BIO Integration

Original Article

Analysis of Quercetin Levels in the Ethanol Extract of Curry Leaves (*Murraya koenigii* L.) as a Potential Animal Feed using High-Performance Liquid Chromatography

Sunaryo Hadi Warsito[1], Mirni Lamid[1,*], Mohammad Anam Al Arif[1], Widya Paramita Lokapirnasari[1], Siti Rani Ayuti[2,3], Aswin Rafif Khairullah[4], Mira Delima[5], Intan Permatasari Hermawan[6], Rakhi Gangil[7] and Zaheer Ahmad[8]

Abstract

Background: Curry leaf (*Murraya koenigii*) is a frequently used medicinal plant. The *M. koenigii* plant is a type of spice in the Rutaceae family. Curry leaf ethanol extract contains flavonoid compounds. Quercetin is a flavonol compound, a flavonoid derivative that has a 3-hydroxyflavone skeleton. There is a need to analyze the quercetin level in curry leaves utilizing high-performance liquid chromatography (HPLC).
Methods: This research was conducted from August to October 2023. A total of 500 g of powdered curry leaves were placed in a maceration vessel. The liquid ethanol extract was mixed with the first re-maceration filtration product. Qualitative analysis was performed by comparing the retention time of the sample solution chromatogram with the reference standard solution of quercetin under identical HPLC conditions.
Results: The quantity of curry leave ethanol extract was 52.613 g. Analysis of the curry leaf extract samples revealed that sample 1 had a quercetin level of 0.080 mg/g, while sample 2 had a quercetin level of 0.079 mg/g. The final analysis of the curry leaf ethanol extract samples showed an average quercetin level of 0.03%.
Conclusion: These results indicate that the curry leave ethanol extract contains flavonoid compounds, especially quercetin.

Keywords

Curry leaf ethanol extract, HPLC, human health, quercetin.

[1]Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

[2]Biochemistry Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia

[3]Research Center of Aceh Cattle and Local Livestock, Faculty of Agriculture, Universitas Syiah Kuala, Aceh, Indonesia

[4]Research Center for Veterinary Science, National Research and Innovation Agency, Bogor, Indonesia

[5]Department of Animal Husbandry Faculty of Agriculture Universitas Syiah Kuala, Aceh, Indonesia

[6]Faculty of Veterinary Medicine, Universitas Wijaya Kusuma, Surabaya, Indonesia

[7]Department of Veterinary Microbiology, College of Veterinary Science and AH MHOW, Nanaji Deshmukh Veterinary University, Jabalpur, India

[8]Faculty of Veterinary and Animal Science, Gomal University, Dera Ismail Khan, Pakistan

*Correspondence to: Mirni Lamid, E-mail: mirnylamid@fkh.unair.ac.id

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Introduction

Plants have long been recognized by the public for their medicinal properties. Traditional medicine is well-known and has been used for generations by Indonesians [1]. Traditional medicine is generally prioritized for maintaining human health, although some applications also aim to treat diseases [2]. One of the medicinal plants frequently used is the curry leaf (*Murraya koenigii*). This plant, a type of spice in the Rutaceae family, is commonly found in Indonesia, especially in the Aceh and West Sumatra regions [3]. *M. koenigii* is known as "Temurui" in Aceh and is used as a spice and food flavoring.

Curry leaves contain several chemical components that have been scientifically shown to have beneficial properties, including antidiabetic, larvicidal, antianxiety, antioxidant, and antibacterial effects. Abeysinghe et al. [4] reported that ethanol, methanol, and chloroform extracts of curry leaves exhibit antibacterial and antifungal activity. Crude extracts from curry leaves using solvents, like methanol, petroleum ether, acetone, ethyl acetate, chloroform, and water, have antibacterial and antifungal activity against *Candida utilis, Shigella sonnei*, *Salmonella typhi*, *Bacillus subtilis*, and *Escherichia coli* [5]. Multiple studies have shown that the diverse bioactive chemicals in curry leaves have therapeutic potential.

