Comparison of Ultrasonic Extraction with Conventional Extraction Methods of Phenolic Compounds in Longan (*Euphoria longana* Lamk.) Seed

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ABSTRACT

Three different extraction methods – hot water (HWE), ethanol (EE) and ultrasonic-assisted water extraction (UAE) – were evaluated for extraction of phenolic compounds in longan (cultivar Edor) seed. The findings indicated that longan seed extracts contained three major phenolic compounds, analyzed by HPLC, including gallic acid, corilagin and ellagic acid. The content of total polyphenolic compounds and antioxidant activities was determined using the Folin-Ciocalteu method and the DPPH assay, respectively. Longan seed extraction by HWE contained the highest yield, total polyphenol content and antioxidant activities (42.80%, 41.250mg gallic acid/g and IC_{50} = 0.017 mg/ml, respectively). UAE produced a higher yield than EE, but lower than HWE, with the lowest extraction time. Moreover, gallic acid content (16.55 mg/g) and corilagin content (35.62 mg/g) extracted by UAE were higher than those of HWE and EE. Ellagic acid content obtained from UAE was significantly ($P<0.05$) higher than that from EE, but not significantly different from that of HWE. The application of UAE could provide an alternative method for extraction of photochemical from longan seeds.

Keywords: Longan extract, Phenolic compound, Antioxidant activity, Extraction method

INTRODUCTION

Longan (*Euphoria longana* Lamk.) is a subtropical fruit widely grown in northern Thailand and is found in Southeast Asia, China and Taiwan. Longan can be consumed as fresh and processed products (Rangkadilok et al., 2005), such as canned longan in syrup and dried fruit, as well as used in cuisine. Most longan, Edor cultivar, is commercially grown for the dried fruit market, either as dried pulp or dried whole fruit. This fruit is said to invigorate the heart and spleen, nourish the blood and have a calming effect on the nervous system; moreover, dried longan is often used as an herbal medicine to cure stomach pain, febrifuge, and as an antidote for poison (Prasad et al., 2009a). Longan has been reported to have pharmacological properties, such as reducing contraction of the blood ves-

sels, radical scavenging activities and anticancer properties (Yang et al., 2011). These beneficial effects of longan are due to the antioxidant activities of phenolic compound (gallic acid, corilagin and ellagic acid) in the seed. A previous study demonstrated that longan seed extract was found to be as effective as Japanese green tea extract in exhibiting radical scavenging activities (Rangkadilok et al., 2007). Zheng et al. (2009) also reported that longan seeds have strong antioxidant activities due to their polyphenols. They further reported that gallic acid isolated from longan seed had the strongest DPPH radical-scavenging activity.

Several studies have compared polyphenolic content and radical-scavenging activities of longan by many extraction methods. Various solvents such as acetone, methanol, ethanol and water were used for the extraction. Usanee et al. (2006) reported that 80% acetone, 70% ethanol or hot water extracts of dried longan seed (Biewkiew cultivar) contain many polyphenol substances, and express more antioxidant activity than that from other parts of longan. Mixing dried longan with a non-toxic solvent, such as hot water, is the common method used for extracting some pharmacological substances in Chinese medicine. Hot water extracts (HWE) of longan seeds contained a higher antioxidant activity than 70% methanol extracts (Rangkadilok et al. 2007). Ethanol is a widely used "green" solvent in extraction of plant material such as grape seed, tamarind seed and sweet basil. Rodriguez-Rojo et al. (2012) also used 96% ethanol to extract antioxidants in Rosemary leaves. In addition, the ultrasound-assisted water extraction (UAE) method has been alternatively used in the food industry to extract bioactive compounds from food components. UAE utilizes ultrasonic waves, with a frequency greater than the upper limit of human hearing (greater than 20 kHz), to enhance the extraction efficiency of organic compounds (Rostagano et al., 2003). The improvement of solvent extraction from plants by UAE is due mainly to the mechanical effects of acoustic cavitation, which enhance both solvent penetration into the plant material and intracellular product release by disrupting cell walls (Wu et al., 2001). UAE was reported to improve the extraction yield of polyphenols from grape marc (Vilkhu et al., 2008). Furthermore, the UAE method potentially enhances extraction of various components compared with conventional extraction. The improved effectiveness of using UAE to extract compounds from the longan pericarp was also reported by Prasad et al. (2009b) and Zhong and Wang (2010).

