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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM RED TURMERIC (*CURCUMA ZANTHORRHIZA)*

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Abstract: *The study was conducted on the basis of conditions of extraction factors including solvents, solvent concentration, solid/solvent ratio, time and extraction temperature which affect total polyphenol content and antioxidant activity of red turmeric (C. zanthorrhiza) extract. The yield of the polyphenols extraction process was determined by the total phenolic compounds (TPC) as well as the antioxidant activity (AC). Generally, the highest TPC and AC of ultrasound-assisted extraction are 130±0.51 mg GAE/g DW and 31.32±0.53 µmol Fe/g DW at the acetone concentration of 60% as the solvent, solid/solvent ratio of 1/35, extraction temperature of 40℃ for 20 minutes. The surface structure of solid before and after treatment changes significantly*

Key words: *Antioxidant, polyphenols, solvent, turmeric, ultrasound.*

1. Introduction

Red turmeric (*C. zanthorrhiza*) belongs to the ginger family (*Zingiberaceae*) and is harvested after about 8 months of cultivation, when the leaves turn yellow. Red turmeric rhizomes have a long cylindrical shape and highly branched,

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orange-brown peel with many burning dark brown. Aromatic rhizomes are found. Inside the turmeric rhizomes, it is an orange colour and a characteristic aroma [10]. They used fresh or boiled in water and dried, then they are ground into a deep orange-yellow powder widely used as flavouring and colouring agent in Vietnam and many other Asia countries. Turmeric also has many valuable ingredients for human health, especially phenolic compounds as curcumin.

Polyphenols are compounds whose molecules contain many benzene rings, including one, two or more hydroxyl groups. The number and characteristics of polyphenols structures are based on the physical, chemical and biological properties of compounds of this class. Polyphenols are found in fruits, berries and vegetables of brilliant colours. These compounds contribute to bitterness, astringent agent, flavour, aroma and stability agent in food [9]. In the food industry, phenolic compounds are also used in food packaging and coating edible films and are enhanced in food products.

Recently, new techniques such as convection extraction (CE), microwave assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), etc. have been used for the extraction of phenolic compounds from plants. Among all of these techniques, UAE was widely used to extract bioactive compounds from plant materials due to the high extraction efficiencies that can be achieved at relatively low temperatures [3]. Ultrasound-assisted extraction was a simple solution, friendly environment and an alternative efficient method to conventional extraction techniques [11]. This method increased the extraction productivity, faster kinetics and can be used with many different solvents [23].

Until now, no studies have presented the UAE method for the extraction of phenolic compounds from red turmeric in Vietnam and evaluate the effects of extraction factors on TPC and AC. Based on the above findings, this study investigated the effect of factors such as solvent, solvent concentration, solid/solvent ratio, temperature and extraction time on the extraction process from turmeric by the UAE method. This study was conducted to determine the TPC and AC of extract.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Red turmeric rhizomes (C. zanthorrhiza) were harvested from Lam Dong province (Vietnam) with the initial moisture content of 86.27±1.64%. Then, they was cut into many small slices from 0.5 to 1 mm thickness and dried at a 60°C within approximately 8 hours until the moisture is lower than 10%. The slices are ground into a fine powder (<0.5 mm), packed in vacuum and then stored in the dark at room condition (25°C) for further use.

2.2. Chemicals and Reagents

Folin-Ciocalteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent were purchased from Merck (Germany). All organic solvents and other chemicals were of analytical reagent grade.

2.3. Extraction Process of Polyphenols

The dried powder was extracted with

various solvents (distilled water, ethanol, methanol and acetone) in an ultrasonic bath (ELMA– S60H type, 37 kHz, 550W, Germany) with the condenser system. The parameter of extraction process contain of extraction times (10-30 minutes), extraction temperatures (30-70°C), the solid/solvent ratio (1/15-1/55, w/v) and solvent concentration (30%-70%, v/v). The mixture was filtered for removal of the residue by means of the vacuum filtration system, and then TPC and AC of extract were analyzed.

2.4. Determination of Total Polyphenol Content (TPC)

The TPC was determined and slightly modified by the Folin-Ciocalteu method with some slight modifications. The absorbance of the solution was measured at 738 nm and gallic acid was used as the standard. TPC were expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW) [19].

