Malaysian Journal of Chemistry, 2024, Vol. 26(3), 389-395
(Special Issue: *3rd Virtual International Symposium on Sciences 2023*)

Determination of Quercetin in *Persicaria odorata* Leaves by Using High-Performance Liquid Chromatography (HPLC)

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Persicaria odorata is also known as Vietnamese coriander or *daun kesum*, an herbal plant enriched with chemical constituents and numerous health benefits associated with its consumption such as a potential drug against COVID-19. Determination of the presence of quercetin in *Persicaria odorata* leaves was optimised by using High-Performance Liquid Chromatography coupled with Ultraviolet (HPLC-UV). In this study, the *Persicaria odorata* leaves were macerated in methanol prior to analysis with the C_{18} column as the stationary phase at 368 nm. The optimisation was achieved with a mixture of methanol-water (70:30) with a flow rate of 0.3 mL/minute, and separation was achieved in less than 7 minutes. A linear concentration range for quercetin determination was obtained in the range of 10-200 ppm with a limit of detection (LOD) and limit of quantification (LOQ) at 18 ppm and 56.32 ppm, respectively. The recovery percentage ranged from 77.6% to 88.9%. The method was simple, accurate, and successfully applied to determine quercetin in *Persicaria odorata* leaves using HPLC.

Keywords: *Persicaria odorata*; High Performance Liquid Chromatography (HPLC); method validation; Quercetin; Vietnamese coriander

Received: October 2023; Accepted: February 2024

Historically, plants have been used as medicine to treat various types of illnesses due to their phytochemical properties [1]. These phytochemicals or active components are chemicals that have been shown to have therapeutic properties in natural products. Therefore, natural products have served as the foundation for creating new drugs [2].

Persicaria odorata (Lour.) or known as Vietnamese coriander, which belongs to the Polygonaceae family, is a delicate perennial plant native in Southeast Asia that grows in wet and shady soil [3]. This plant is 30-35 cm tall, with long pointed leaves (6-15 cm) and a distinct dark purple pattern in the middle [4]. It has a unique taste that is pungent and spicy [5]. In Malaysia, it is known as *daun kesum* and commonly used as a flavouring agent in food preparation [6].

Persicaria odorata has been extracted to produce an essential oil and most of the phytochemical constituents present in the leaves have been identified. For example, Sasongko *et al.* [5] found that the volatile compounds from the essential oil of *Persicaria odorata* contain 28% decanal, 44% dodecanol, and 11% decanol. Other chemical compositions such as sesquiterpenes (α-humulene and β-caryophyllene) were also found in the essential oil [3, 7]. In addition, ferulic acid, quercetin, gallic acid, p-coumaric acid, and ellagic acid were the bioactive compounds that have been identified using High-Performance Liquid Chromatography (HPLC) analysis [8]. Moreover, another research conducted by Pawlowska *et al.* [9] revealed the presence of several phenolic compounds in the methanolic-aqueous extract of *Persicaria odorata* by using HPLC coupled with diode-array detection and electrospray ionisation tandem mass spectrometry (HPLC-DAD-MS). The chemical compounds reported were methyl gallate, (+)–catechin, quercetin 3-O-β-D-glucuronide, quercetin 3-O-β-D-rhamnoside, sinapic acid hexoside, (epi)catechin gallate, tetrahydroxy-flavonol derivative, quercetin sulphate, and kaempferol sulphate.

A class of organic compounds with varying phenolic structures found in fruits, vegetables, grains, barks, roots, stems, leaves, and flowers are called flavonoids. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-Hchromen-4-one) is a plant pigment of flavonoid that has a flexible antioxidant with the ability to guard against tissue damage brought on by different medication toxicities [10]. These phytochemical constituents are well known for their beneficial effects on health, and efforts are being made to extract the components known as flavonoids [11]. The chemical structure of quercetin is shown in Figure 1.

