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Effects of Solvent and Time on Extraction of Bioactive Compounds from Cambodia Black Turmeric Using Ultrasound-Assisted Extraction

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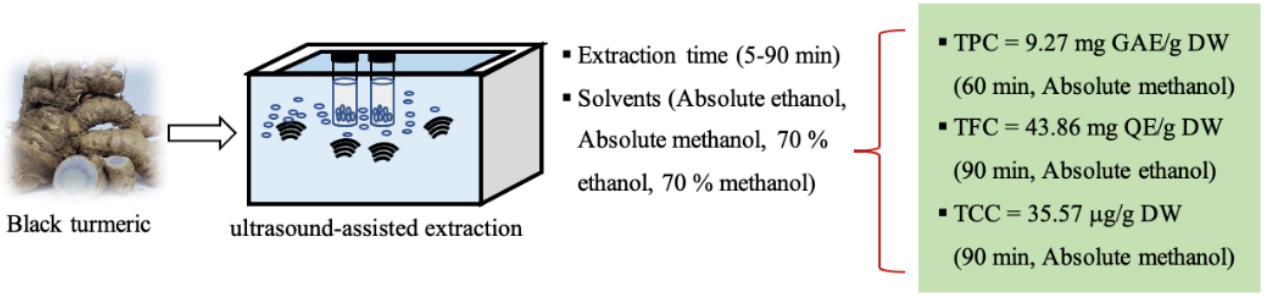
Abstract: *Herbs and spices are known to have high biological activities such as antimicrobial, antioxidant, and so on. However, it depends on the types of bioactive compounds present in the extracts. The quality of the extracts is strongly dependent on the extraction method and extraction conditions. In this study, we focused on the investigation of the effect of extraction time and solvent types on the extraction yield of bioactive compounds such as total phenolic contents (TPC), total flavonoid contents (TFC), and total curcuminoid contents (TCC) extracted from Cambodia black turmeric. The extractions were performed by ultrasound-assisted extraction method using different solvents (absolute methanol, absolute ethanol, aqueous methanol, and aqueous ethanol), and various extraction times (5 to 90 mins). Results from this experimental study indicated that these extraction parameters could affect the extraction yield of the bioactive compounds from black turmeric. Absolute ethanol reveals the best solvent for extraction of TFC (43.86 mg QE/ g DW), whereas absolute methanol is the best solvent for extractions of TPC (9.66 mg GAE/g DW) and TCC (35.57 µg/g DW). Regardless of the effect of solvent type, increasing extraction time leads to an increase in the extraction yield of bioactive compounds. However, in the case of TPC, the extraction yield tended to decrease at 90 min of extraction time, which could be linked to the degradation and condition of ultrasonic. The results in this study can be useful information for further improving ultrasound-assisted extraction conditions for black turmeric and other plant materials to obtain a high yield of bioactive compounds.*

Keywords: Extraction solvent; Extraction time; Bioactive compounds; Black turmeric; Ultrasound-assisted extraction

Graphical abstract:



1. INTRODUCTION

Extraction of bioactive compounds has been done by conventional methods including maceration, solvent extraction, Soxhlet extractions, and so on. However, these methods have some challenges such as longer extraction time, lower efficiency, a large amount of toxic solvent used, and high process costs for some cases [1]. To overcome these limitations, it is necessary to find a better extraction method to enhance extraction yields. Emerging new and developed extraction technologies could be

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an option to improve extraction efficiency, reduce time consumption, and save energy [2].

These developed extraction methods included ultrasound-assisted, supercritical fluids, microwave-assisted, and subcritical water extractions. Among these extraction methods, ultrasound-assisted extraction (UAE) has been widely known as one of the extraction methods which is possible to decrease extraction time, extraction temperature, and solvent consumption, compared to the conventional extraction methods. The main principle of ultrasound-assisted extraction is the cavitation phenomenon. This phenomenon creates a fast order of alternating compression and expansion waves close to the solid matrix surface [2]. Moreover, the decompression from cavitation increases the formation of large air bubbles that ultimately collapse and implode which releases the accumulated energy in the form of waves. The mechanisms influence the solid sample tissues because of the formation of microscopic channels which facilitate solvent penetration, promote the release of the desired compounds from the solid tissues. On the other hand, the intensity of cavitation and the extraction process mainly rely on different factors such as the physical properties of solvents, ultrasonic power, extraction temperature, and extraction time.

