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ULTRASOUND-ASSISTED EXTRACTION (UAE) OF PHYTOCHEMICALS WITH RESPONSE SURFACE METHODOLOGY(RSM) IN *CURCUMA XANTHORRHIZA*

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Peer-review under responsibility of 4th Asia International Multidisciplinary Conference 2020 Scientific Committee
http://connectingasia.org/scientific-committee/

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Abstract

The Curcuma genus belongs to the Zingiberaceae family is commonly known for its usage as spice and medicine.it is widely used in pharmaceutical, food supplement, cosmetic industries lead to economic benefits. Recently, noticing to an eco-friendly extraction approach which provides the extraction efficiency is considered that in the extraction process, exploring the optimum condition of extraction parameters. This study is performed to improve the bioactive compounds extraction from C. xanthorrhiza by ultrasound-assisted extraction (UAE) through Response Surface Methodology (RSM). Three independent variables: extraction time (5-20 min), temperature (30-50° C) and liquid-solid ratio (6-10 mL / g) are applied using Box-Behnken (BBD) design. Quantification of curcumin and xanthorrhizol are carried out using reversed-phase high-performance liquid chromatography (RP HPLC) equiped by a photodiode array detector. Extraction time 20 minutes, temperature of 50°C, and LS ratio 8 mL/g are found as the optimum condition of extraction yield and quantification of xanthorrhizol. Curcumin 's optimum state, however, is observed at extraction time 12.50 minutes, temperature 30 ° C, and liquid-solid ratio of 10 mL / g. This study suggests that there are favorable potential using method of ultrasound-assisted extraction under specific parameters in the extraction process which is advantageous for advanced research.

Research Highlights

This study conducted on optimization procedure of the Ultrasound-Assisted Extraction (UAE) components extracted from C. xanthorrhiza by the Response Surface Methodology (RSM). In the best of my knowledge in this study, the desirability of the effect of parameters on the yield responses, the quantification of phenolic and sesquiterpenoid compounds, were established. Consequently, the highest percentage of yield (72.20 %) and the concentration of xanthorrhizol (85.68 % in %w/w) were obtained at the extraction time of 20 mins, temperature of 50°C, and 8 mL/g LS ratio. Nevertheless, a large difference in curcumin concentration (39.73 % in % w / w) with extraction temperature of 30° C, 12.50 minute and 10 mL/g LS ratio has been observed. The experimental values have been displayed closed values with the predicted values. The determination coefficient (R^2) of extraction yield, xanthorrhizol, curcumin [4] was 0.9990, 0.9986, and 0.9993 respectively. The analysis of variance (ANOVA) showed a significant statistical and model fitting of the quadratic model. From the numerical data, the result suggested the RSM method's effectiveness in optimizing phytochemical compounds of C. xanthorrhiza [3]. This research has been shown that the considerable potential to be utilized in the extraction under specific parameters can be obtained via ultrasound-assisted extraction. This extraction method can also enhance the efficacy impact on the yield and quantification of phytochemical compounds.

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Graphical Abstract

Experimental Design

1 g of C. xanthorrhiza + 6, 8, 10 ml\L methanol solvent

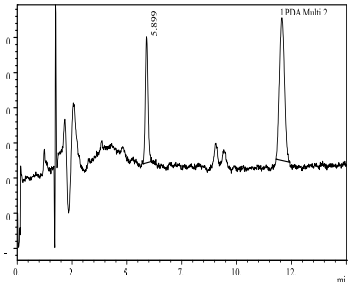
Ultrasonic bath
Time; 5 min-20 min
Temperature: 30 ºC -50ºC
Liquid-solia ratio: 6 mL/g-10 mL/g