†Paper presented at the 3rd Virtual International Symposium on Sciences (3rd VISoS 2023)

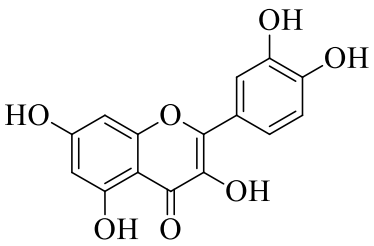

Figure 1. Chemical structure of quercetin.

Quercetin can act as an antibacterial agent, an antioxidant, a plant metabolite, and an antineoplastic agent. In addition, quercetin is used to treat diverse conditions including cardiovascular disease, hypercholesteromia, infection, and cancer [10]. The recent research conducted by Saeedi-Boroujeni *et al.* [12] states that quercetin is a potential drug that can treat COVID-19 patients due to its ability to inhibit the inflammatory response of SARS-CoV-2 virus. Based on the clinical trial result, quercetin inhibits Interluekin-17 (IL-17) leading to a positive outcome in the treatment of COVID-19.

In the present study, the developed method was optimised by two parameters (mobile phase composition and flow rate) of separation of quercetin in *Persicaria odorata* leaves in conjunction with High-Performance Liquid Chromatography (HPLC) coupled with the C_{18} column. Method validation of HPLC was performed with a good sensitivity of the limit of detection and limit of quantification.

EXPERIMENTAL

Chemicals and Materials

Methanol was purchased from R&M Chemicals while quercetin standard ($C_{15}H_{10}O_7$) was purchased from Sigma-Aldrich (M) Sdn Bhd. All other chemicals used in this research were of analytical grade.

Plants Materials

Persicaria odorata or *daun kesum* leaves were purchased from a local market in Kuala Pilah, Negeri Sembilan, Malaysia. The leaves were peeled from the stems and washed using tap water, then sterilized with distilled water. Then, the leaves were dried in the oven at 50°C so that all the leaves become well-dried for grinding. The leaves were then ground into fine powder using a grinder and transferred to an airtight container [19].

Extraction of the Leaves

For extraction, 45 grams of the leaves' powder from the previous procedure was macerated in 450 mL methanol for 24 hours. Then, the solvent was filtered with Whatman (No. 1) filter paper and poured into a round-bottomed flask. The solvent was evaporated using a rotary evaporator. Through this process, a neat semi-solid plant extract was achieved [19]. The pure extracted leaves of *Persicaria odorata* were placed in a beaker and weighed on the analytical balance. The dried extract was kept in the refrigerator at 4°C for the next procedure.

Preparation of Stock Solution

About 25 mg of standard quercetin was weighed and transferred to a 50 mL volumetric flask. The quercetin was dissolved in methanol with a standard quercetin concentration of 500 ppm [20]. The standard solution was filtered using 0.45 μm membrane filter.

Preparation of Sample Solution

A total of 50 mg of dried fraction was weighted using an analytical balance. The samples were then dissolved and diluted with methanol. Next, the samples solution was filtered and sonicated before being injected into the HPLC system.

Calibration Curve

The standard quercetin powder was accurately weighed using an analytical balance and dissolved in 50 mL of methanol to get a concentration of 500 ppm, making it a standard quercetin solution [21]. The standard quercetin solution was then diluted with distilled water until reaching the calibration mark of 25 mL volumetric flask to give serial concentrations within the range of 10-200 ppm. The calibration curve was established by injecting 20 μL of each of the concentrations (10, 50, 100, 150, and 200 ppm) into the HPLC system. The peak area of the standard solutions obtained was placed at the y-axis while the concentrations were placed at the x-axis of the calibration curves. The peak was identified based on the retention time (Rt) and the area below the peak.

HPLC Conditions

An Agilent 1260 Series system consisting of a manual injector, binary pump, column compartment (25°C), and ultraviolet detector were used in determining the presence of quercetin in the *Persicaria odorata* leaves extract. A Zorbax Eclipse Plus C18 column at 4.6 x 250 mm, 5 μm particle size was used to separate the sample. The mobile phase was prepared using two different solvents (methanol as solvent A and water as solvent B). Each solvent was filtered through 0.45 μm membrane filter and sonicated for 15 minutes to avoid the interference caused by dissolved gas. Mobile phases were employed in an isocratic elution method.