From this point of view, the selection of solvent is important for the extraction of bioactive compounds from solid matrices. Solvent choice in UAE is driven by the solubility of the target metabolites and physicochemical properties of the solvents such as viscosity, surface tension, and vapor pressure [1]. Those physicochemical properties influence the acoustic cavitation phenomenon and more specifically cavitation threshold. An increase of viscosity, or surface tension, led to an increase in these molecular interactions, thereby significantly raising the cavitation threshold. Moreover, a solvent with low vapor pressure is preferred in UAE, as the collapse of cavitation bubbles is more intense compared to solvents with high vapor pressure. However, vapor pressure depends on the temperature in the liquid medium because at higher temperature led to decrease viscosity and surface tension, facilitating a rapid penetration of solvent into solute that releases the target compounds [1]. Besides, the extraction time is also considered an important parameter that can reduce the energy cost. However, extending the extraction time led to an increase in the oxidization of bioactive compounds as well as degradation [3]. Therefore, the effect of extraction time in the extraction should be investigated to preserve the high extraction yield of bioactive compounds.

Black turmeric has an abundant source of phytochemical compounds existing with huge biological activities such as antioxidant, antibacterial, and antifungal [4]. Correspondingly, phenolic compounds or polyphenols (phenolic acids, flavonoids, curcuminoids, and) have been paid considerable attention and conducted more research to seek its natural antioxidants. Bioactive compounds such as phenolic acids and flavonoids are the dominant groups in plants which have been increasing interest by many researchers due to free radical scavenging properties. These bioactive compounds vary in type, number, and position of functional groups, resulting in variations in chemical properties which can influence the solubility of these compounds in different solutions.

Ultrasound-assisted extraction of bioactive compounds from herbs and spices has been acquired many interests from many researchers [1]. Meanwhile, extraction of phenolic compounds from various species of *Curcuma* by ultrasound-assisted extraction has been done by several studies. However, literature reporting about the extraction of bioactive compounds from black turmeric (*Curcuma caesia*) is still limited. Moreover, scientific research on the extraction of bioactive compounds such as total phenolic compounds, total flavonoids, and total curcuminoids from Cambodia black turmeric (Neang Kong) has not been conducted yet.

Therefore, this work is the first to study the extraction of bioactive compounds from Cambodia black turmeric. The investigation of the effects of solvent type and extraction time on the extraction yield of bioactive compounds (total phenolic contents, total flavonoids contents, and total curcuminoid contents) are specifically evaluated in this study.

2. METHODOLOGY

2.1 Chemicals

All chemicals used in this study were analytical grade. Folin-Ciocalteu's reagent and Quercetin (> 95%) were obtained from Sigma Aldrich, (Switzerland). Gallic acid (> 98%) was bought from Himedia, (India). Sodium carbonate was purchased from Merck (Denmark). Aluminum chloride was obtained from Acros, (Germany). Sodium Nitrite was purchased from Fisher Scientific. Sodium hydroxide was bought from Merck, (Germany). Ethanol and methanol were acquired from Merck, (Germany). Properties of solvents used in this study such as ethanol, methanol, and water are shown in Table 1.

2.2 Sample preparation

The Black turmeric (Khmer cultivar) was collected from Phnom Kulen, Siem Reap province in February 2021. Black turmeric was peeled and washed with distilled water. Then, it was sliced and dried using an oven dryer for 16 hours at 50 °C. The dried sample (Moisture 10.18 ± 0.55%) was stored in the Ziplock plastic and kept in the refrigerator. Subsequently, the dried black turmeric sample was ground and sieved then brought toward the extraction process.

2.3 Ultrasound-assisted extraction

Fig. 1 shows the extraction procedure of ultrasound-assisted extraction (UAE) in this study. Black turmeric powder (0.5 g) was mixed with 10 ml of extraction solvent using a vortex mixer. UAE was carried out at a frequency of 40 kHz based on the study of Chemat et al. [1],

Table 1 Properties of solvents at 25 °C used in this study

Solvents	Dielectric constant[1]	Dissipation factor[1]	Density [Kg. m^{-3}]*	Viscosity [Pa.S]*	Molecular weight [g. mol^{-1}]*	Boiling points [°C]*
Water	78.3	1570×10^{-4}	1000	0.89	18.01	100
Ethanol	24.3	2500×10^{-4}	789	0.69	46.07	78
Methanol	32.0	6400×10^{-4}	792	0.54	32.04	65