The study of extraction methods of phenolic compounds in longan seeds poses an interesting challenge for further development of functional foods or supplements to promote health. Therefore, this study focused on determining the efficiency of different methods for extracting the phenolic compounds from longan seed; three different extraction methods including HWE and 70% ethanol extraction (EE), both conventional methods, and UAE, an alternative method, were evaluated.

MATERIALS AND METHODS

Plant material

Fresh longan seeds, a by-product from dried longan fruit processing, were obtained from Thongpoon Food Ltd., Part. in Lamphun Province, Thailand. The seeds were dried at 75ºC for 24 h in a hot air oven and ground into powder (80 mesh).

Longan seed extraction

The polyphenolic compounds were extracted by three different methods: HWE, EE, conventional methods and an alternative method, UAE. The HWE method followed the procedures described by Rangkadilok et al. (2007). The longan seed powder was mixed with hot water (75ºC) at a ratio of 1:4 w/v, left for 24 h, then filtered. The residue was then re-extracted by hot water twice and all extracts were combined. The EE method was modified from Rangkadilok et al. (2005) by combining 70% ethanol with the seed powder (5 ml ethanol: 0.4 g seed) in tubes. The mixture was shaken and then left at room temperature for 24 h. The seed residue from first extraction was re-extracted with 5 ml 70% ethanol using the same method. The extract was centrifuged at 3600 rpm at 25ºC for 3 min and then the supernatant was collected. Both EE Longan seed extracts were combined. The UAE method was modified from Yang et al. (2008). The seed powder (4 g) was added into an Erlenmeyer flask with 100 ml of hot water (70ºC) and then extracted by an ultrasonic bath (Crest, USA) with water at 120 W ultrasonic power, 70ºC for 20 min. The extract was then filtered through a Whatman No.1 filter paper. The extracts from the different extraction methods were concentrated using a rotary evaporator and lyophilized in a freeze-dryer.

Determination of total polyphenol content

The total polyphenol content was determined by using the Folin-Ciocalteu method adapted from Waterman and Mole (1994). Gallic acid solutions at concentrations of 50, 100, 200, 300, 400, 500, 600 ppm were prepared in 95% ethanol as standard solutions. 0.25 ml of the seed extracts (0.01 g/ml) was mixed with 0.30 ml of deionized water, 0.25 ml of Folin-Ciocalteu reagent and 2.50 ml of 7% sodium carbonate. The mixture was then incubated in the dark at room temperature for 30 min before the absorbance was measured at 760 nm by a spectrophotometer (UV-vis model 1601, Shimadzu, Kyoto, Japan). The results were expressed in mg gallic acid per g extract.

High performance chromatography (HPLC) analysis

The freeze-dried seed extracts (25 mg) were dissolved in 3.0 ml hot water (75ºC), vigorously shaken and left at room temperature until cooled and then filtered through a 0.45 µm Nylon membrane (Orange Scientific, Belgium). The resulting extract (10 µL) was injected into an HPLC system to determine the content of gallic acid, corilagin and ellagic acid. The HPLC technique was carried out by the method of Rangkadilok et al. (2007). A reversed phase HPLC system (HP1100) consisted of a diode-array detector and a binary pump. LiChrospher RP-18 column (Macherey-Negel, Germany), 25 ºC, was used for separation with UV

detection at 270 nm. Solvent gradients were formed by solvent A (0.4% formic acid) to solvent B (methanol) at a flow rate of 1.0 mL/min. The solvent gradient system (total 20 min) started from 100%A at 0 min, 95%A at 2 min, 70%A at 5 min, 66%A at 8 min, 45%A at 14 min and 100%A at 17 min. Calibration curves were created from gallic acid (98% purity) and ellagic acid standard dissolved in a HPLC grade of methanol at five concentrations, 0.008, 0.03, 0.06, 0.1, 0.14 mg/mL gallic acid, 0.006, 0.012, 0.063, 0.125, 0.63 mg/mL corilagin, and 0.005, 0.01, 0.03, 0.06, 0.1 mg/mL ellagic acid.