Optimisation of Chromatographic Condition

Several parameters have been optimised to enhance the separation process. In order to do this, a variety of mobile phase compositions was made along with the eluent flow rate as shown in Table 1 below.

Table 1. Optimisation of chromatographic condition.

Mobile phase composition, % (methanol: water)	Flow rate (mL/min)
40:60	0.8
50:50	1.0
60:40	1.0
70:30	1.0
80:20	1.0
70:30	0.5
70:30	0.3

Method Validation

The process of validating an analytical technique entails using laboratory testing to ensure that the performance properties meet the requirements for its intended use [13]. Parameters including linearity, accuracy, precision, recovery, limit of detection (LOD), and limit of quantification (LOQ) were validated using calibration standard quercetin in sample matrices.

Linearity

Linearity refers to the ability of an analytical technique to yield test results that are directly proportional to the analyte concentration within a certain range [13]. This study evaluated linearity at five different concentrations of quercetin ranging from 10 to 200 ppm. Then, using a least square regression analysis, the corresponding peak area was plotted against the concentration to determine the linearity. The regression equation can be calculated using the formula of Y = mX + c, where Y represents the peak area or absorbance, and X represents the concentration (ppm).

Accuracy and Recovery

Accuracy is the difference between the mean value discovered and the true value. It was determined by applying the method to samples containing known amounts of analyte [13]. Recovery values can be determined by spiking samples with a known representative concentration. In this analysis, a concentration of 100 ppm was spiked into the sample solution and the percentage recovery was calculated using the formula below.

$$\textbf{Recovery}\ (\%) = \frac{\textbf{Sspiked} - \textbf{Rreal}}{\textbf{Sspiked}} \times \textbf{100}\% \qquad \textbf{(1)}$$

Where;

S_{spiked} = known concentration of quercetin spiked to the sample

R_{real} = concentration of quercetin in real sample solution

LOD and LOQ

Limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected but not always measured under the test's specified parameter. Meanwhile, limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be identified under the specified test circumstances with sufficient precision and accuracy [15]. The LOD and LOQ of quercetin in the developed method were studied using the signal-to-noise ratio of 3 for estimating LOD and 10 for estimating LOQ. The LOD and LOQ were determined by using formula (2) below.

$$\text{LOD} = \frac{3.3 \times \sigma}{\text{slope of the calibration curve}} \qquad \textbf{(2)}$$

$$\text{LOQ} = \frac{10 \times \sigma}{\text{slope of the calibration curve}}$$

Where;

σ = the standard deviation of the response

Statistical Analysis

Linearity, slope, and regression coefficients were determined by linear regression. Microsoft Excel 2010 was used for all statistical analyses.

RESULTS AND DISCUSSION

Extraction Yield of the Leaves Extract of *Persicaria odorata*

Maceration is the most basic and affordable traditional extraction technique which requires a container for the extraction process [2, 16]. The maceration technique was employed in this study to extract phytochemicals from the leaves of *Persicaria odorata*. During the maceration, the leaves of *Persicaria odorata* were ground into powder form to increase the surface area so that the powdered materials and the solvent can be properly mixed. The powdered leaves were immersed in the solvent at room temperature to extract the constituent materials. Methanol was used as an extraction solvent because it has high polarity and could produce high extraction yield [17]. The crude extract obtained was calculated to the weight of the dried plant leaves to determine the percentage yield of the plant as shown in the formula below.

$$\textbf{Yield}\ (\%) = \left(\frac{\textbf{Amount of extract yield}}{\textbf{Amount of dried plant used}}\right) \times \textbf{100}\% \qquad \textbf{(3)}$$

Table 2. Percentage of extraction of *Persicaria odorata* leaves.

