Ultrasound-Assisted Extraction of Phenolic Compounds from *Alpinia galanga* (L.) Willd. rhizome

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Summary

In this study, the main purpose is to determine the ultrasound-assisted extraction (UAE) conditions for extracting phenolic compounds obtained from *Alpinia galanga* (L.) Willd. rhizome. The influences of extraction factors (type of solvent, solvent concentration, solid-to-liquid (SL) ratio, time and temperature) on the total polyphenol content (TPC) and 2,2-diphenyl-1-picrylhydrazyl free radical scavenging capacity ($DPPH_{RSC}$) were investigated. The findings pointed out that all of these factors significantly affected TPC and $DPPH_{RSC}$. The highest TPC and $DPPH_{RSC}$ obtained from *A. galanga* root extract were 7.49 ± 0.19 mg GAE g^{-1} DM and 90.34 % at the acetone concentration of 60 % (*v/v*), SL ratio of 1:25, extraction time of 20 min and extraction temperature of 40°C. The microstructure of the sample before and after extraction significantly changed under ultrasound radiation.

Key words

extraction, galangal, polyphenols, rhizome, ultrasound

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Introduction

Alpinia galanga (L.) Willd. was commonly known as greater galangal and lesser galangal in English, respectively. It belongs to Zingiberaceae family (ginger) and is a perennial aromatic rhizomatous herb. This plant is widely distributed over Asia (Devi et al., 2018), especially Vietnam, Laos, Thailand and Malaysia. Although galangal rhizome is bitter, acrid and thermogenic, the local citizens in Vietnam widely used it as spices for flavoring food. In addition, dried rhizomes of galangal have diverse pharmacological properties and high medicinal values because of their rich source of bioactive compounds. Thus, they are important herbal drugs in the folk medicine systems of China and India. This plant is used to treat loss of appetite, upper abdominal pain and sluggish digestion. It combats inflammation and has stress-reducing properties, antiallergic/anticancer/hypolipidaemic activity, gastroprotective effect, etc. (Ravindran et al., 2012). Some studies recorded that this material contained many bioactive compounds, for instance, essential oil (Akhtar et al., 2010), flavonoids, tannins (Ravindran et al., 2012), etc. In there, phenolic compounds in galangal rhizome are useful for the human health but they have rarely been studied in many recent years.

In fact, many previous studies reported that phenolic compounds from herbal plants were extracted by different methods, for instance, Quoc and Muoi (2015) optimized the extraction parameters to extract the maximum TPC and AC from *Polygonum multiflorum* Thunb. roots by microwave-assisted extraction (MAE), Alessandro et al. (2012) extracted phenolic compounds by ultrasound-assisted extraction (UAE). Besides, other isolation methods such as Soxhlet, heated reflux extraction, maceration (Khoddami et al., 2013), enzyme-assisted extraction (MAE) (Quoc and Muoi, 2017), etc. were also used to recover these compounds from solid samples. Essentially, most of the extraction methods cause the potential environmental pollution, require large volumes of organic solvent, have low extraction yield and long extraction time, especially conventional methods (Dai and Mumper, 2010). Meanwhile, ultrasound-assisted extraction (UAE) is a new technique that has been developed in recent years, can overcome the mentioned obstacles and has many other advantages, for instance, the extraction system is simple and inexpensive. In addition, it is completely suitable for small and large scale in extraction factory (Lee and Lin, 2007).

In relation to galangal rhizome, there are few studies that determine bioactive compounds (Jaju et al., 2009; Devi et al., 2018). Moreover, using ultrasound to extract phenolic compounds from this material has not yet been well investigated. Thus, the most important aim of the present study is to clear up the influence of the major extraction factors (type of solvent, SL ratio, solvent concentration, extraction time and temperature) on the total polyphenol content (TPC) as well as antioxidant capacity (AC) ($DPPH_{RSC}$). At the same time, the obtained TPC and AC should be as high as possible.

Materials and Methods

Sample Preparation

A. galanga rhizome grown in the region of Lam Dong province (Vietnam) was used. The rhizomes were stored at room temperature (29 ± 2°C). No physical damage and no pest contamination of the plants were observed. Then, samples were cleaned under tap water and the outer skin was removed. After that, they were sliced and dried at 60°C for hours until the moisture was lower than 8%. The dried samples obtained were powdered by a grinder (Panasonic MX-V310KRA, China) for a few minutes to collect the specimens that could pass through a sieve (the holes' diameter size was 0.5 mm). Finally, dried galangal powder was vacuum-packed in PE bags and stored at room temperature.