DPPH radical-scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay for analysis of antioxidant activities in longan seed extracts was modified from Zheng et al. (2009). Determination started by mixing 1.0 ml of the DPPH solution (0.1 mM) with 1.0 ml (0.1, 0.05, 0.01, 0.005, 0.002 mg/ml) of the extracts and/or ascorbic acid as a positive control. The mixtures were incubated in darkness for 30 min before measuring the absorbance of samples at 517 nm using a spectrophotometer. The inhibition of DPPH radical scavenging activity was calculated by the equation: %DPPH scavenging activity = [(absorbance of sample – absorbance of blank)/absorbance of control] x 100. The control contained DPPH solution and methanol whereas blanks included methanol and the extracts. The DPPH antioxidant activities were shown as IC_{50} (the concentration that scavenges 50% of DPPH).

Statistical analysis

Samples of the extracts were prepared and analyzed in three replications. One-way analysis of variance and Duncan's New Multiple-range test were done using Minitab 16.1.1 to determine the differences among the means. A probability value of $P<0.05$ was used to determine statistical significance.

RESULTS

Longan seed extraction

Different extraction methods were used to extract phenolic compounds in seeds of longan cultivar Edor. Table 1 shows the longan extraction times and yields of the methods compared in this study. The EE method required the longest extraction time and produced the lowest yield. The HWE method produced the highest longan extract yield, 42.8%, while the yield of UAE method, 35%, was intermediate between the other two treatments, but used the shortest time.

Table 1. Extraction time and yield of longan extracts by various extraction methods.

Extraction method	Extraction time (min)	Extraction yield (%)
Ethanol (EE)	2880	27.7 ± 1.5c
Hot water (HWE)	180	42.8 ± 1.2 a
Ultrasonic-Assisted (UAE)	20	35.0 ± 1.9 b

Note: *Mean ± standard deviation (S.D.) within the column followed by different letters were significantly different by DNMRT at P<0.05.

High performance liquid chromatography (HPLC) analysis

Figure 1 presents the chromatograms of longan seed extracts by various extraction methods. The findings indicated that longan seed extracts contain three major phenolic compounds at the identified peak: gallic acid, corilagin and ellagic acid (retention times of 3.6, 7.8 and 13.2 min, respectively). The contents of gallic acid, corilagin and ellagic acid of longan seed extracts by three extraction methods are presented in Table 2. The gallic acid, corilagin and ellagic acid content varied from 1.20–16.55 mg/g, 8.30–35.62 and 1.05–7.02 mg/g, respectively.

Table 2. The content of gallic acid, corilagin and ellagic acid in longan seed extracts.

Extraction method	Gallic acid (mg/g)	Corilagin (mg/g)	Ellagic acid (mg/g)
Ethanol (EE)	1.20 ± 0.73 ^{c}	8.30 ± 0.09 ^{c}	1.05 ± 0.98 ^{b}
Hot water (HWE)	4.33 ± 0.39 ^{b}	26.40 ± 0.22^{b}	6.05 ± 0.67 ^{a}
Ultrasonic-Assisted (UAE)	16.55 ± 0.42 ^{a}	35.62 ± 0.35 ^{a}	7.02 ± 1.32 ^{a}

Note: * Mean ± standard deviation (S.D.) within the column followed by different letters were significantly different by DNMRT at P<0.05.

The total polyphenol content

Table 3 shows the total polyphenol content of longan seed extracts by the Folin-Ciocalteu Method. The total polyphenols by the different extraction methods varied from 11.717–41.250 mg GAE/ml, with HWE producing the highest amount.

DPPH radical-scavenging activity

The DPPH-radical scavenging activity of longan seed extracts expressed as unit of IC_{50} is shown in Table 3. Longan seed extraction by HWE exhibited high antioxidant activity, requiring the lowest seed extract concentration, but was not significantly different from UAE. Antioxidant activity appears to be related to total polyphenol content as seen in Table 3.