[1]Veggi et al. [5]; * NIST database

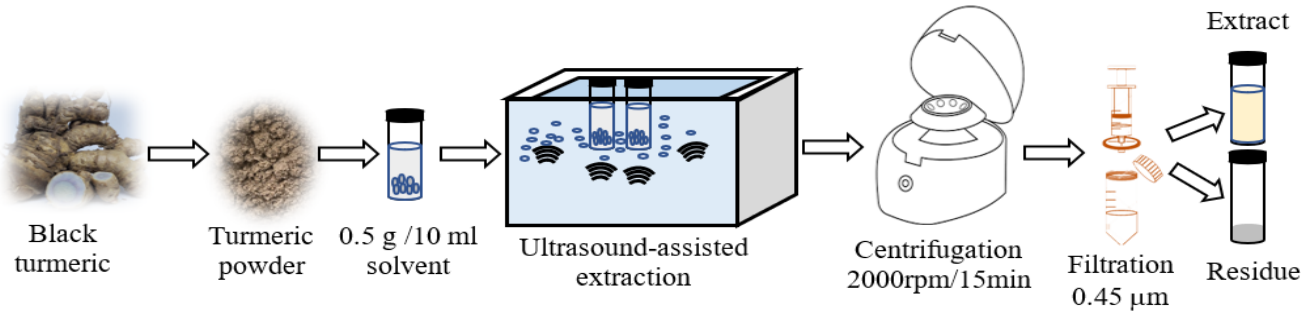

Fig. 1. Schematic diagram presenting extraction procedure in this study

at temperature of 50 °C, and different extraction times of 5 to 90 min. The summary of extraction conditions used in this study for investigating the effect of solvent type and extraction time is given in Table 2.

Table 2 Experimental conditions designed in this study

Investigation parameters	Fixed conditions
Extraction time (5, 10, 20, 35, 45, 60 to 90 min)	Extraction temperature: 50 °C, solid-to-solvent ratio: 0.5 g / 10 ml, absolute methanol, absolute ethanol
Solvents (absolute ethanol, absolute methanol, 70 % ethanol, and 70 % methanol)	Extraction time: 30 min, Temperature: 50 °C, Solid-to-solvent ratio: 0.5 g/ 10 ml

The test tubes containing samples and solvents were placed in the ultrasonic water bath (Diahan Scientific, South Korea) at the designated condition. After extraction, the suspension was centrifuged (Rotofix 32A, Germany) at 2000 rpm for 15 min. The clear supernatant was filtered by a syringe nylon filter with a diameter of 0.45 µm. The extract was placed in an amber bottle and stored in the refrigerator for further analysis. The experiments were duplicated runs which is enough to get the standard error and evaluate the effect of the investigated parameters.

2.4 Analysis of extracts

2.4.1. Analysis of total phenolic contents

Total phenolic contents (TPC) were analyzed by Folin-Ciocalteu assay with some modification from Sepahpour et al. [6]. Gallic acid was used as a standard solution and the calibration curve was conducted by different diluted concentrations from the stock solution of 1 mg/g. To determine the total phenolic content, a volume of 0.5 ml of extracts or standard solution of gallic acid were mixed with 2.5 ml Folin-Ciocalteu's reagent (FCR) diluted at 1:10 v/v, then after 5 minutes, 2 ml of 7.5 % sodium carbonate solution was added and homogenized using Voltex mixer for around 30 second. Then, the solution was allowed to stand for 1h at room temperature and dark place. The absorbance of the sample was measured against the blank at 765 nm using a UV spectrophotometer (UV-1280, Shimadzu, Japan). The total phenolic content was expressed as milligram of Gallic acid equivalent per gram of dry weight sample (mg GAE/g DW).

2.4.2 Analysis of total flavonoids contents

Total flavonoid contents (TFC) were analyzed by the Aluminum chloride colorimetric assay with slight modification [7]. Quercetin was applied as a standard solution and the calibration curve was conducted by different diluted concentrations from the stock solution of 1 mg/g. To measure the total flavonoid contents, a volume of 0.5 ml of extracts or standard solution of quercetin were mixed with 2 ml of distilled water and 0.15 ml of 5 % $NaNO_3$ was added, then it was kept 5 minutes in a dark place, 0.15 ml of 10 % $AlCl_3$ was added. After that it was kept for another 5 minutes, 1 ml of 1M NaOH was

added, and the volume was made up to 5 ml by distilled water (1.2 ml). The absorbance of the sample was measured against the blank at 415 nm using a UV spectrophotometer (UV-1280, Shimadzu, Japan). The total flavonoids content was expressed as milligram of Quercetin Equivalent per gram of dry weight sample (mg QE/ g DW).

