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Research Article

Comparison of Maceration and Ultrasound-Assisted Extraction Method in Determining Quercetin Content of *Clitoria ternatea* L. Flowers

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ABSTRACT

Butterfly pea flowers (*Clitoria ternatea* L.) contain the compound quercetin which has been studied extensively. Butterfly pea flowers have shown various pharmacological activities. The extraction method has an impact on the amount of active compound extracted. The aim of this research was to determine the effect of different extraction methods on quercetin content of *C. ternatea* L flowers using the High-Performance Liquid Chromatography (HPLC). The HPLC system uses an ODS-3 column stationary phase; a mobile phase mixture of 0.1 % orthophosphoric acid and methanol (36:64); a flow rate of 1.2 mL/min; and a PDA-UV detector. The extraction methods used were maceration, and ultrasound-assisted extraction (UAE) using methanol as a solvent. The results showed that the quercetin content of *C. ternatea* L flower extract with maceration method results was 1.0669 ± 0.0283 mg/g and with UAE method was 1.3915 ± 0.1789 mg/g. Statistical test results showed that differences in both extraction method did not have a significant effect on quercetin content. However, the UAE method is considered more efficient in terms of extraction time, so the UAE method can be used as an alternative for extracting quercetin from *C. ternatea* L. flowers.

Keywords: *C. ternatea* L.; Flowers; Maceration; Quercetin; UAE

INTRODUCTION

Clitoria ternatea L. (*C. ternatea* L.) flowers are widely used in traditional medicine. This part has been used to treat several diseases. The pharmacological activities of *C. ternatea* L. flowers extract was an antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-arthritic, analgesic, and cytotoxic (Jeyaraj et al., 2022; Shinde & Khemnar, 2024). *C. ternatea* L. flowers have striking colors, so they are often used as a natural food and coloring agent for drinks (Gamage et al., 2021; Rahma et al., 2024). The ethanol extract of *C. ternatea* L. flowers contain alkaloids, flavonoids, resins, tannins, polyphenols, saponins, and triterpenoids (Rahma et al., 2024; Jayanti et al., 2021; Sarma et al., 2023; Yee & Than, 2020).

The widely known active compound in *C. ternatea* is quercetin. According to Jeyarai et al. (2020), phytochemical compounds that have been isolated from *C. ternatea* L. flowers were quercetin and myricetin glycosides known as anthocyanins. Furthermore, quercetin 3-(2G)-rhamnosylrutinoside)s, quercetin 3-neohesperidoside, quercetin 3-rutinoside, quercetin 3-glucoside, myricetin 3-neohesperidoside, myricetin 3-rutinoside, and myricetin 3-glucoside were found in *C. ternatea* L. flowers (Al-Snafi et al., 2016; Mahajan et al., 2022). Quercetin is one of the natural bioactive products from the flavonol family, which is widely studied because it has pharmacological activities such as anticancer, anti-inflammatory, anti-obesity, preventing coronary heart disease, and

preventing disease asthma and lungs, neuroprotective, treatment of skin disorders, antiaging, and preventing diabetes complications (Gupta et al., 2016; Alizadeh et al., 2022).

Quercetin is bound to five hiydoxyl groups and contains a chromophore group, which can be analyzed using high performance liquid chromatography (HPLC) equipped with a UV-Vis detector (Wang et al., 2022; Briliantama et al., 2022; Nammatra et al., 2021). The content of quercetin 3-rutinose in the methanol-water extract of *C. ternatea* L. flowers extracted using the ultrasound-assisted extraction (UAE) method and analyzed using HPLC with water: acetonitrile mobile phase was 0.492 mg/g while quercetin 3-glucose was 0.174 mg/g (Briliantama et al., 2022). According to Pandey and Tripathi (2014), the extraction method and type of extraction solvent greatly influence the amount and quality of extracted secondary metabolites. According to Manzoor et al., (2021) that quercetin content in extract was affected with extraction method. Therefore, the quercetin content of *C. ternatea* L. flowers were determined using two different extraction methods using methanol solvent (Mehmood et al., 2019; Briliantama et al., 2022).

METHODS

Equipments

The equipment used in this research was an HPLC instrument (Jasco®), column (Inertsil®) ODS-3 (5 µm, 4.6 µm x 150 mm), UV-Vis Detector (Jasco® UV-4075), Photo- Diode Array detector (PDA) (Jasco® MD-4010), micropipette, syringe filter 0.22 µm, millipore 0.45 µm, oven, sonicator (Branson®), analytical balance (And®), and a set of laboratory glassware.