Amount of fresh leaves (g)	Amount of crude extract (g)	Percentage of yield (%)
45.0508	7.433	16.47

In this study, the results are shown in Table 2 and the percentage yield obtained is 16.47%.

The first step in isolating the desired products from raw materials or natural sources is extraction [2]. The universal solvents commonly used for phytochemical screening are ethanol and methanol [2]. The small particle size enhanced the extraction efficiency as it was able to penetrate solvents and diffuse into solutes. This can reduce the time required for maximal phytochemical extraction [17, 18]. Apart from that, modern extraction techniques such as microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction can help reduce extraction time and increase selectivity.

A recent study stated that the extraction method using ethanol was able to yield high antioxidant activity from the *Persicaria odorata* leaves extract [14]. Ethanol was chosen for extraction due to its low toxicity and low cost. In addition, Sasongko *et al*. [5] determined the volatile compounds of the *Persicaria odorata* leaves extracted using ethanol which are beta-caryophyllene, dodecanal, ethyl hexadecanoate, and neophytadiene. Maceration requires a lengthy extraction time whereby the *Persicaria odorata* dried samples were macerated with methanol for 72 hours. The results showed that the *Persicaria odorata* contain the desired phytochemical constituents such as dodecanal, decanal, α-humulene, cyclodecane, β-eoclovene, trans-caryophyllene, and cyclododecane [7].

Optimisation of Chromatographic Conditions

Several parameters have been optimised to enhance the separation process in a reasonable run time. Quercetin was observed in the range of 360 – 400 nm on the UV spectra chromatograms with a monitoring wavelength for quantitative determination at 368 nm. In addition, a variety of mobile phase compositions was made along with the flow rate of the eluent (Table 1). Thus, the best separation was achieved with the composition of methanol: water (70:30) flowing at 0.3 mL/min. The standard quercetin and sample injected in the HPLC system was 20 µL and a total run time of 10 minutes as the peak of quercetin appeared less than 9 minutes.

Method Validation

Linearity

The highest and lowest analyte concentrations at which adequate linearity was obtained were used to determine the analytical range. Quercetin's correlation coefficient value (R^2) was more than 0.99, indicating that they are linear in the 10 – 200 ppm range.

Accuracy

Each concentration of the standard quercetin solution (10-200 ppm) was injected into the HPLC systems for three replicates to observe the consistency of the peak area and retention time. The percentage recovery (%RSD) of the sample was less than 5% because smaller relative standard deviations result in more accurate data measurement. The results of recovery studies are shown in Table 3.

Table 3. Accuracy of standard quercetin.

Concentration (ppm)	Average of peak area	Standard deviation	%RSD
10	1964.256023	54.818019	2.79
50	2965.939757	73.3326001	2.47
100	3926.257813	91.870502	2.34
150	4750.890983	116.101278	2.44
200	5770.996420	132.786137	2.38

Recovery and Level of Quercetin in the Sample

The recovery of the developed method was measured using accuracy studies. The standard quercetin of 100 ppm concentration showed the lowest percentage recovery (%RSD) among the five series concentrations of standard quercetin solution. Thus, the sample of *Persicaria odorata* was spiked with 100 ppm of standard quercetin and injected into the HPLC system for three trials. The peak area for each sample injected was compared to the calibration curve of standard quercetin. The percentage recovery was calculated using the known concentration of quercetin spiked into the sample minus the concentration of quercetin in the real sample over the known concentration of quercetin spiked into the sample multiplied by 100 percent (Equation 1).

Table 4 shows the recovery and level of quercetin in the real sample in ppm. The blank and the standard samples were analysed. The chromatogram of standard quercetin solution at 100 ppm concentration and sample solution was spiked with the standard of quercetin as shown in Figure 2. The chemical structure of quercetin can predict its affinity for low-polarity environments and at the same time, the presence of five hydroxyl groups implies a predisposition for polar environments [22]. Therefore, the quercetin in the sample solution showed a longer retention time as it attracted to the polarity of the column which was C_{18}. This may have happened due to the presence of other compounds such as gallic acid, catechin, and rutin in the sample solution. The LOD and LOQ of quercetin in the developed method were studied using the signal-to-noise ratio of 3 for estimating LOD and 10 for estimating LOQ with LOD and LOQ estimated at 18.03 ppm and 18.32 ppm, respectively.