2.4.3 Analysis of total curcuminoid contents

To determine total curcuminoid contents (TCC), approximately 3 ml of extract was taken to analyze by UV spectrophotometer (UV-1280, Shimadzu, Japan). Curcumin was utilized as a standard solution and the calibration curve was conducted by various diluted concentrations from the stock solution of 60 µg/g. The absorbance of the sample was measured against the blank at 424 nm. The total curcuminoid content was expressed as microgram per gram of dry weight sample (µg/g).

2.4.4 Calculation of bioactive compounds yield

To compute the yield of bioactive compounds (total phenolic contents (TPC), total flavonoid contents (TFC) and total curcuminoid contents (TCC) is used the equation below:

$$Y_i = (C_i \times m_{Ext} \times D)/(m_s \times 1000) \quad \text{(Eq. 1)}$$

Where:

Y_i = Yield of TPC, TFC and TCC
C_i = Concentration of TPC, TFC and TCC (µg/g)
m_{Ext} = Mass of extract (g)
D = Dilution factor
m_s = Mass of sample (g)
1000 onverted factor from µg to mg.

3. RESULTS AND DISCUSSION

In this study, the effects of solvent types and extraction time on extraction yields of bioactive compounds such as total phenolic contents, total flavonoid contents, and total curcuminoid contents were investigated. The experimental results presenting the effect of those extraction parameters are discussed in the following sections.

3.1 Effect of solvent types

3.1.1 Total phenolic and total curcuminoids contents

As presented in Fig. 2 and 3, the yield of total phenolic contents (TPC) and total curcuminoid contents (TCC) extracted from black turmeric were obtained the highest yield when absolute methanol was used for the extraction (9.66 mg GAE/g DW for TPC and 35.57 µg/g for TCC), followed by absolute ethanol (5.93 mg GAE/g DW for TPC and 29.85 µg/g for TCC). However, the yields of TPC and TCC extracted by aqueous ethanol (70 % ethanol) were 4.38 mg GAE/g DW and 8.16 µg/g, respectively, and 4.47 mg GAE/g DW and 6.26 µg/g, respectively for aqueous methanol (70 % methanol). These results indicated that methanol could extract a high amount of phenolic acids and curcuminoids compared to ethanol which is probably due to the high polarity index of methanol.

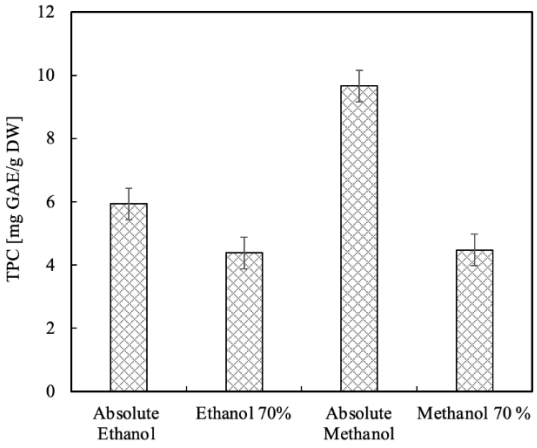

Fig. 2. Effect of solvent types on the extraction yield of total phenolic contents (TPC)

According to the theory of similarity and inter-miscibility states that when the solute and the solvent have similar polarity, the solute will be dissolved in the solvent with greater ease [8]. It may be because of the high polarity of phenolic acids and curcuminoids in the black turmeric are high polar compounds that require high polar solvents to extract them. Moreover, the presence of water in either aqueous methanol or aqueous ethanol increases the polarity of the solvent mixture leading to reducing the yield of bioactive compounds.

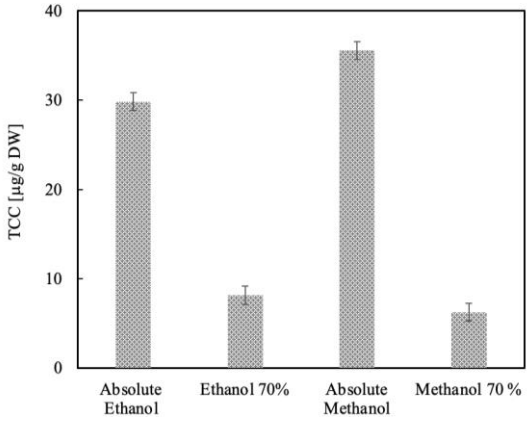

Fig. 3. Effect of solvent types on the extraction yield of total curcuminoid contents (TCC)

3.1.2 Total flavonoids contents

In this work, the extraction yield of total flavonoid contents (TFC) extracted from Cambodia black turmeric using different solvents presents in Fig. 4. The effect of solvent on the yield of TFC is in contrast with the case of TPC.