Aziman et al. [6] identified flavonoid compounds in the ethanol extract of curry leaves. These substances have the potential to be utilized in the food and pharmaceutical industries due to their recognized therapeutic effects. The various secondary metabolite compounds found in curry leaves have the potential to be further developed as sources of medicinal ingredients [4]. Flavonoids, a broad class of plant polyphenolic chemicals, are found in a wide range of food items [7]. Flavonoids are composed of 15 carbon atoms in the basic carbon skeleton that form a C6-C3-C6 configuration with 2 benzene rings (C6) joined to a propane chain (C3) [8]

Flavonoids act as antibacterials by interfering with the function of bacterial microorganisms. Flavonoids slow bacterial growth by altering the permeability of lysosomes, microsomes, and bacterial cell walls [9]. Quercetin, a flavonol compound and flavonoid derivative, has a 3-hydroxyflavone skeleton. The presence of a hydroxyl group (-OH) in the quercetin structure gives rise to various bioactivities, including antioxidation [10]. Specifically, the radical scavenging mechanism occurs by donating hydrogen atoms from the hydroxyl group, giving compounds with hydroxyl groups antioxidant bioactivity [11].

High-performance liquid chromatography (HPLC) is a technological advance that has facilitated quercetin analysis. HPLC is an instrument that is used in qualitative separation analysis techniques [12]. Given the potential pharmacologic activity of quercetin, there is a need to analyze the quercetin level in curry leaves using HPLC. Therefore, this research aimed to provide scientific data regarding the use of curry leaves as an herbal medicinal ingredient.

Materials and methods

Research design

This study was conducted from August to October 2023. The curry leaf extract was prepared in the Pharmacology Laboratory (Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia). Analysis of quercetin using HPLC was performed in the Pharmacology Laboratory (Faculty of Pharmacy, Universitas Airlangga).

Preparation of curry leaf ethanol extract

Five hundred grams of powdered curry leaves were placed in a maceration vessel. Then, 2000 ml of 96% ethanol were added until the simplicia was submerged. The mixture was kept in a closed vessel for 3 d, protected from direct sunlight, and stirred occasionally. Filtration was performed every morning, afternoon, and evening (3 × 24 h) to obtain the liquid ethanol extract. The residue from the maceration process was re-macerated using 1500 ml of 96% ethanol, stirred, and left for 3 d.

The obtained liquid ethanol extract was mixed with the results of the first re-maceration filtration. The residue from the first re-maceration was re-dissolved in 1200 ml of fresh 96% ethanol, stirred, and left for 3 d. A thick ethanol extract was produced by combining the filtered product and evaporation using a rotating vacuum evaporator.

Determination of the quercetin level in curry leaf ethanol extract using HPLC

The maximum wavelength of quercetin was determined using a UV-Vis spectrophotometer (HACH DR6000, Germany) to run a quercetin solution at a concentration of 3 ppm in the 200–700 nm wavelength range, yielding a maximum wavelength of 260 nm. Qualitative analysis was performed by comparing the retention time of the sample solution chromatogram with the reference standard solution of quercetin under identical HPLC conditions. The quercetin standard was weighed at 10 mg and dissolved in 10 ml of methanol (1000 ppm stock solution). Then, 0.25 ml was pipetted and diluted to the desired concentration (50 ppm) in a 5-ml measuring flask using methanol as the solvent.

The 100-ppm comparison standard solution was prepared by pipetting 0.6, 0.8, 1, 1.2, and 1.4 ml into separate 5-ml measuring flasks and diluted with solvent to the mark line, yielding concentrations of 5, 15, 25, 50, and 100 ppm, respectively. Each solution was passed through a 0.45-µm filter membrane and sonicated for 20 min. Next, 60 µl of each solution was injected into the HPLC apparatus at room temperature using a 50:50 methanol mobile phase at a flow rate of 1 ml/min. The chromatogram was recorded and a calibration curve was created between the peak area and concentration.

A total of 0.25 g of curry leaf ethanol extract was carefully weighed and placed in a 100-ml round bottom flask. The sample was then ultrasonicated for 2 h at room temperature, followed by the addition of 5 ml of 6 M HCl and refluxed for 2 h at 90 °C. After cooling to room temperature, the sample was filtered through a 0.45-µm nylon membrane into an HPLC vial. The quercetin content in the sample was then calculated.

Results and discussion

This study aimed to determine the quercetin level in curry leaf ethanol extract using HPLC. An extraction process was carried out to extract quercetin from the curry leaf samples. Curry leaf simplicia was extracted using the maceration method with 96% ethanol as the solvent. The use of 96% ethanol is based on the similar solubility properties with quercetin [13], which is a polar polyphenolic compound that requires a polar solvent for dissolution.

