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Comparison of Quercetin Yield Obtained Using Convectional and Ultrasound-Assisted Extraction from *Chromolaena Odorata*

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ABSTRACT

Chromolaena odorata is believed to have great potential in the pharmaceutical industry due to the presence of phytochemical components such as alkaloids, flavonoids, and phenolics. In this study, two extraction methods, include ultrasound-assisted extraction (UAE) and conventional method were performed to extract an interesting phytochemical compound which was quercetin compound. Quercetin is one of the most abundant dietary flavonoids with high anti-inflammatory and antioxidant properties. UAE was chosen because it was famous with giving high extraction yield at shorter extraction time compared to conventional method. In this study, the effect of UAE method and conventional method towards quercetin yield was observed and compared at different extraction times (20-60 minutes). The analysis of quercetin yield was done using High Performance Liquid Chromatography (HPLC) and Field Emission Scanning Electron Microscopy (FESEM). Moreover, phytochemical screening was done as preliminary study to identify the presence of quercetin in *Chromolaena odorata*. The phytochemical screening showed dark green colour was observed with indicated the presence of flavonoid in *Chromolaena odorata*. It was found that maximum quercetin yield was obtained with the extraction yield of 0.3795±0.0028% at 60 min for UAE, while only 0.0668±0.0006% at 60 min for the conventional method. FESEM image of UAE showed greater void and pore was observed compared to FESEM image of conventional method. These results concluded that UAE was found to be more efficient in extraction of quercetin from *Chromolaena odorata* compared to conventional method.

Keywords: Ultrasound-assisted extraction; conventional; quercetin; *Chromolaena odorata*

1. INTRODUCTION

Chromolaena odarata is one of the phytochemical plants containing some organic compounds that provide definite physiological action on the human body. Previous scientific studies found that *Chromolaena odorata* is rich in many pharmacological activities such as antibacterial, antimalarial, wound healing, blood coagulation, antipyretic, and many more (Chakraborty et al., 2011). These powerful pharmacological activities attributed from bioactive compound of *Chromolaena odorata* such as flavonoid, alkaloid, tannin and many more. The main focus in this study is quercetin compound.

Quercetin is major compound of flavonoid class with overwhelming anti-inflammatory properties. It has three structural groups in the quercetin compound, which are the B ring o-dihydroxyl groups, the 4-oxo group in conjugation with the 2,3-alkene, and the 3- and 5-

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hydroxyl groups. These structural groups are important in determining the pharmacological activities of the plant such as antioxidant activity (Bentz, 2017). Furthermore, quercetin also can scavenge free radicals and bind transition metal ions. Consequently, these characteristics allow in the prevention of some diseases such as chronic inflammation, cancer, atherosclerosis, cardiovascular, and neurodegenerative diseases (Bentz, 2017). Besides, findings from Saeedi-Boroujeni and Mahmoudian-Sani, (2021) showed that quercetin can be a potential treatment for COVID-19 patients since it has high binding power to SARS-CoV-2 targets and can suppresses the NLRP3 inflammasome by inhibiting their regulators.

Extraction process is a primary step in obtaining the quercetin compound from *Chromolaena odorata*. There are two extraction methods involved in this study which are conventional and ultrasound-assisted extraction (UAE) method. In this study, maceration extraction was chosen as the conventional method. Maceration is an extraction process that involves placing untreated powder plant in a container with the solvent. Firstly, plant material is ground into coarse or powdered form to increase the surface area of the plant sample. Secondly, the solvent is added into the container containing the plant sample for the maceration process. The heat from the hotplate and frequent agitation are also provided. Lastly, the filtrate from the mixture is obtained. This process is much convenient in extracting bioactive compounds as it helps to soften and break the plant's cell wall to release the bioactive compound (Azwanida, 2015; Azmir et al., 2013). However, conventional method has numerous drawbacks such as longer extraction time, high chemical usage, evaporation of the solvent, low extraction selectivity, and degradation of thermolabile compounds (Mandal et al., 2015).