Chemicals and Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu (FC) reagent and gallic acid were supplied by Sigma-Aldrich (Germany). All organic solvents and other chemicals were of the reagent class of analysis.

Ultrasound-assisted extraction (UAE) Methodology

Ultrasound-assisted extraction was performed in an Erlenmeyer flask (150 mL) kept in an ultrasonic bath (Bath power of 150 W, frequency of 40 kHz, Elma-S60H, Germany). The Erlenmeyer flask was placed at the depth of 2.5 cm above the bottom of the bath. The powder (2 g) was taken in the Erlenmeyer flask and the phenolic compounds from the sample were extracted using a series of extraction media (60% acetone, 60% ethanol and 60% methanol, v/v), various solvent concentrations (40% - 70%, v/v), SL ratios (1:15-1:30, w/v), extraction times (10-40 min) and temperatures (30-60 °C). The solvent was heated to the desired temperature and the ultrasonic bath temperature was also maintained through the time period of the extraction process. In order to avoid the loss of solvent, the Erlenmeyer flask was covered with a lid before it was placed in an ultrasonic bath. Then, the extract was filtered through Whatman filter paper (No. 4) to remove insoluble solid and then the clear filtrate made up 100 mL by adding extraction solvent. TPC and AC were determined.

Determination of Total Polyphenol Content (TPC)

Total polyphenol content (TPC) was determined according to the procedure of Siddiqua et al. (2010) with some minor modifications. Briefly, 0.3 mL of diluted extract reacted with 1.5 mL of 10% FC reagent for 4 min, then 2 mL of 7.5 % Na_2CO_3 solution was added to the mixture and made up 10 mL by adding distilled water. The mixture obtained was slightly shaken and kept for 1 hour in dark at room temperature. The absorbance of the solution was measured at 760 nm and TPC of samples was expressed as mg gallic acid equivalent per g dry matter (mg GAE g^{-1} DM).

Determination of Antioxidant Capacity (AC)

The antioxidant capacity (AC) was determined according to the procedure of Aguiar et al. (2020) with some slight changes. The amount of galangal powder extract (150 µL) was added to 2850 µL of a solution of DPPH in ethanol (10^{-4} M). The mixture was kept for 30 min in dark at room temperature. Ethanol DPPH solution without the sample was employed as a control. The absorbance of the solution was measured at 517 nm and the antioxidant capacity (DPPH radical scavenging capacity, $DPPH_{RSC}$) of the extract was calculated according to the following formula:

$$DPPH_{RSC}\ (\%) = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Scanning Electron Micrographs (SEM)

The structural morphology of the sample was determined by a scanning electron microscope (Hitachi S-4800, Japan) under a high vacuum condition and an accelerating voltage of 10 kV. SEM micrographs were undertaken at various magnifications.

Data Analysis

All experiments were carried out in triplicates and the results were expressed in the form of a mean ± standard deviation (SD). Statistical analysis was performed using the Statgraphics Centurion XV (version 15.1.02, Statgraphics Technologies, Inc., USA). The significance of differences was calculated using a degree of confidence of 95%.

Results and Discussion

Effect of the Type of Solvents on the TPC and AC of Galangal Extract

Table 1 shows the influence of different solvents on the polyphenols extraction yield while the other extraction factors (SL ratio of 1:25, extraction time of 20 min and extraction temperature of 40°C) remained unchanged. The obtained results indicated that the solvent dramatically affected TPC of the extract and the values of $DPPH_{RSC}$ were relatively equally represented in all samples. Totally, the aqueous acetone extract (60%, v/v) had the best results (TPC and $DPPH_{RSC}$ were 7.53 mg GAE g^{-1} DM and 91%, respectively), followed by 60% ethanol extract, while 60% metanol extract showed the lowest TPC. In fact, some previous documents reported that phenolic compounds were extracted by various solvents (water, ethanol, methanol, acetone, ethyl acetate, etc. or the mixture of solvent and water at different proportions) (Zhang et al., 2014). The difference in polarities of different solvents strongly influences the solubility of bioactive compounds in a sample and its extraction yield. Thus, the choice of an appropriate solvent system is one of the important steps in enhancing the recovery of antioxidant compounds from a sample (Sulaiman et al., 2011). In other words, the phenolic compounds extracted from galangal powder have the same polarity with the solvent used (aqueous acetone, 60%).