2.5. Determination of Antioxidant Capacity (AC)

The AC was determined and slightly modified by the Phen assay of the 1.10-phenanthroline solution in methanol. The reaction between Fe (II) and 1,10-phenanthroline forms complex orange-red complexes. AC was measured by a standard curve obtained at a wavelength of 510 nm. AC was expressed as µmol Fe per gram of dry weight (mmol Fe/g DW) [18], [21].

2.6. Scanning Electron Micrographs (SEM)

Solid powder before and after treatment was observed by SEM (Jeol/JSM-6480LV, Japan) to indicate changes of material in morphological characteristics at various magnification.

2.7. Data Analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean±standard deviation (SD).

3. Results and Discussions

3.1. Effect of Solvent Type on Extraction of Phenolic Compounds

In fact, there are many various organic solvents used in the extraction of phenolic compounds from plants depending on its different polarities, for instance, acetone, hexane, ethanol, methanol, deionized water, etc. In this case, there are only four aqueous solvents such as 50% ethanol, 50% methanol, 50% acetone and deionized water used to extract phenolic compounds from turmeric extract at extraction temperature of 50°C, solid/solvent ratio of 1/25 (w/v) for 20 minutes. The effect of solvent type on the TPC and the AC of extracts has a significant difference ($p<0.05$).

Table 1 showed that aqueous acetone solvents had the best extraction yield (including both of the TPC and AC), TPC and AC values peak at 106.10±9.46 GAE/g DW and 19.82±0.94 µmol Fe/g DW, respectively. While using distilled water for extraction obtained the lowest yield.

This can be explained that water can only dissolve highly-polar polyphenols [22] while aqueous acetone solution can dissolve many types of phenolic compounds mainly because its polarity is suitable to that of solvent. This shows that the combinations of organic solvents with water can improve the extraction yield of phenolic compounds [5].

Effect of solvents on TPC and AC Table 1

Solvents	Deionized water	50% Acetone	50% Ethanol	50% Methanol
TPC (mg GAE/g DW)	17.72±1.16[a]	106.10±9.46[b]	45.60±0.91[c]	38.19±2.49[c]
AC (µmol Fe/g DW)	2.97±0.12[a]	19.82±0.94[b]	9.87±0.58[c]	5.16±0.11[d]

Different lowercase letters in the same row indicate a statistically significant difference ($p<0.05$)

The result of this study is similar to many other studies, the authors extract the phenolic compounds from plants with aqueous acetone as the solvent, for instance, *Polygonum multiflorum* Thunb. roots [17], soybean [15] etc. The aqueous acetone is a good solvent for dissolving polar and non-polar polyphenols and some polyphenols have special linkage such as polyphenol-protein complexes, since they made the degradation of the polyphenol–protein complexes [1], [8]. Based on the above results, an aqueous acetone concentration of 50% was chosen for further experiments.

3.2. Effect of Solvent Concentration on Extraction of Phenolic Compounds

The effects of solvent concentration on the extraction of phenolic compounds were shown in Table 2 and they significantly affect TPC and AC ($p<0.05$). The TPC and AC values increase to 107.91 GAE/g DW and 29.44 µmol Fe/g DW at a solvent concentration of 60%, respectively. Then, the TPC and AC values decrease steadily during the rest of the scale. The acetone concentration of 40% had the minimum values (51.79±1.56 GAE/g DW for TPC and 8.33±0.52 µmol Fe/g DW for AC).

This can be explained that red turmeric contains many polyphenols with the polarity equivalent to the polarity of the solvent acetone of 60%, so they can dissolve better. Increasing the amount of water in the solvent (low solvent concentration) can increase the diffusion of water into the plant cells, the bioactive substances are easily transported into the solvent. Besides, a large amount of water can dissolve many different organic compounds such as sugars and proteins that can affect the accuracy of the measurement of TPC and AC [8]. Increasing the acetone concentration in solvents will reduce the polarization of the solvent. In addition, this can breakdown membranes of the cell, promotes diffusion abilities of solvent into a solid mixture [27]. However, the high acetone concentrations can cause protein denaturation, prevent the dissolution of polyphenols and then affect the extraction process [25]. With the goal of maximizing the ability to acquire compounds with antioxidant properties, acetone concentration of 60% was choices for further studies.