Consequently, these numerous challenges raise another alternative and innovative technique to enhance the extraction, which is ultrasound-assisted extraction (UAE). UAE was proven more effective compared to conventional method since it used ultrasound probe during extraction process. The ultrasound probe is a powerful piece of equipment with an electric generator that increases the yield of extraction by agitating the particles in the sample and disrupting the plant cell wall (Chemat et al., 2011). Moreover, there have been no studies on extraction of quercetin from *Chromolaena odorata* obtained using different extraction methods. Therefore, the study herein investigates the comparison of quercetin yield obtained using conventional method and ultrasound-assisted extraction from *Chromolaena odorata.*

2. MATERIAL AND METHODS

2.1 Chemicals and Sample Preparation

Chemicals used for this experiment were ammonia solution, concentrated hydrochloric acid and in analytical grade. Quercetin standard was purchased from Merck Sdn Bhd. Chemicals for HPLC purpose were HPLC water, acetic acid and acetonitrile. *Chromolaena odorata* plant was collected around Universiti Malaysia Kelantan, Jeli, which included the leaves, stem, and roots. The plant was washed thoroughly 2-3 times with running tap water and then with distilled water to remove extraneous materials. Next, the plant was dried for 2 weeks under the shade in a clean environment until completely dried. The dried plant leaves were milled using a blender and sieved to obtain a powder with 425 μm size. After that, the respective powder was placed inside the zip-lock bag and stored in a desiccator at room temperature to prevent the powder from gathering moisture until further analysis.

2.2 Phytochemical Screening

The preliminary screening for quercetin was based on Ugwoke et al., (2017). Few drops of concentrated ammonia were added into 2 mL sample extract until it mixed. Formation of a yellow solution indicated the presence of quercetin. The confirmatory test was conducted by adding few drops of concentrated hydrochloric acid into the yellow solution. The change of yellow coloration to white precipitate confirmed the presence of quercetin in the extract.

2.3 Conventional Method

A simple conventional method was conducted using 1:10 sample to water ratio (Salehan and Sulaiman, 2015) for extraction of bioactive compounds. Sample were mixed with water and placed in hotplate for 20, 40 and 60 minutes at temperature 50°C. Centrifugation was done at 5800 rpm for 15 min to obtain aqueous extract of *Chromolaena odorata.*

2.4 Ultrasound-assisted Extraction

Ultrasound-assisted extraction was used to extract quercetin compound from *Chromolaena odorata* at 20 until 60 min. A beaker with the mixture of sample and distilled water was used. With a sample-to-water ratio 1:10 (g/mL). Then, the sample was placed below the ultrasound probe. The tip of 15 mm diameter agitates the solution in the designated reactor throughout the experiment. The tip was submerged at half the height of the sample solution. The optimum values of the parameters were used as constant parameters as shown in Table 1. All experiments were done in triplicate.

Table 1: Constant Parameters throughout the Extraction

Parameters	Value
Sample size	425 μm
Sample to water ratio	1:10
Sample weight	5 g
Volume of solvent	50 mL
Sonication frequency	20 kHz
Centrifugation temperature	21°C
Centrifugation speed	5800 rpm
Centrifugation time	30 min

2.5 High-Performance Liquid Chromatography (HPLC)

HPLC was performed using Shimadzu Corporation, prominence autosampler (SIL-20A), consisted of quaternary pump connected to vacuum degasser DGU 20A5 with integrator CBM 20A, temperature-controlled sample trays, Software LC S=solution 1.22 SP1, and diode-array detector (DAD). A reversed-phase chromatography analysis was performed under gradient conditions using a C18 column (5 μm particle size, 4.6 mm internal diameter x 250 mm length) for separation of analytes at ambient temperature of 30°C. All the tests were carried out in duplicate.