Table 1. Effect of type of solvents on the TPC and AC of galangal extract

Solvents	60% Ethanol	60% Methanol	60% Acetone
TPC (mg GAE g^{-1} DM)	7.19 ± 0.04^{b}	6.36 ± 0.03^{a}	7.53 ± 0.19^{c}
$DPPH_{RSC}$ (%)	91 ± 0.79^{a}	90.83 ± 0.47^{a}	90.79 ± 0.32^{a}

Different lowercase letters in the same row denote significant difference ($P < 0.05$) between various solvents according to confidence interval

The same solvent (acetone) was used by a previous study for the extraction of bioactive compounds from galangal (Akhtar et al., 2010) but the yield obtained was quite low, while Devi et al. (2018) used 60% aqueous methanol, 60% aqueous ethanol and distilled water to extract polyphenols from this material. However, TPC and $DPPH_{RSC}$ in their study were lower than those in our results for all rhizome, except for methanol extracts at an extract concentration of 18 mg/mL (($DPPH_{RSC}$ is 92.4%). This can be explained due to the different extraction methods and the source of initial materials. Thus, based on the results achieved, 60% aqueous acetone was selected for the next experiments in this study.

Effect of Acetone Concentrations on the TPC and AC of Galangal Extract

To determine the most appropriate acetone concentration to recover phenolic compounds, acetone concentrations ranging from 40% to 70% were investigated, whereas the SL ratio, the extraction time and temperature were kept the same, as shown in Table 2. An increase in acetone concentrations can lead to an increase in the TPC and $DPPH_{RSC}$ of the extract. For 60% aqueous acetone (*v/v*), the TPC and $DPPH_{RSC}$ peaked at 7.49 mg GAE g^{-1} DM and 90.41%, then they dropped quickly in the rest concentrations, especially TPC value.

The presence of some water in the solvent will result in the bulge of the sample by water, so the exposure of surface area between the sample matrix and the solvent was enhanced. Thus, the extraction efficiency was improved (Addai et al., 2013). In addition, adding water can change the polarities of the initial pure solvent. These combinations can improve the extraction of phenolic glycosides which are more water-soluble. However, if the ratio of water to solvent is too big, the extraction yield is not effective because water can dissolve many other components in samples, for instance, sugar, protein, other inorganic substances, etc. These components strongly affect the results obtained (Chirinos et al., 2007). This acetone concentration also is similar to that used to extract some other materials, for instance, *Polygonum multiflorum* Thunb. roots (Quoc and Muoi, 2016), *Styrax officinalis*, *Rosmarinus officinalis*, etc. (Proestos and Komaitis, 2008). Hence, a suitable acetone concentration of 60% was selected for the next step.

Effect of SL Ratios on the TPC and AC of Galangal Extract

The dried powder samples were extracted with various SL ratios (1:15, 1:20, 1:25 and 1:30) in order to select a proper SL ratio to enhance the extraction yield, while acetone concentration, extraction time and temperature were 60%, 20 min and 40 °C, respectively. The TPC and $DPPH_{RSC}$ increased as the amount of solvent increased. They significantly increased from 4.56 to 7.37 mg GAE g^{-1} DM for TPC and from 89.52 to 90.54% for $DPPH_{RSC}$, with the increase in SL ratios from 1:15 to 1:25, respectively. By increasing the SL ratios from 1:25 to 1:30, the TPC and $DPPH_{RSC}$ slightly decreased (Table 3).

Essentially, the effects of SL seemed to be the most influential factor in the polyphenols extraction from galangal powder. If the amount of solvent is too small, phenolic compounds in initial material cannot be completely extracted. If the amount of solvent is too big, it may promote the compounds which diffuse easily into the solvent and will cause high process costs (Cacace and Mazza, 2003). The amount of optimal solvent in this study (SL ratio of 1:25) is higher than that in the result of Meregalli et al. (2020), who extracted polyphenols from *Psidium cattleianum* Sabine peels by the conventional and UAE method (SL ratio of 1:10). It also is lower than that in the result of Quoc (2017), where the author

recovered polyphenols from banana seeds by UAE method (SL ratio of 1:50). The potential reasons for these different results are due to different materials or extraction methods. Consequently, the SL ratio of 1:25 was considered as suitable to gain maximum TPC and AC for the polyphenols extraction from galangal.