The maceration procedure is particularly advantageous because in addition to being inexpensive and simple to apply heat-sensitive compounds are preserved [14]. This method is modified to account for the chemical and physical characteristics of flavonoids, the class of compounds found in curry leaves [15]. Quercetin, a member of the flavonol group, is a flavonoid that is easily oxidized at high temperatures and lacks heat resistance [16].

A rotary vacuum evaporator was used to evaporate the maceration results until a thick extract was produced. As shown in **Table 1**, the thick extract obtained weighed 52.613 g, yielding a soaking result of 9.50%. The percent immersion method calculates the amounts of secondary metabolites carried by the solvent, not the type of chemical carried [17].

The first step in the HPLC measurement of quercetin in curry leaf ethanol extract is to ascertain the maximum wavelength, which is the wavelength at which the substance absorbs light the most [18]. The maximum wavelength

Table 1 Percentage Yield of Curry Leaf Ethanol Extract

Sample	Simplicia Weight (g)	Extraction Yield (g)	Amount of Ethanol Solvent (ml)	Extract Yield (%)
Curry leaves	500	52.613	4700	9.50%

measured was 260 nm, which was previously determined using a UV-Vis spectrophotometer operating in the 200–700 nm range.

Quantitative analysis begins with preparing a standard quercetin solution at a concentration of 50 ppm, which is then diluted to create a series of concentrations (5, 15, 25, 50, and 100 ppm). Each standard solution was filtered with a 0.45-µm filter membrane and sonicated to remove air bubbles. Then, 60 µl of each solution was injected into the HPLC apparatus at room temperature with a flow rate of 1 ml/min using a 50:50 methanol mobile phase at a wavelength of 260 nm. The chromatogram was recorded and a calibration curve was created between the peak area and concentration. The results of standard quercetin measurements are shown in **Table 2**.

Table 2 Standard Measurements of Quercetin

Standard Solution (µl)	Added µl	PPM	Area
5	1000	5.0898	163.48793
15	1000	15.2694	873.67419
25	1000	25.4490	1352.55347
50	1000	50.8980	3310.36597
100	1000	101.7960	7602.09912

The analysis of curry leaf extract samples revealed that samples 1 and 2 had a quercetin level of 0.080 mg/g 0.079 mg/g, respectively. The average quercetin level in the ethanol extract was 0.03% (**Table 3**).

The retention time (RT) was obtained in repeated HPLC measurements of the reference compound and quercetin was detected in the chromatogram from the ethanol extract of curry leaves. The chromatogram showed that quercetin in sample 1 had an RT of 6.584 min (**Figure 1**), while quercetin in sample 2 had an RT of 6.634 min (**Figure 2**). These results indicated that the curry leave ethanol extract contains flavonoid compounds, especially quercetin.

Flavonoid compounds, such as quercetin, are powerful antioxidants [16]. The ability of antioxidants to scavenge free radicals is associated with an ability to donate protons [19]. The quantity and orientation of the aromatic or hydroxyl groups in the phenolic component affect the number of hydrogen protons that can be donated [20]. The more aromatic hydroxyl groups present, the more effective the aromatic hydroxyl groups are at inhibiting chain reactions in the oxidation process by donating hydrogen atoms or acting as free radical acceptors [21, 22].

As an antioxidant, quercetin can be used in the treatment of cancer and heart disease [23, 24]. Antioxidant activity is found in the leaves, stems, and roots of plants [25, 26]. Curry leaves were selected due to the high chlorophyll content, which is believed to provide potent antioxidant properties [27, 28]. Additionally, quercetin has diverse pharmacologic activities, such as coronary artery dilation, blood fat level reduction, and

Table 3 Results of Quercetin Measurements from Curry Leaf Ethanol Extract Samples

Sample	Retention Time	Sample Area	Sample Weight (mg)	Sample ppm	Quercetin Level (mg/g)	Quercetin Content (%)	Average (%)
Curry leaf extract	6.584	218.98546	258.8	8.033	0.080	0.03	0.03
	6.634	207.46503	250.6	7.887	0.079	0.03	