Mobile phase A was 1% acetic acid in HPLC water solution. Mobile phase A (1% acetic acid in water) and B (acetonitrile HPLC grade) were filtered using a 0.45 μm membrane. The mobile phase was run using gradient elution as follows: 30, 60, and 90% of solution B at 5, 7, and 10 min, respectively. The wavelengths detection used were 280 nm and 360 nm, with the flow rate of 1.2 mL min^{-1} as tabulated in Table 2. For sample preparation, 1 mL of sample extract was diluted in 10 mL of HPLC grade water. After that, the sample solution was filtered using a 0.45 μm syringe filter before injected into the vial. The injection volume of sample was 20 μL.

Table 2: HPLC parameters for analysis quercetin

HPLC Parameters	Chromatographic Conditions
Detector	Detector DAD
Column	MetaChem C18 (5 μm, 4.6 mm x 250 mm)
Column temperature	30°C
Mobile phase: Solvent A	HPLC water/acetic acid (99:1 v/v)
Solvent B	Acetonitrile (HPLC grade)
Wavelength detection	280 nm - 360 nm
Flow rate	1.2 mL/min
Running time	5 min
Injection volume	20 μL

For standard preparation, 20 mg of quercetin standard was weighed into a 20 mL volumetric flask for preparation of 1 mg/1mL stock solution. Methanol was added to the mark. A series of standard dilutions was prepared from stock solution, which was 0.01, 0.05, 0.10, 0.15, and 0.20 mg/mL and diluted with methanol up to 20 mL. Each dilution was filtered using a 0.45 μm syringe filter before injected into the vial for HPLC analysis. Lastly, the sample peak was compared with standard calibration curve of quercetin to calculate the concentration of quercetin. Extraction yield of quercetin was measured as below:

$$\text{Extraction yield}(\%) = \frac{\text{concentration of quercetin}}{\text{dried sample weight}} \text{x}100 \qquad \text{(Equation 1)}$$

2.6 Field Emission Scanning Electron Microscopy (FESEM)

Field emission scanning electron microscopy (FESEM) was used to observe the surface morphology (texture) of plant. A field emission scanning electron microscopy (Fei, Quanta FEG 450) was used to examine the micro-structures of sample plant. The micro-structures plant after conventional method was examined and compared with the micro-structures plant after UAE extraction process. All samples were dried using oven to prevent any charging occur during scanning. The sample was placed onto the metal support and sputter-coated with gold (sputtering apparatus Leica EM) to ensure no interruption charges occur. Magnificent depth was examined at 5000x (Khadhraoui et al., 2019). The rupture and perforation of sample plant were observed.

3. RESULTS AND DISCUSSION

3.1 Preliminary Screening Analysis

Ammonia and hydrochloric acid were used to investigate the presence of quercetin in sample extract. The addition of ammonia produced yellow colour and the sample turned into white precipitate by the addition of hydrochloric acid. Based on the theory, when flavonoid (quercetin) dissolves in alkalis (ammonia), a yellow solution (phenates) forms and changes into colourless on the addition of acid. In this study, flavonoid reacted with ammonia to turn into quinoid. Quinoid in β-ring with longer conjugated double bond is responsible for the appearance of yellow colour (yellow arrow) after addition of ammonia in sample extract (Adhani, 2017) as shown in Figure 1 (a). Next, the confirmation step was done by addition of hydrochloric acid onto the same extract. A white precipitate (blue arrow) appeared after hydrochloric acid was added confirmed the presence of flavonoids in *Chromolaena odorata* extract. The yield of flavonoids depends on the amount of white precipitate as shown in Figure 1(b). A greater amount of white precipitate exhibited high yield of flavonoids. Hence, this indicated the presence of quercetin in *Chromolaena odorata.*