Materials

The research material was used *C. ternatea* L. flowers obtained from Ciapus Village, Ciomas District, Bogor Regency, and was determined at the National Research and Innovation Agency (BRIN) Cibinong, Bogor. The chemicals used were distilled water, formic acid, orthophosphoric acid, ethyl acetate, quercetin (Sigma-Aldrich, Singapore), HPLC grade methanol (Merck KGaA, Germany), toluene, and silica gel 60 GF_{254} plates (Merck KGaA, Germany).

Extraction of *C. ternatea* L. Flowers

Harvested *C. ternatea* L. flowers were dried in the oven at 40 ºC. The dry sample obtained was then powdered and filtered using a 40-mesh sieve and then extracted using maceration and ultrasound-assisted extraction (UAE) methods, respectively. A total of 50 g of powder was macerated with 300 mL of methanol for 48 hours and then remacerated with 200 mL.

Meanwhile, for the UAE process, 50 g was used in an Erlenmeyer, then 300 mL of methanol was added. The mixture of dry sample and methanol was extracted with a Sonicator (Branson®) at 50 ℃ in 15 minutes. The extract is then filtered, and the residue was re-extracted with 200 mL of methanol. The extraction was carried out in triplicate. All filtrates were collected and concentrated with a rotary evaporator at 64 ℃, respectively.

Identification of Quercetin Using Thin Layer Chromatography (TLC)

Identification of quercetin using TLC according to research by Doshi and Une (2015). The TLC system used consists of a stationary phase of silica gel 60 GF_{254} plates and a mobile phase of toluene, ethyl acetate, formic acid (5:4:0.2). Quercetin standards and extracts were dissolved in methanol (10 mg/mL). The sample was filtered, and the chamber was saturated for 30 minutes. The quercetin standard and extract were spotted using a capillary tube on a silica gel plate. The spot results were dried and put into a chamber. After elution was complete, the plate was removed and viewed under λ254 nm UV light. The RF (retention factor) value was calculated.

Quercetin Analysis with HPLC

Preparation of Quercetin Stock Solution and HPLC Methode Optimization

The stock solution of quercetin was made at 500 ppm by weighing 25 mg of quercetin and then dissolving it in a 50 mL volumetric flask using methanol to the limit mark. Instrument optimization was carried out by searching for the maximum wavelength of quercetin using a PDA-UV detector and selecting the mobile phase. The search for the maximum wavelength was carried out by injecting the quercetin standard and observing the absorption pattern. The mobile phase was selected using the composition of a mixture of 0.1 % orthophosphoric acid (in water) and methanol (46:54, 41:59, and 36:64),

which provided optimal separation. Mobile phase elution was isocratic at a flow rate of 1.2 mL/min.

Validation of Analysis Methods

Partial validation analysis method were performed for parameters such as linearity, LOD (limit of detection), LOQ (limit of quantification), precision, and accuracy (Marson et al., 2020).

Analysis of Quercetin Content ofs in Extracts

The *C. ternatea* L. flowers extract was weighed at 100 mg and dissolved in a 10 mL flask using methanol to the limit mark. The solution was ultrasonicated for 15 minutes. The solution was pipetted to 2.5 mL and diluted with methanol to 5 mL. The solution was filtered using a 0.22 µm syringe filter and put into a vial. The extract sample solution was degasified for 15 minutes, and 20 µL was injected into the HPLC system under optimum conditions.

RESULTS AND DISCUSSION

Extraction and Identification of Quercetin Using TLC

The maceration method produced an extract yield of 28.00 ± 0.5291 %, while the UAE extract yield was 21.26 ± 0.7023 %. This was thought to be due to the extraction time factor, which is a total of 96 hours, so it is possible that the compound withdrawal process was more optimal compared to the UAE method. Extraction time will affect the amount of extract yield. Identification of quercetin in *C. ternatea* L. flowers extract was carried out using TLC. The TLC test results showed a brown spot on the plate when observed under 254 nm UV light (Figure 1).

Rf value of quercetin standard was 0.53, while Rf quercetin from macerattion and UAE extract were 0.52. The Rf value provides an initial identification of the similarity between the standard and the sample (Doshi & Une, 2015). The differences in Rf value between the quercetin standard and the extract sample was considered small, so it can be concluded that the sample contains quercetin.