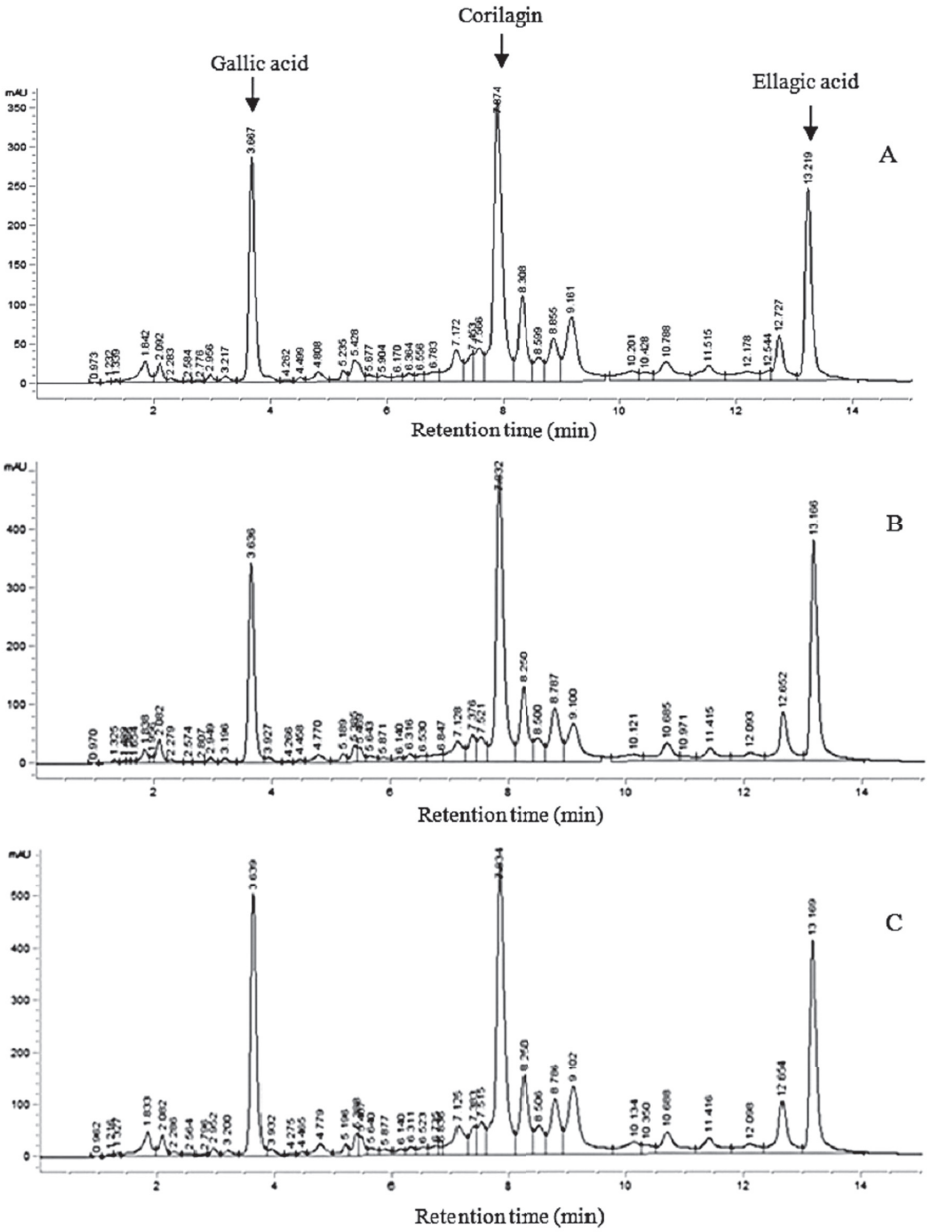

Figure 1. HPLC chromatograms of gallic acid, corilagin and ellagic acid in longan extracts by different extraction methods: (A) Ethanol (EE), (B) Hot water (HWE), (C) Ultrasonic-assisted (UAE).

Table 3. Total polyphenol content and antioxidant activity (DPPH assay) by ethanol, hot water and ultrasonic extraction methods.