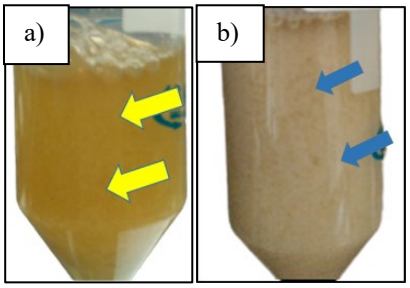

Figure 1: Phytochemical screening of quercetin: a) yellow solution and b) white precipitate

Table 3 showed the quercetin result using conventional and UAE methods from 20 until 60 min. Based on the results, all extract showed positive result of quercetin as shown in Figure 1. For conventional method, slight opacity of white solution were observed from 20 until 60 min. There was not much difference can be observed in amount of quercetin when using conventional method. For UAE, higher quercetin was observed as the extraction time increased from 20 until 60 min. At 60 min, the presence of white precipitate was observed which indicated higher amount of quercetin.

Table 3: The phytochemical result for bioactive compounds

Extraction time (min)	Quercetin from conventional method	Quercetin from UAE method
20	+	+
40	+	++
60	+	+++

Note:(-): Absence of turbidity/ flocculation/ precipitation
(+): Slight opacity
(++): If the reactive product and not turbidity flocculation
(+++): Present of precipitate/ flocculation heavy

3.2 Effects of Different Extraction Methods on the Yield of Quercetin

Figure 2 shows the extraction yield of quercetin using conventional and UAE method at 20 until 60 min. Based on the data, the extraction yield increased when the extraction time increased from 20 to 60 min for conventional and UAE method. This indicated that the effect of extraction time was significant on the yield of quercetin despite the extraction method used.

From Figure 2, 60 min extraction time showed the highest yield of quercetin with the extraction yield of 0.3795±0.0028% when using UAE. Besides, the highest yield of quercetin using conventional method was only 0.0668±0.0006% at 60 min extraction time.

This obtained yield concluded that quercetin yield was five time higher using UAE compared to conventional method due to their different principles. Similar finding from Khawory et al (2021) found that quercetin yield from *Carica papaya* leaves was two time higher using UAE (6.76%) compared to conventional method (3.22%) even though the extraction time for conventional method (3 hours) was longer than UAE method (1 hour). Conventional method happened based on the principle of slow diffusion by agitation and heat transfer using hotplate and magnetic stirrer (Naviglio et al., 2019). For UAE, ultrasound probe was used during extraction process. The energy from the ultrasound probe is directly focused on the localized sample zone and produces an effective cavitation effect during the extraction process. Cavitation is a phenomenon in which the bubbles in the liquid form, grow in size and collapse (Chemat et al., 2011). The collapse of bubble created tremendous local shock wave in the solution which then disturbing the plant cell wall and enhance the extract of bioactive compound. Overall, higher quercetin was obtained using UAE than the conventional method.

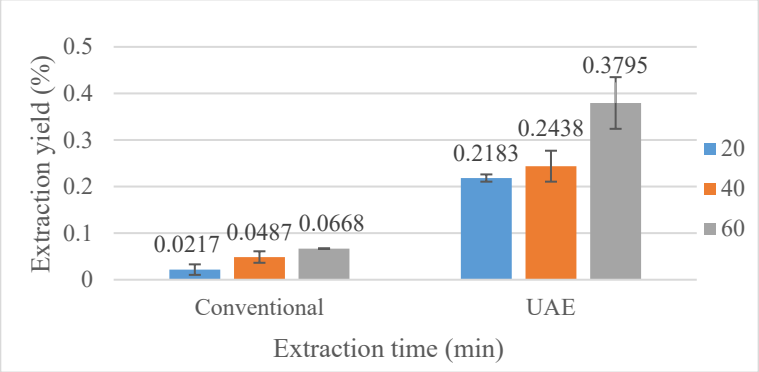

Figure 2: Extraction yield of quercetin with different extraction times using conventional and UAE methods.