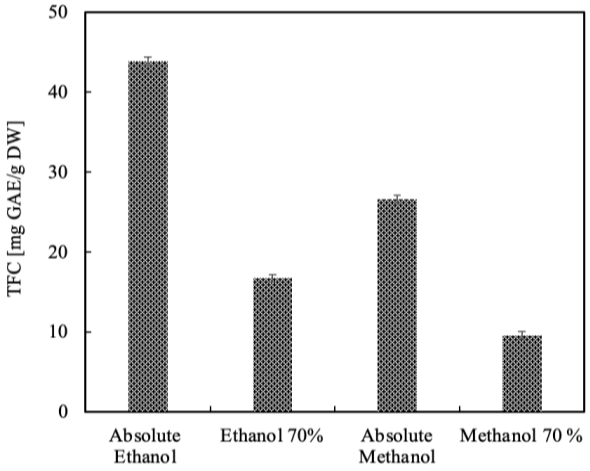

Fig. 4. Effect of solvent types on the extraction yield of total flavonoid contents (TFC)

As seen in Fig. 4, it is observed that using absolute ethanol as the solvent resulted in the highest yield of TFC (43.86 mg QE/g DW) compared to other solvents used in this study. In the case of aqueous alcohols, it is also found that using aqueous ethanol (70 % ethanol) led to providing an extraction yield of TFC higher than that using aqueous methanol. These results may attribute to a good affinity of ethanol with flavonoids compounds presented in the black turmeric. Based on the physical property of the solvents given in Table 1, it should be noticed that the polarity of ethanol is lower than methanol, and flavonoids molecules are generally less polar compared to phenolic acids molecules. Therefore, the extraction yield of TFC obtained from ethanolic extracts was higher than those obtained from methanolic extracts, based on the polar-like-polar principle [9].

3.2 Effect of extraction time

3.2.1. Total phenolic contents

Fig. 5 shows the effect of extraction time on the yield of TPC from black turmeric using absolute methanol and ethanol by UAE. As seen the Fig. 5, the yield of TPC increased from 5.54 mg GAE/g DW to 9.27 mg GAE/g DW for methanol, and it increased from 3.41 mg GAE/g DW to 7.26 mg GAE DW for ethanol, with increasing extraction time from 5 min to 60 min. This trend may attribute to expanding extraction time provided enough time for the solvent to contact with the sample, and then it can remove much amount of the solutes from the cell of black turmeric leaching into the solvents.

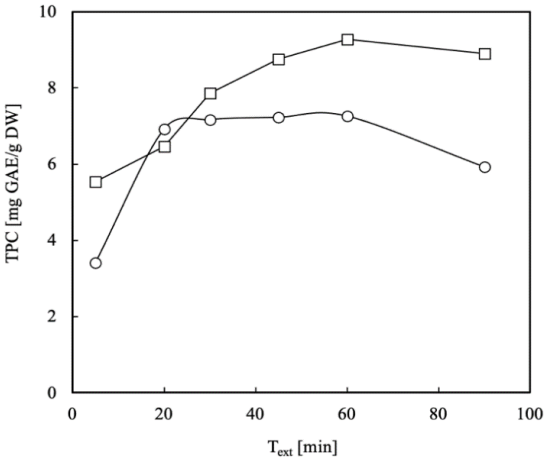

Fig. 5. Effect of extraction time on the extraction yield of total phenolic contents (TPC); □ Methanol and ○ Ethanol

However, there was a reduction in the yield of TPC (8.90 mg GAE/g DW) with prolonging extraction time to 90 min. This phenomenon can be explained that longer extraction may cause the decomposition of phenolic compounds [3]. This study obtained TPC yield higher than the study of Devi et al. [10] which total phenolic content was found to be 0.68 mg GAE/g DW. The results indicated that ultrasound-assisted extraction provided better extraction yield and consumed shorter extraction time than Soxhlet extraction that used longer extraction time from 12 h to 14 h in the study of Devi et al. [10].