Effect of Extraction Temperatures on the TPC and AC of Galangal Extract

The influence of the temperature on TPC and $DPPH_{RSC}$ is shown in Table 4. The TPC and $DPPH_{RSC}$ at different temperatures have significant differences ($P < 0.05$). With an increase in extraction temperature from 30 to 40°C, the results showed a significant increase in TPC, whereas $DPPH_{RSC}$ was almost unchanged. As the temperature increased beyond this point (40°C), the yield reduced in all cases. At 40°C, the best TPC and $DPPH_{RSC}$ obtained were 7.52 mg GAE g^{-1} DM and 90.28%, respectively.

Basically, phenolic compounds, in this case, were quite sensitive to high temperatures and they were degraded at 50°C. The suitable extraction temperature (40°C) in this study is similar to that of Meregalli et al. (2020), who isolated bioactive compounds from red araca peel; and lower than that of Wang et al. (2008), where phenolic compounds were extracted from wheat bran by UAE method (60°C). According to the report of Wang et al. (2008), an increase in temperature can lead to a decrease in the viscosity of extract. Besides, the solubility of bioactive compounds in the sample could be enhanced, accelerating the whole extraction. Therefore, an extraction temperature of 40°C is the best choice for the next experiment.

Effect of Extraction Times on the TPC and AC of Galangal Extract

As well as the temperature, extraction time also affects the efficiency of the extraction process. The results shown in Table 5 have significant differences ($P < 0.05$) between the extraction periods. The TPC and $DPPH_{RSC}$ tended to increase as temperature increased ranging from 10 to 20 min. However, a further increase in the extraction time from 20 to 40 min led to a decrease in TPC and $DPPH_{RSC}$. In general, the TPC and $DPPH_{RSC}$ reached maximum values (7.49 mg GAE g^{-1} DM and 90.34 %) for 20 min under ultrasound radiation.

Table 2. Effect of acetone concentrations on the TPC and AC of galangal extract

Acetone concentrations (%)	40%	50%	60%	70%
TPC (mg GAE g^{-1} DM)	2.79 ± 0.03^{a}	6.01 ± 0.08^{b}	7.49 ± 0.06^{c}	5.64 ± 0.38^{b}
$DPPH_{RSC}$ (%)	88.42 ± 0.3^{a}	89.87 ± 0.5^{a}	90.41 ± 0.33^{b}	89.58 ± 1.32^{a}

Different lowercase letters in the same row denote significant difference ($P < 0.05$) between various solvent concentrations according to confidence interval

Table 3. Effect of SL ratios on the TPC and AC of galangal extract

SL ratio (g mL^{-1})	1:15	1:20	1:25	1:30
TPC (mg GAE g^{-1} DM)	4.65 ± 0.34^{a}	5.34 ± 0.03^{b}	7.37 ± 0.05^{d}	6.56 ± 0.04^{c}
$DPPH_{RSC}$ (%)	89.52 ± 0.2^{ab}	89.36 ± 0.3^{a}	90.54 ± 0.15^{c}	89.98 ± 0.35^{b}

Different lowercase letters in the same row denote significant difference ($P < 0.05$) between various SL ratios according to confidence interval

Table 4. Effect of extraction temperatures on the TPC and AC of galangal extract

Temperature (°C)	30	40	50	60
TPC (mg GAE g^{-1} DM)	4.51 ± 0.23^{a}	7.52 ± 0.21^{d}	6.45 ± 0.09^{c}	5.62 ± 0.27^{b}
$DPPH_{RSC}$ (%)	89.12 ± 0.45^{b}	90.28 ± 0.67^{b}	81.79 ± 1.46^{a}	80.06 ± 0.89^{a}

Different lowercase letters in the same row denote significant difference ($P < 0.05$) between various extraction temperatures according to confidence interval

Table 5. Effect of extraction times on the TPC and AC of galangal extract

Time (min)	10	20	30	40
TPC (mg GAE g^{-1} DM)	6.89 ± 0.08^{a}	7.49 ± 0.23^{b}	6.7 ± 0.2^{a}	6.66 ± 0.03^{a}
$DPPH_{RSC}$ (%)	84.36 ± 0.13^{a}	90.34 ± 0.4^{c}	87.49 ± 1.19^{b}	85.36 ± 0.27^{a}

Different lowercase letters in the same row denote significant difference ($P < 0.05$) between various extraction times according to confidence interval