In conclusion, based on the results, significant amounts of flavonoids (quercetin) exist in the *Persicaria odorata* leaves sample. Therefore, including *Persicaria odorata* leaves in one's diet may have beneficial health effects.

Table 4. Recovery of quercetin in *Persicaria odorata* leaves sample. ***(N=3).***

Sample	Real concentration of quercetin in the sample (ppm)	Recovery (%)
1	22.4	77.6
2	11.1	88.9
3	18.8	81.2

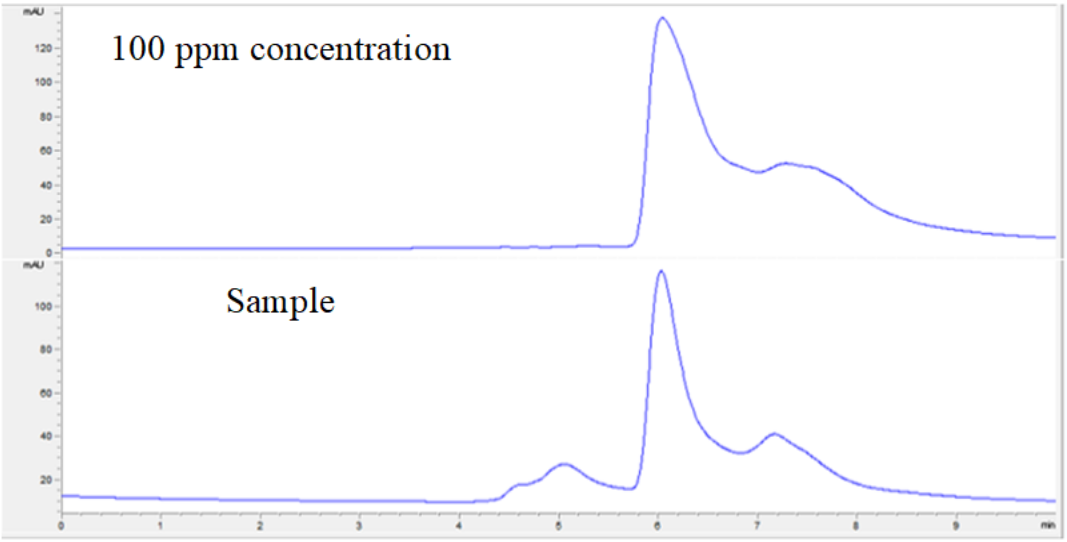

Figure 2. Comparison between standard quercetin (100 ppm concentration) with quercetin in the *Persicaria odorata* extract with methanol composition: water (70:30) flowing at 0.3 mL/min.

CONCLUSION

The method was simple, accurate, and has been successfully applied for the determination of quercetin in *Persicaria odorata* leaves by using High-Performance Liquid Chromatography coupled with ultarviolet (HPLC-UV). The determination of quercetin in *Persicaria odorata* leaves extract was achieved with the composition of methanol: water (70:30) flowing at 0.3 mL/min. This study validates a relatively simple and sensitive method with LOD and LOQ at 18.03 ppm and 18.32 ppm, respectively, with 88.9% recovery. The presence of quercetin Persicaria odorata leaves was detected in the range of 18.8-22.4 ppm. Further research and exploration into the specific mechanisms and bioavailability of these compounds can provide valuable insights into their role in promoting health and well-being.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Faculty of Applied Sciences, Universiti Teknologi Mara (UiTM), Cawangan Negeri Sembilan, Kampus Kuala Pilah Campus for the facilities and services provided for the analysis.

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