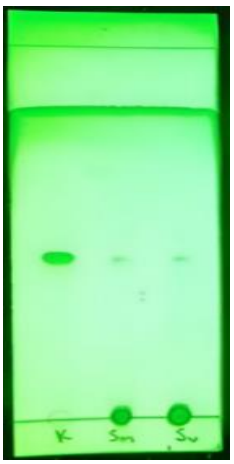

Figure 1. Chromatogram Thin Layer Chromatography of Quercetin in UV 254 nm Wavelength.
Note: *K = quercetin standard solution, Sm = maceration extract solution, Su = UAE extract solution*

Optimization of the HPLC instrument

Optimization of the HPLC instrument was carried out by measuring the maximum wavelength using a PDA-UV detector (Figure 2). The results obtained were 368 nm and almost the same at the maximum wavelength from the other literature (Umer et al., 2024). The selection of the mobile phase is carried out to obtain the separation characteristics of standard compounds and samples to obtain an optimal analytical system. The mobile phase composition used was a mixture of 0.1 % orthophosphoric acid and methanol (36:64, 41:59, and 46:54), with a reverse phase HPLC system using an ODS-3 column. Optimization results can be seen in Table 1.

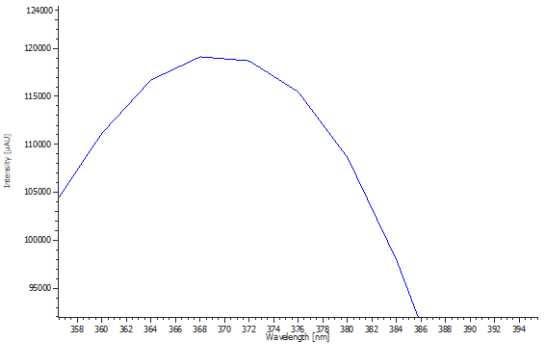

Figure 2. Quercetin Maximum Wavelength.

Table 1. Profile Chromatogram of *C. ternatea* Flower Extracts

	Eluen ratio	tR (minute)	Rs	NTP	HETP (mm)	Symmetri factor
Quercetin standar	36-64	3.292	1.1467	735	0.2040	1.384
Quercetin in maceration extract		3.242	2.909	555	0.2702	1.959
Quercetin in UAE extract		3.275	2.825	612	0.2450	1.612
Quercetin standar	41-59	4.925	-	596	0.2516	1.714
Quercetin in UAE extract		4.625	-	611	0.2454	1.705
Quercetin standar	46-54	7.708	-	550	0.2727	1.896
Quercetin in UAE extract		7.242	-	818	0.1833	1.912
Qualify		<10	>1.5	>2000	0.01-1.00	1

Note: *eluent composition was 0.1 % orthophosphoric acid and methanol. tR= time retention. Rs=resolution. NTP= number of theoritical plate. HETP=height equivalent to a theoretical plate*

A good chromatogram can be seen from the resolution value. symmetry factor. and retention time. The eluent composition with 0.1 % orthophosphoric acid and methanol in a 36:64 ratio has the highest NTP and HETP value and a good resolution value (Table 1) (Schieppati et al., 2021). Meanwhile, the eluent compositions 41:59 and 46:54 do not have a good Rs value (Figure 3 & 4), which may indicate that the separation is less than optimal under certain conditions. Optimization of the HPLC system and mobile phase is necessary for good and reproducible separation of the components in the sample. It is also important to check for a symmetry factor close to 1 to ensure a good peak shape. Based on optimal conditions for the analysis of quercetin compounds. it was achieved 0.1 % orthophosphoric acid and methanol in a 36:64 ratio.

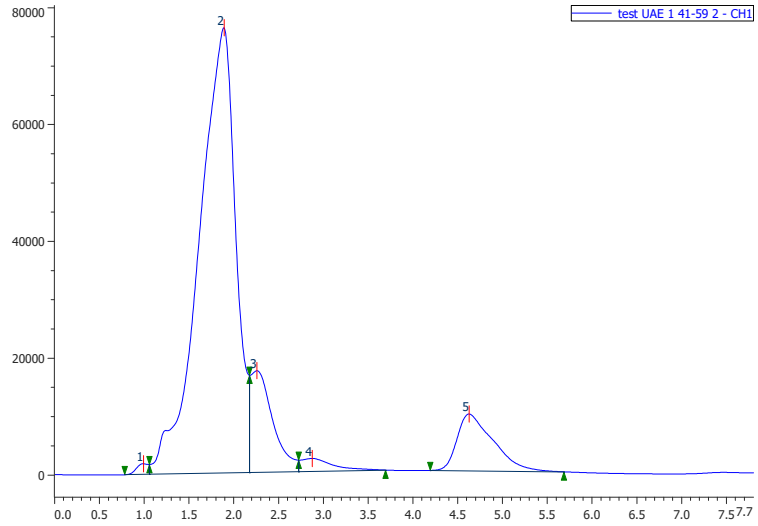

Figure 3. Chromatogram Quercetin in Extract UAE
Note: eluent composition was 0.1 % orthophosphoric acid and methanol (41 : 59), flow rate of 1.2 mL/min, and PDA-UV detector.