Some previous reports proved the fact that, below a certain limit, long extraction time might enhance the extraction yield. However, beyond the limit, longer extraction time causes the degradation or transformation of phenolic compounds due to its prolonged exposure to temperature, light or oxygen in the ambient environment (Naczk and Shahidi, 2004). Compared to the other extraction method, extraction time in UAE was usually shorter than that in the conventional method (2 hours) (Meregalli et al., 2020) or pectinase-assisted extraction (80 min) (Quoc and Muoi, 2017) and longer than that in the MAE (5 min) (Švarc-Gajic et al., 2013). For the same extraction method (UAE), extraction time in this study (20 min) is quite close to that of 21 min from Liu et al. (2013) and 25 min from Cheok et al. (2013). This is also the optimum value for the extraction process.

Effect of UAE on Microstructure of Material

SEM analysis of galangal rhizome powder before and after extraction under ultrasound radiation studied morphological changes. As seen in Fig. 1a, the initial dried powder consists of many cell wall and starch. The surface of the cell wall is undisturbed and smooth in shape while the starch shape is long oval. Fig. 1b shows materials exposed to ultrasound radiation, the microstructure of the cell wall is disturbed, sticky and flaky in shape.

In addition, the sample after UAE treatment has many wrinkles on the surface. The ultrasound radiation can destroy the biological cell walls, facilitating the release of bioactive compounds (Khoddami et al., 2013). This also demonstrated that the microstructure of the sample was dramatically affected by UAE methodology. These findings in this study are similar to those in other studies, for instance, Zhang et al. (2013) and Quoc (2017) extracted phenolic compounds from Semen Astragali Complanati and banana seeds by UAE, respectively.

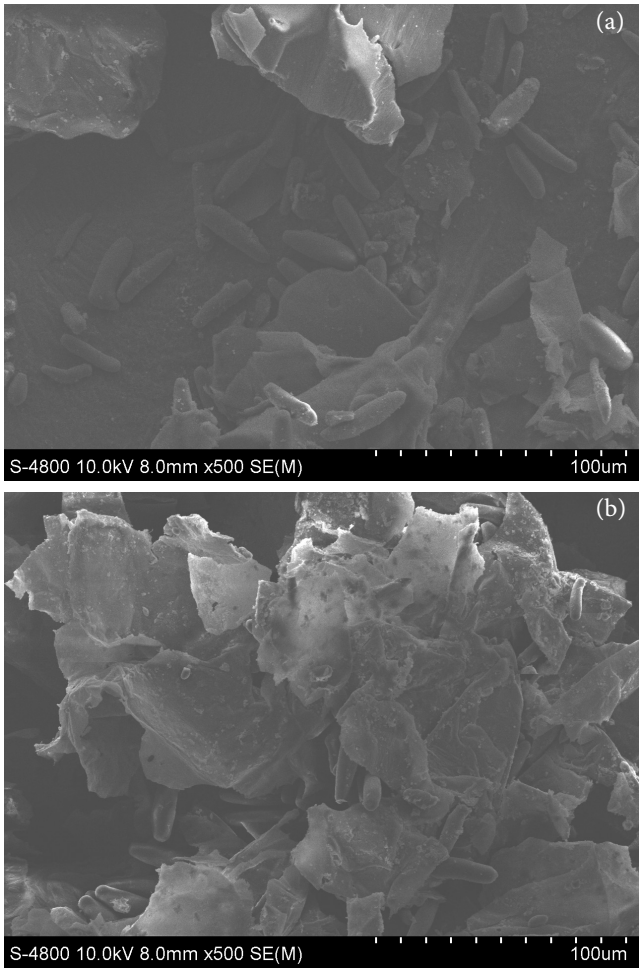

Figure 1. Scanning electron microscopy for samples before (a) and after (b) extraction under ultrasound radiation

Conclusions

In the present study, the ultrasound-assited extraction of phenolic compounds from *A. galanga* rhizome was investigated. Through the research results, all factors (type of solvent, SL ratio, solvent concentration, extraction time and temperature) strongly affected the polyphenol extraction process. The maximum values of TPC and $DPPH_{RSC}$ were obtained under the following extraction conditions: acetone concentration of 60% (*v/v*), SL ratio of 1:25 (w/v), extraction time of 20 min and extraction temperature of 40°C. The microstructure of the sample completely changed under ultrasound treatment.

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