Extraction method	Total polyphenol content (mg GAE/g)	DPPH assay IC_{50} (mg/ml)
Ethanol (EE)	11.717 ± 0.001 [c]	0.042 ± 0.009 [a]
Hot water (HWE)	41.250 ± 0.008 [a]	0.017 ± 0.002 [b]
Ultrasonic-Assisted (UAE)	26.908 ± 0.018 [b]	0.031 ± 0.012 [ab]

Note: *Mean ± standard deviation (S.D.) within the column followed by different letters were significantly different by DNMRT at $P<0.05$.

DISCUSSION

Different extraction methods for phenolic compounds in longan seeds were evaluated, including HWE, EE and UAE. For extraction yield and time, HWE had the highest extraction yield, whereas the lowest extraction time was found with UAE. Mason et al. (1996) reported that the use of ultrasound significantly improved the solvent extraction of organic compounds in plants and seeds due to its mechanical effects. The effect of ultrasound theoretically provides a greater penetration of solvent into cellular materials and improves mass transfer. UAE was used to extract polyphenols from grape seed, and compared with conventional extraction methods (Porto et al., 2013). Porto et al. (2013) concluded that UAE could shorten extraction time and reduce consumption of extraction solvent. Moreover, Prasad et al. (2009a) reported that the extraction yield of longan pericarp extract by UAE was similar to conventional extractions, but needed a shorter time.

High performance chromatography (HPLC) has been used to determine phenolic compounds in cherry wines, green and black tea, and plant extracts, especially parts of longan (Rio et al., 2004; Rangkadilok et al., 2007; Sun et al., 2011). HPLC chromatrograms of the extracts in this study are similar to those of Rangkadilok et al. (2007), who reported that longan seed extracts contained the same major phenolic compounds at higher levels than those in pulp. In addition, the extraction methods affected these phenolic contents of longan seed extracts. Extraction by UAE produced the highest content of the compounds. The lowest content of compounds were found in longan seed extracted by EE. The content of gallic acid, corilagin and ellagic acid by HWE were higher than by EE due to multiple extraction and mechanical processes used.

Fruit seeds contain many phenolic compounds capable of protecting them from oxidative damage and defending them against yeast, fungi, virus and bacteria that might inhibit their germination (Soong and Barlow, 2005). These compounds also act as antioxidant agents. The antioxidant activities of longan seed extracts were correlated with their polyphenolic compounds content. The extracts contained high levels of polyphenolic compounds that were composed of one or more aromatic rings bearing one or more hydroxyl groups, thus they are capable of direct free radical scavenging and inactivation (Navipa, 2010). Our results show that HWE was an efficient method on extracting phenolic compounds in longan seed. The content is higher than the polyphenols content in Argentina green tea

(0.210–0.143 mg GAE/ml) (Anesini et al, 2008). Moreover, polyphenol concentrations in this study were comparable to a previous studied by He et al., (2009), who determined the total polyphenols in Chinese longan seeds.

To compare the effectiveness of the methods for extracting the phenolic compound from longan seed, free-radical scavenging activity evaluation is required. DPPH radical-scavenging activity has been extensively used to examine antioxidant capability in foods and plant extracts. The DPPH method uses a 2, 2-diphenyl-1-picrylhydrazyl solution as a stable radical to react with the extract or specific compounds. Color change from purple to yellow during the reaction influences its absorbance at the characteristic wavelength absorbs at 515 nm (Brand-Williams et al., 1995). In this study, longan seed extracts by HWE exhibited the highest antioxidant activity as indicated by the lowest seed extract concentration (IC_{50}).

CONCLUSION

The efficiency of the longan seed extraction method depends on extraction time, extraction yield, total polyphenol content, content of the major phenolic compounds and free radical scavenging ability. The highest content of total polyphenol and extraction yield was observed with the hot water extraction, whereas the opposite was observed for the ethanol extraction. The use of ultrasonic-assisted water extraction provided the highest content of gallic acid, corilagin and ellagic acid. In addition, the UAE method is eco-friendly due to reduced time of extraction and energy consumption. Therefore, extraction of phenolic compounds by UAE could be an alternative method for extractions of phytochemicals from longan seeds.

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