3.3 High-Performance Liquid Chromatography

A sensitive and accurate method coupling high performance liquid chromatography (HPLC) with diode array detector (DAD) was developed for the detection of quercetin in *Chromolaena odorata* extract. Figure 3 shows chromatogram of optimum quercetin obtained from UAE method. The retention time for quercetin was 2.201 min as shown in Figure 3. The peak obtained was an ideal chromatography peak with a Gaussian peak. Gaussian peak is a nice sharp shape without peak splitting, broadening and shoulder peak (Rüger et al., 2021). This concluded that an ideal HPLC method was developed for extraction of quercetin from *Chromolaena odorata*.

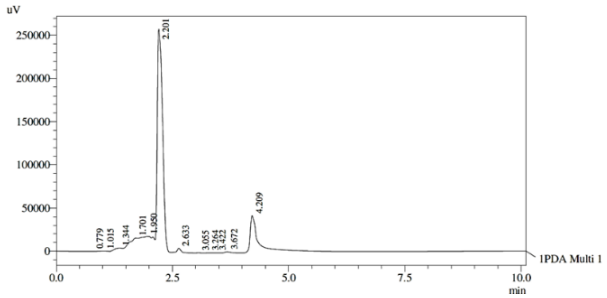

Figure 3: Chromatogram of optimum quercetin from UAE method.

3.4 Field Emission Scanning Electron Microscopy (FESEM)

The purpose of FESEM was to observe the surface morphology of sample plant after extraction process. Figure 4 (a) illustrates the FESEM image of sample after using conventional method while Figure 4 (b) showed the FESEM image of sample after UAE method. The images were captured at 5000x magnification. Based on Figure 4 (a), the surface of plant was smooth (red arrow) with a very little rupture (blue arrow). This proved that conventional method does not disturb well the sample plant. Then, the result was less permeable of cell wall and less quercetin content can be released.

Besides, a noticeable difference can be observed from Figure 4(b). After UAE, the plant showed larger void and greater rupture (yellow arrow) compared to conventional method. This was due to the cavitation effect during extraction process. A cavitation effect can produce strong local shock wave in the solution. This ultrasound wave can destruct the lignin and cellulose bond, effect the changes in chemical composition, resulting higher permeable of cell wall (Angelina et al., 2018) and increasing the release of quercetin content from *Chromolaena odorata.* This results also support the result from HPLC analysis, which showed a higher quercetin yield of UAE than the conventional method.

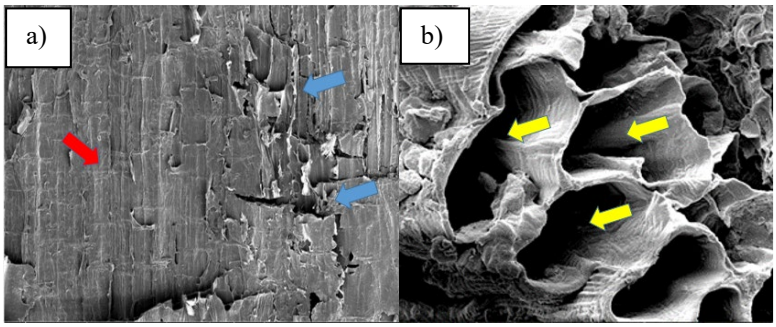

Figure 4: FESEM images of a) conventional method and b) ultrasound-assisted extraction

4. CONCLUSION

The objective of this study was achieved. The extraction of quercetin from *Chromolaena odorata* was accomplished. The comparison between two extraction methods were successfully evaluated. From preliminary screening analysis, UAE method gave higher amount of white precipitate at 60 min compared to conventional method. HPLC result showed that UAE are five-time power than conventional method. From HPLC, the highest amount of quercetin also observed at 60 min using UAE. Besides, FESEM image also support these results by illustrating higher rupture and perforation was observed on sample plant after using UAE compared to conventional method. Overall, this study proved that UAE was much better than conventional method.

5. ACKNOWLEDGMENT

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