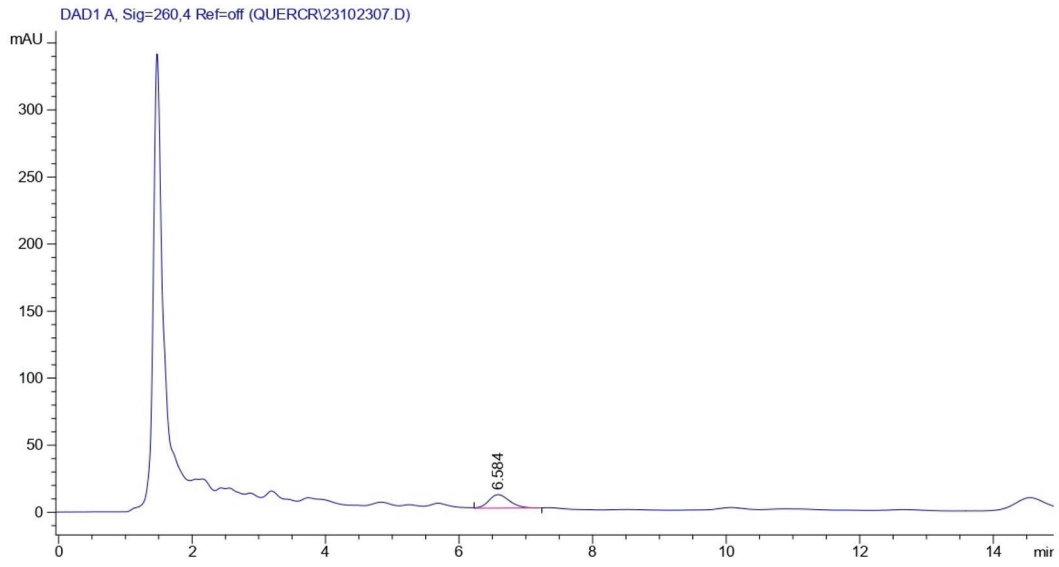

Figure 1 Spectrum analysis results using HPLC from curry leaf ethanol extract sample 1.

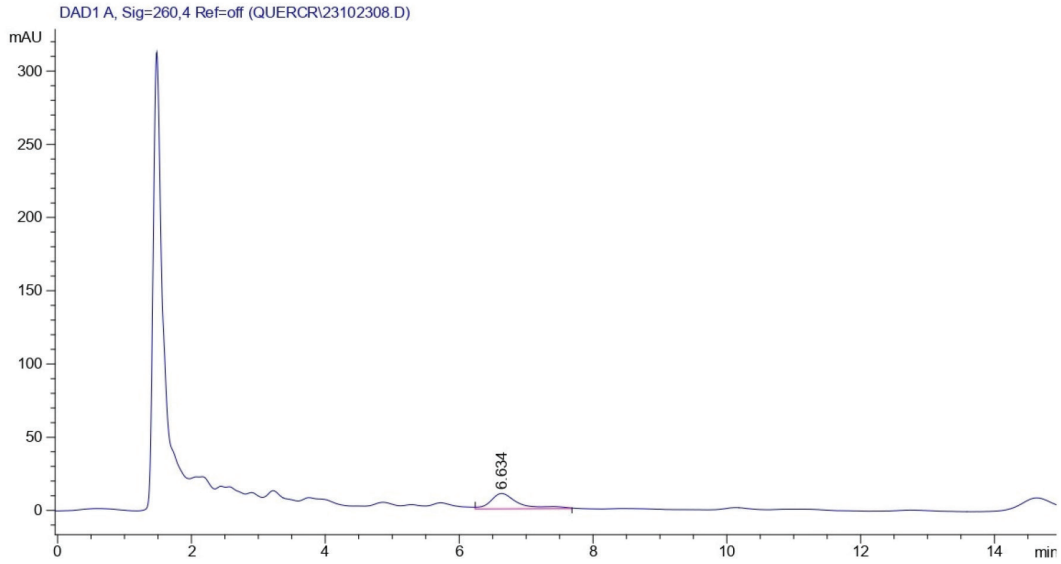

Figure 2 Spectrum analysis results using HPLC from curry leaf ethanol extract sample 2.

anti-platelet, anti-cancer, antioxidant, anti-anemia, anti-inflammatory, and anti-anaphylaxis effects [29, 30].

The results of the analysis with respect to the quercetin level in curry leaf ethanol extract are essential for the development of future medicines and public knowledge. The results of this study provided a basis for further research to expand the use of medicinal plants, especially curry plants, in the future.

Conclusion

The research findings indicated that quercetin is present in the ethanol extract of curry leaves. The average quercetin content in the extract was 0.03%.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Ethics statement

This study involved the use of curry leaves, so ethical approval was not required.

Authors' contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved the manuscript for publication.

Data availability

We do not wish to share our data before we have thoroughly analyzed it. All data sources described in this study are directed at the corresponding author.

Disclosure

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