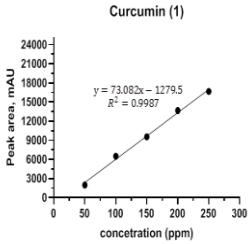
Filtration

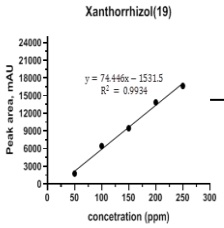
HPLC

51 vials collected

Vacuum Rotary Evaporator







Verified the Results by ANOVA

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Research Objectives

The objectives of this research are:

a. To optimize the extraction parameters from *Curcuma xanthorrhiza* via Ultrasound-Assisted Extraction (UAE) using Response Surface Methodology (RSM).

b. To quantify the presence of yield, xanthorrhizol**,** and curcuminof the extract from *Curcuma xanthorrhiza* via RP HPLC-PAD.

c. To verify obtained result from percentage yield, and quantification of xanthorrhizol**,** and curcumin of the extract from *Curcuma xanthorrhiza* by ANOVA.

Significance of this Research are:

This study provides a simple method regarding Ultrasound-Assisted Extraction (UAE) optimization for the extraction of phytochemicals from the rhizome of *C. xanthorrhiza* and extraction of essential oil from fresh rhizomes of *C. xanthorrhiza* [1]. Indeed, with the growth of "Green Chemistry" over the past few years, environmentally friendly approaches are becoming ever more desirable. Ultrasonically assisted extraction of bioactive compounds is regarded as important extraction methods that can highly provide reproducibility in a shorter period, easier handling, decreased solvent consumption and temperature, and lower energy intake.

Nevertheless, the economic viability of an industrial procedure often demands work to obtain high extraction quality which is achieved to affect the output of extraction. Several factors have been recently identified to affect the extraction efficiency, including extraction time, extraction approaches, extraction temperature, the form of solvent, and solvent concentration. Response surface methodology (RMS) which has been used in this study allows the evaluation of the effects of variables on response variables and their interactions [2].

Moreover, in terms of the percentage of yield, this study can provide the desired outcome to give high quality of the final product and preserves the amount of nutrient content in the plants via optimization of the extraction method [5]. Besides, belonging to the standardization of extraction able to apply, this leads to raising the production supplement of food from natural resources. Economically, increasing demand from pharmaceutical industrials such as cosmetics and medicines can accelerate the production using the standard operating procedure of extraction.

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Methodology

Experimental Procedure

Ultrasonic cleaning bath equiped via a power of 750 W and a incidence of 60 kHz, armed with time and temperature regulator was used. The extraction of *C. xanthorrhiza* was performed by 17 runs. Powder of C. xanthorrhiza rhizome (1 g) has been weighed into a conical flask containing various liquid-solid ratio which was 6, 8, and 10 mL/g respectively. The conical flask was put in a medium frequency ultrasonic bath at 30° C to 50°C for duration of 5 to 20 minutes' extraction time. The flask containing xtract was filtered and concentrated with vacuum rotary evaporator to achieve a waxy crude extract of *C. xanthorrhiza*. The extracts were kept in a refrigerator until further use. All trials were carried out in triplicate.

HPLC Analysis of Phytochemicals

For further examination, an RP-HPLC Agilent Series 20 equipped with a Photo Detector Array (PDA) with the autosampler was used. This instrument was used to analyze and identify the presence of the phytochemical compounds. Separation of phytochemical compounds was carried out on a reversed-phase HPLC, Tokyo, Japan model:(Shimadzu – Nexera LC-20 ADXR) instrument which armed with Luna® column (description: Luna® 5µm C18(2) 100 Å), (Size: LC Column 150 × 4.6 mm) and UV flash as a detector. The elution solvents were methanol and water as solvent A and solvent B respectively with the flow rate at 1 mL/min. 10 µl was used for injection volume. The compounds were described by comparing the observed retention times at 270 nm (0 – 7 min) and 270 nm (7 – 15 min) to those of the reference standards.