3.2.2 Total flavonoid and total curcuminoid contents

In this study, the effect of extraction time on the yield of TFC and TCC from black turmeric using absolute methanol and ethanol as extraction solvents by UAE is shown in Fig. 6 and 7, respectively. It is observed that either using methanol or ethanol as the extraction solvents, increasing extraction time from 5 to 90 min led to an increase in the extraction yield of TFC and TCC.

In common principle, extraction time is the contact duration between sample and solvent in which longer time means longer contact period of solutes and solvent. The extraction time generally can affect the mass transfer rate between solvent and sample. Longer extraction time can allow the complete mass transfer resulting in the increase of extraction yield. However, if we compared the results at 60 to 90 min of extraction time, the trend of the yield of TFC and TCC is opposite from that in the case of TPC. This result probably means that the mass transfer between flavonoids molecules, solid matrix, and solvent requires extraction time longer than that between phenolic acids molecules, solid matrix, and solvent. However, further experiments on optimization can be done to confirm the mechanism.

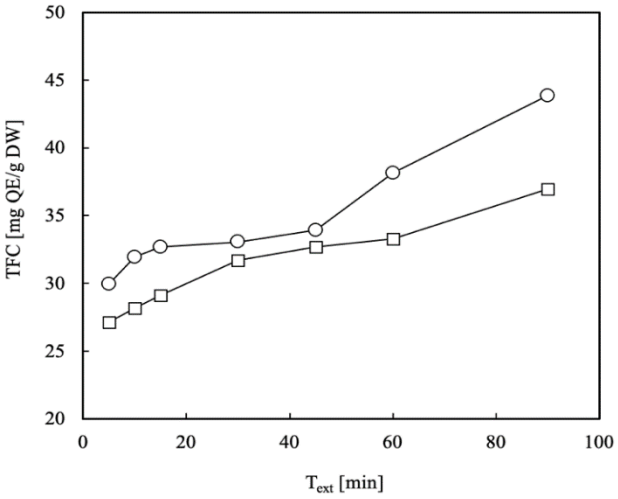

Fig. 6. Effect of extraction time on the extraction yield of total flavonoid contents (TFC); • Methanol and ○ Ethanol

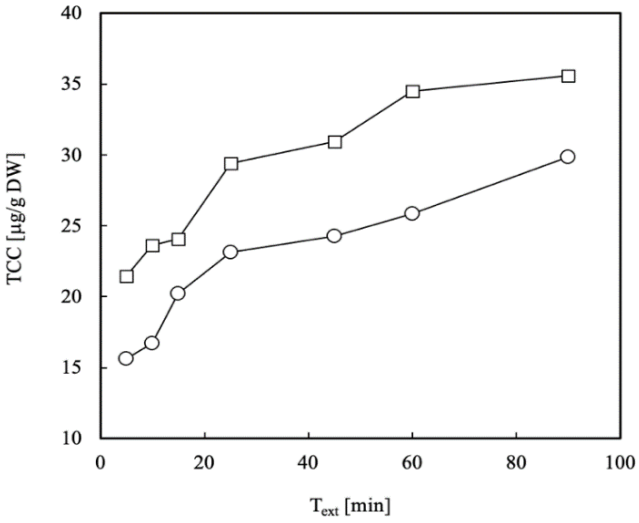

Fig. 7. Effect of extraction time on the extraction yield of total curcumin contents (TCC); • Methanol and ○ Ethanol

2. CONCLUSIONS

Ultrasound-assisted extraction (UAE) was applied to extract bioactive compounds from Cambodia black turmeric. The effect of extraction parameters such as solvent type and extraction time on the yield of total phenolic contents (TPC), total flavonoids contents, and total curcuminoids contents (TCC) was examined. As result, the yield of TPC extracted from black turmeric was 9.27 mg GAE/g DW using methanol as solvent and an extraction time of 60 min. Similarly, the yield of TCC was highest (35.57 µg/g DW) at extraction time 90 min using methanol as the extraction solvent. Whereas, using ethanol and extraction time of 90 min resulted in the highest yield of TFC (43.86 mg QE/g DW) extracted from the black turmeric. Since extraction efficiency links to many factors, further research on optimization of UAE conditions for bioactive compounds from Cambodia black turmeric should be conducted to find the optimum condition of UAE parameters to get the maximum yield of bioactive compounds. Moreover, Ethanol is recommended to use in the extraction of bioactive compounds from plants in terms of environmental-friendly and it also provides a similar extraction yield of bioactive compounds for the case of black turmeric.

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