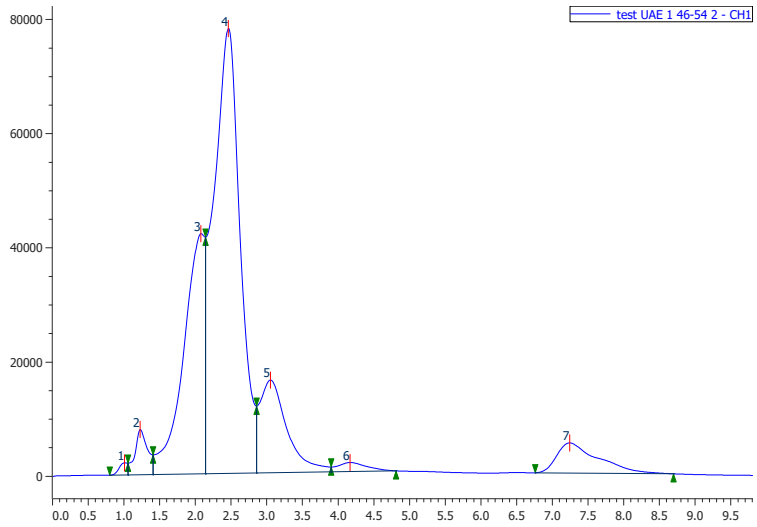

Figure 4. Chromatogram Quercetin in Extract UAE
Note: eluen composition was 0.1 % orthophosphoric acid and methanol (46 : 54), flow rate of 1.2 mL/min, and PDA-UV detector.

Validation Method Analysis of the Quercetin Compound

Linearity test was carried out with an external standard calibration curve. The calibration curve was obtained by measuring the standard series of quercetin concentrations at 5; 10; 20; 40; and 80 µg/mL. Based on the results of determining the area of the standard quercetin solution at 5 different concentrations. The linear regression equation was y = 51288x + 21316 with a correlation coefficient (R^2) of 0.9998. This R^2 value was close to 1, which indicates that the regression equation was linear. The LOD and LOQ values were achieved at 1.5572 ppm and 5.1906 ppm. The results of the validation method analysis method using the spike method can be seen in Table 2. and showing that the analysis method was valid.

Qualitative and Quantitative Test of Quercetin with HPLC

Qualitative tests were carried out by comparing the tR of the analyte from the sample with a standard quercetin. The tR measurement results from the quercetin standard with the mobile phase 0.1 % orthophosphoric acid and methanol (36:64) were 3.175 minutes; in the macerated extract. there was a peak with tR 3.242 minutes; and in the UAE extract there was a peak with tR 3.275 minutes (Figure 5). The extract chromatogram has a difference time of 0.1 minute and is generally considered a very small difference in HPLC analysis. This may occur due to variations in analysis conditions in retention time measurements. and in most cases. these small differences will not significantly affect the analysis results. so the extract sample was identified as containing the compound quercetin.

The quantitative test for the determination of quercetin content *C. ternatea* L. flowers extract was calculated using the linear regression. The results of determining the quercetin content in macerated *C. ternatea* L. flower extract was 1.0669 mg/g while in UAE extract was 1.3915 mg/g (Figure 6). These results showed that the UAE extraction method dissolves more amount of quercetin than the maceration method. but based on statistical analysis using one-way ANOVA and paired samples, there is no significant difference between the two methods on quercetin content.

Table 2. The Results of Validation Method Analysis

Concentration (µg/mL)	RSD	% Recovery
64	1.0824	97.8143–100.716
80	0.9840	97.9258–100.44
96	0.9063	97.9898-100.182
Qualify	≤ 2.00 (Snyder, 2010)	80-110 (AOAC, 2013)