Effect of solvents concentration on TPC and AC Table 2

Solvent concentration [%]	40	50	60	70	80
TPC (mg GAE/g DW)	51.79±1.56^{a}	99.73±7.07^{c}	107.91±2.52^{d}	93.43±4.47^{bc}	89.12±3.73^{b}
AC (µmol Fe/g DW)	8.33±0.52^{a}	20.71±0.88^{b}	29.44±0.70^{d}	27.50±0.18^{c}	26.37±0.97^{c}

Different lowercase letters in the same row indicate a statistically significant difference ($p<0.05$)

3.3. Effect of Solid/Solvent Ratio on the Extraction of Phenolic Compounds

The solid/solvent ratio is one of the most important factors affected strongly the extraction yield. The results of these effects were shown in Table 3. There are the significant difference ($p<0.05$) between the solid/solvent ratios. At the solid/solvent ratio of 1/35, the maximum TPC and AC values are 127.39±3.81 mg GAE/g DW and 30.36±0.48 µmol Fe/g DW, respectively.

The optimum solid/solvent ratio in this study is similar to that of the study of Quoc and Muoi [17], they extracted phenolic compounds from *Polygonum multiflorum* Thunb. roots at the solid/solvent ratio of 1/30. The high solvent ratio increases the diffusion rate of solvents, enhances the ability of exposure to solid, dissolves the bioactive compounds into solvents, thus the extraction yield is improved. This process occurs continuously until the concentration equilibrium is reached [13]. However, the correlation between TPC and AC is quite complicated; it does not follow any rules. A high solid/solvent ratio is not enough to motivate the osmosis process, the solvent cannot completely extract phenolic compounds from materials. Besides, it will be quite difficult to filter if the viscosity of extract is high. On the contrary, a small solid/solvent ratio means the amount of solvent is higher and the dissolved oxygen into the solvent is larger. The presence of oxygen not only reduces TPC but also weakens the AC of polyphenols [14]. Therefore, the solid/solvent ratio of 1/35 is chosen to conduct the next experiments.

Effect of solid/solvent ratio on the TPC and AC Table 3

Solid/solvent ratio [w/v]	1/15	1/25	1/35	1/45	1/55
TPC (mg GAE/g DW)	87.02±3.18^{a}	108.84±2.54^{b}	127.39±3.81^{c}	108.66±2.02^{b}	84.29±1.77^{a}
AC (µmol Fe/g DW)	21.32±1.10^{b}	28.24±0.92^{c}	30.36±0.48^{d}	28.12±0.49^{c}	17.47±0.71^{a}

Different lowercase letters in the same row indicate a statistically significant difference ($p<0.05$)

3.4. Effect of Time on the Extraction of Phenolic Compounds

The timelines of the extraction process were investigated from 10 to 30 minutes.

Table 4 shows that the best TPC and AC values are 126.60±1.56 mg GAE/g DW and 30.45±0.55 µmol Fe/g DW at extraction time of 20 minutes, respectively. The results have a significant difference ($p<0.05$) between the various extraction times. The TPC and AC value tend to increase with increasing extraction time

from 10 to 20 minutes then reduces slowly during the rest of timelines.

In fact, it cannot completely extract polyphenols into the solvent in a short time. The bioactive ingredients do not have enough time to fully diffuse into the solvent. Conversely, the extraction time increases with increasing the polyphenols reduction because these compounds easily expose to unfavourable factors for a long time such as light, oxygen and temperature. Besides, it also takes time and does not bring economic efficiency. The optimal extraction time of this experiment was 20 minutes, lower than that of polyphenols extraction by the UAE methos from *Astragalus complanatus* R. Br. (30 minutes) [26] and higher than that of polyphenols extraction by MAE method from citrus mandarin peels (49 seconds) [12]. This can be explained that the different extraction time depend on many factor including the structure of material, type of phenolic compounds, type of solvent, extraction methods, etc. Based on the results obtained, the extraction time of 20 minutes is used to conduct the next experiments.

Table 4

Effect of the extraction time on the TPC and AC

Extraction time [min.]	10	15	20	25	30
TPC (mg GAE/g DW)	88.41±13.79^{a}	99.60±5.67^{a}	126.60±1.56^{b}	114.26±1.54^{b}	89.55± .89^{a}
AC (µmol Fe/g DW)	22.32±0.73^{a}	26.98±1.15^{b}	30.45±0.55^{d}	29.08±0.31^{c}	27.61±0.49^{b}

Different lowercase letters in the same row indicate a statistically significant difference ($p<0.05$)

3.5. Effects of Temperature on the Extraction of Phenolic Compounds

The effect of extraction time on TPC and the AC of extracts has a significant difference ($p<0.05$) and these results are shown in Table 5. The extraction yield reached the maximum values at 40°C, the TPC and AC values were 130±0.51 mg GAE/g DW and 31.32±0.53 µmol TE/g DW, respectively.

