes with ethanol 60% v v^{-1}.

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