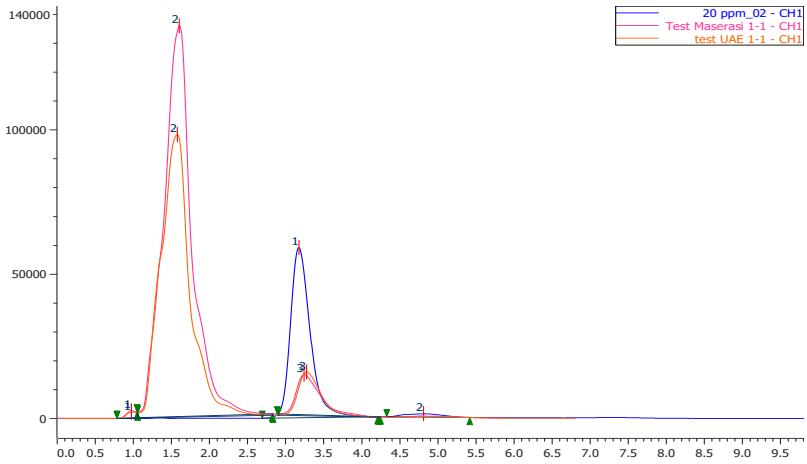

Figure 5. Chromatogram overlaid of quercetin solution standard and quercetin solution in extract.

Although the extraction method does not make a significant difference to the quercetin content of *C. ternatea* L. flowers, it can be considered in terms of time efficiency. The UAE method requires a faster total extraction time than the maceration method. Ultrasonic waves cause an increase in the diffusion process between material and solvent, causing the compound withdrawal process to speed up. Higher temperatures in UAE extraction also increase the solubility of quercetin in methanol solvents (Abubakar & Haque., 2020; Hasni et al., 2021; Manzoor et al., 2021; Mousavi et al., 2022).

The maceration and UAE extraction methods have different mechanism processes. According to Abubakar and Haque (2020), the extraction process using maceration method occurs due to breaking up the walls and membranes of *C. ternatea* L. flowers dry sample cells by the pressure difference between the outside and inside of the cell. So, the metabolite compounds will be pulled out and dissolved in the methanol solvent. The longer of the extraction time, the greater the solvent penetration, and the more solvent used, the greater the pressure on the cells until the maximum metabolite compounds are extracted. Meanwhile, according to Manzoor et al., (2021) in the UAE method, which uses ultrasonic waves, exposure to ultrasonic waves helps the formation of cavitation bubbles, which produce mechanical pressure and can help break up cells and dissolve metabolite compounds in the solvent. However, exposure to ultrasonic waves over a long period of time can cause damage to the structure of the solute and reduce the extraction yield. If ultrasonic waves are not controlled properly, the cavitation bubbles that occur can also cause unwanted damage to the structure of the substance. This reason may cause there to be no significant difference between the UAE and the maceration method.

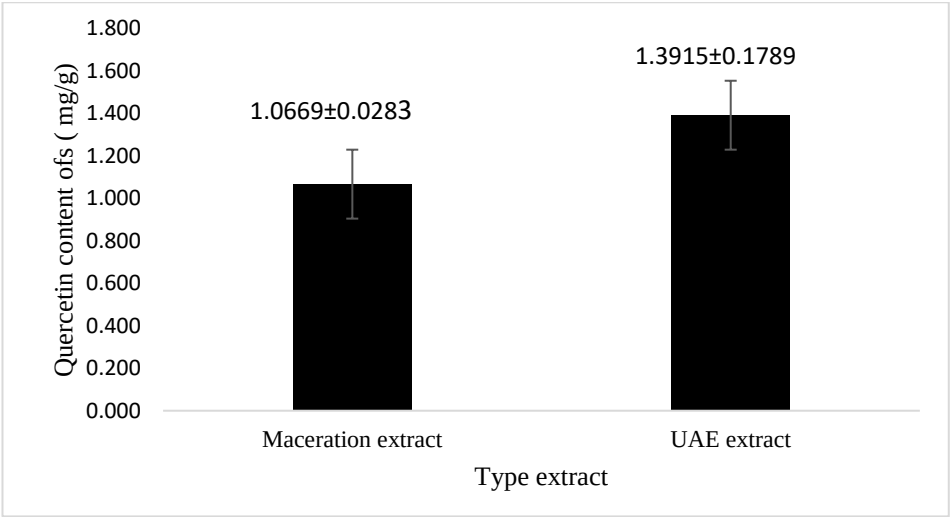

Figure 6. Quercetin content in maceration and UAE extract of *C. ternatea* L. flower.

CONCLUSION

The maceration extraction method of *C. ternatea* L. flowers can produce quercetin content of 1.0669±0.0283 mg/g and the UAE method produces quercetin content of 1.3915±0.1789 mg/g. UAE quercetin content was greater than maceration. But it was concluded that the maceration and UAE extraction methods did not significantly influence to quercetin content.

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CONFLICT OF INTEREST

All authors declarated that there was no conflict of interest.

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