The best extraction temperature in this study is lower than that of other studies from various materials such as banana seeds (50°C) [16], *Polygonum multiflorum* Thunb. roots (60°C) [17], *Astragalus complanatus* R. Br. (50°C) [26]. Generally, the temperature has a positive effect on the extraction of polyphenols compounds from plants [20]. As extraction temperature increases from 30°C to 40°C, the TPC and AC values also increase. The high temperature extraction can reduce the viscosity of the solvent, promotes the diffusion of phenolic compounds [24], breaks polyphenols molecular bonding, affects the structure of cell walls [2]. In addition, it can open the cell matrix and easily release phenolic compounds into solvent [26]. However, the high extraction temperature could promote the decline or even decomposition of the remaining phenolic compounds in plant cells. In addition, it may enhance solvent losses through evaporation and augment the cost of the extraction process [6]. Therefore, the suitable extraction temperature was 40°C for the extraction process.

Table 5

Effect of the extraction temperature on the TPC and AC

Extraction temperature [^{o}C]	30	40	50	60	70
TPC (mg GAE/g DW)	107.65±4.19^{b}	130.±0.51^{c}	126.41±1.17^{c}	108.30±3.36^{b}	87.67±2.43^{a}
AC (µmol Fe/g DW)	25.89±0.29^{a}	31.32±0.53^{c}	28.97±1.21^{b}	26.19±0.41^{a}	25.86±0.06^{a}

Different lowercase letters in the same row indicate a statistically significant difference ($p<0.05$)

3.6. Effect of Ultrasound–Assisted Extraction on Structure of Material

The surface structures of the material before and after the treatment by the UAE method at the optimum conditions were examined by SEM and their micrographs are shown in Figure 1. The particles of the initial sample are not sticky and incoherent, whereas the structure of residue changes strongly, they are sticky, appear few creases and debris. This result is in agreement with the study of Quoc and Muoi [17], they extract polyphenols from *Polygonum multiflorum* Thunb. roots by the UAE methods because the localized temperature and pressure increase dramatically by the UAE-generated cavitation bubbles, the thin cell walls were broken and damaged. In addition, the ultrasound also breakdowns the cuticular layer [4, 7] leading to phenolic compounds was easily released into the solvent.

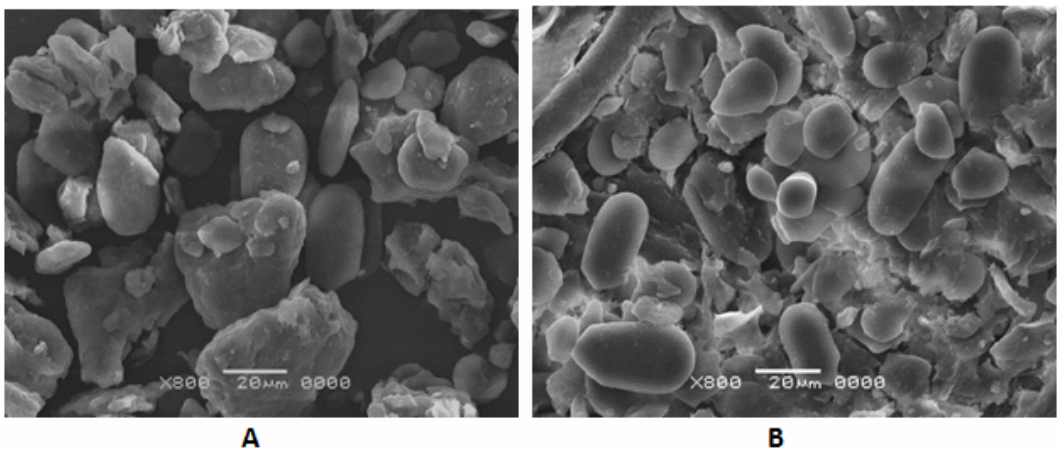

Fig. 1. *Structure of material before (A) and after (B) treatment by UAE*

4. Conclusion

Through the research results, all of the extracting factors affect the polyphenols extraction process including solvent type, solvent concentration, solid/solvent ratio, and extraction temperature and time. The best TPC and AC values obtained corresponding to the optimal extraction conditions such as acetone concentration of 60% (v/v), solid/solvent ratio of 1:35 (w/v), extraction temperature of 40°C and extraction time of 20 minutes. The UAE method affected strongly the surface structure of the material.

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