Results

This study conducted on optimization procedure of components extracted from *C. xanthorrhiza* using the Ultrasound-Assisted Extraction (UAE) via the Response Surface Methodology (RSM). In the best of my knowledge in this study, the desirability of the impact of parameters on the extraction yield responses, the quantification of phenolic and sesquiterpenoid compounds, were established. Consequently, the highest percentage of yield (72.20 %) and the concentration of xanthorrhizol (85.68 % in %w/w) were obtained at the extraction time of 20 minutes, temperature of 50°C, and 8 mL/g LS ratio. Nevertheless, a large difference in curcumin concentration (39.73 % in % w / w) with extraction temperature of 30 ° C, 12.50 minute and 10 mL/g LS ratio has been observed. The experimental values have been displayed closed values with the predicted values. The determination coefficient (R^2) of extraction yield, xanthorrhizol, curcumin was 0.9990, 0.9986, and 0.9993 respectively. The analysis of variance (ANOVA) showed a significant statistical and model fitting of the quadratic model. From the

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numerical data, the result suggested the RSM method's effectiveness in optimizing phytochemical compounds of C. xanthorrhiza. This research has been shown that the ultrasound-assisted extraction approach is considered to have favorable potential to be implemented under different conditions in the extraction process. This extraction method can also enhance the efficacy impact on the yield and quantification of phytochemical compounds.

Findings

This study was done to find optimum condition of extraction parameters from *C. xanthorrhiza* via Ultrasound-Assisted Extraction (UAE) using Response Surface Methodology (RSM). The 17 experiments are developed by three independent variables: temperature (30, 40, 50°C), time (5, 12.50, 20 min), and LS ratio (6, 8, 10 mL/g) using Box-Behnken design (BBD). The effect of parameters on the responses of extraction yield, quantification of xanthorrhizol (19), and curcumin (1) are determined. Using RP-HPLC equiped with a photodiode array detector, xanthorrhizol (19) and curcumin (1) were quantified in the extracts. At the range including extraction temperature of 50°C, time of 20 mins and 8 mL/g LS ratio, the highest percentage of yield (72.20%) and the concentration of xanthorrhizol (19) (85.68% w/w) was observed. Although, at the extraction temperature of 30°C, time of 12.50 mins, and 10 mL/g LS ratio, the highest concentration of curcumin (1) was obtained. The experimental values are closed in agreement with the values predicted. The determination coefficient (R^2) of extraction yield, xanthorrhizol (19), and curcumin (1) are 0.9960, 0.9983, and 0.9990, respectively. The variance analysis (ANOVA) showed a good quadratic model fit statistic and model fit. The numerical data suggested the success of the RSM method in optimizing the phytochemicals extraction parameters from *C. xanthorrhiza*. This research proposed the use of other environmentally friendly methods of extracting essential oils from various parts of the plant; such as microwave-assisted extraction and steam distillation. Besides, This study suggested that the ultrasound-assisted extraction method would have a ideal potential to be utilized for extraction , which can also improve the effectiveness of the extraction method on yield and quantification of phytochemicals.

Acknowledgement

We thank the Ministry Of Higher Education for the financial support under Research University Grant (Q. J130000. 2526.12H36) and the Faculty of Science, Universiti Teknologi Malaysia for offering the research equipments. Also, Sincere appreciation and gratefulness to my supervisors, Dr. Norazah Binti Basar and Dr. Susilawati Binti Toemen, for their unmeasurable professional guidance, encouragement, valuable advice and constructive criticism from the beginning of this research to the final review of the study.

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Najiba Azemi *obtained her B.Sc in organic chemistry in 2012 from Balkh University and Master degree in organic chemistry (2020) from Universiti Teknologi Malaysia (UTM), Malaysia. Her MSc research was in the area of Natural Products Chemistry. She completed her master in Organic Chemistry at the Universiti Teknologi Malaysia (UTM). Najiba started her career as an assistant lecturer at the Department of Chemistry, Faculty of Education, Balkh University in July 2013 and currently, she is working as a Senior Lecturer. She actively involved in research area and teaching activities in Chemistry with two indexed published papers (Scopus) during her master.*

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