

Phytochemical Analysis of Curry Leaf Extract (*Murraya koenigii* L.) as a Potential Animal Feed and Medicinal Ingredient

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ABSTRACT

Herbal plants have been used for centuries as traditional medicine to treat various diseases. Green plants generally contain phytochemical compounds, such as vegetables and plants that add aroma to dishes, one of which is curry leaves (*Murraya Koenigii*). This research aims to identify the phytochemical compounds contained in curry leaves. This research was carried out from August 2023 to October 2023. The curry leaves that were obtained were converted into extract form and then the extract was tested for the content of alkaloids, flavonoids, saponins, phenolics and tannins. The research results showed that curry leaf extract contained 23.73% alkaloids, 1.24% flavonoids, 8.74% saponins, 4.4% phenolics, and 5.2% tannins. Alkaloids in plants have a role as a defense against biotic and abiotic disorders. The benefits of flavonoids in plants include anti-mutagenic, anti-inflammatory, antioxidant and anti-carcinogenic. Saponins have various benefits in the health sector, including being able to reduce cholesterol concentrations in the blood. Polyphenols have good antioxidant power because this group can provide electrons to neutralize free radical electrons formed in the body. Tannins also consist of polyphenolic compounds which have antibacterial, antioxidant and astringent activities. The results of the analysis regarding the content of secondary metabolite compounds in curry leaf extract play an important role in the development of future medicines and need to be carried out to provide knowledge to the public. This study can be a basis for bioactive content for further research to expand the use of medicinal plants in the future, especially curry plants.

Keywords: Curry leaf extract, Medicine, Phytochemicals, Plant, Human health.

INTRODUCTION

Research in the field of phytochemistry continues to develop from year to year for the sustainability of human health. Thousands of new compounds have been discovered every year in the development of drugs from natural ingredients, especially plants.¹ Plants can create secondary metabolites that can be used as pesticides, fragrances, colors, antioxidants, food enhancers, and medications, including ones that treat hypertension.² There are 150,000 secondary metabolites that have been identified and 4000 new secondary metabolites per year. Phytochemicals are natural bioactive compounds that produce physiological actions in the human body, interacting with nutrients and fiber will protect the human body against disease.³ Phytochemicals that are highly significant include alkaloids, flavonoids, tannins, saponins, and phenols.

Green plants generally contain phytochemical compounds, such as vegetables and plants that add aroma to dishes, one of which is curry leaves (*Murraya Koenigii*).⁴ Temurui or what is known as the curry plant (*Murraya koenigii* (L.) Spreng) belongs to the Rutaceae family.⁵ This plant comes from India and Sri Lanka and thrives in tropical climates. The curry plant is a plant typical of India, Sri Lanka and several regions in Southeast Asia such as Indonesia.⁶ These curry leaves are often found in Aceh Province, where they are known in the regional language as "temurui leaves". The majority of Acehnese people use the curry plant as a cooking spice. Traditionally, curry plant has also

been used as a treatment for rheumatism, wounds, dysentery, diarrhea and snake bites.⁷

Even though this plant is found quite often, in Indonesia this plant has not been widely used by the public. Its use is often found in the people of Aceh, West Sumatra, and to a small extent in Java.⁸ For Indonesia, another challenge is that most of the raw materials for this medicine still depend on imports. Nearly 95% of medicinal raw materials are imported from various other countries.⁹ A number of studies have proven that there are various bioactive compounds contained in curry leaves which provide medicinal properties. Exploration carried out by Jelita et al. (2018) found alkaloid, flavonoid, saponin, phenolic and tannin compounds in the ethanol extract of curry leaves.¹⁰ These compounds are known to act as medicinal ingredients so they have the potential to be used in the food and pharmaceutical industries. The various secondary metabolite compounds explored in curry leaves have the potential to be further developed to find sources of medicinal raw materials.

Many chemical compounds found in *M. koenigii* leaves have been reported to have benefits as bioactive compounds, such as antidiabetic, larvicidal activity, antianxiety, antioxidant and antimicrobial activity.⁴ Curry leaves (*Moringa koenigii* (L.) Spreng) are compound leaves and the leaf shape is pinnate. The shape of curry leaves is almost the same as bay leaves, only the size is smaller and the smell is sharper than bay leaves. Curry trees have small, white flowers, blackish-brown fruit, long stalks with an odd number of leaves on each stalk, and a

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maximum height of 4-6 meters. Curry leaf stems have a dark brownish green color, while immature leaves are light green and fully grown leaves are dark green in color.¹¹

Curry leaf extract as a producer of bioactivity has been widely known and reported in developed countries and is known to be active as an antitumor, antioxidant, antimutagen, anti-inflammatory, antidiabetic, antidysentery, stimulant and antibacterial. This research aims to identify the phytochemical compounds contained in curry leaves. It is believed that this study's findings would help curry leaves become a more viable traditional therapeutic component.

MATERIALS AND METHODS

Research design

This research was carried out from August 2023 to October 2023. The preparation of curry leaf extract was carried out in the pharmacology laboratory, Faculty of Veterinary Medicine, Airlangga University, while the phytochemical analysis of curry leaf extract was carried out in the pharmacology laboratory, Faculty of Pharmacy, Airlangga University.

Curry leaf extraction

Extraction of the curry leaves used comes from Sungai Pauh Langsa Barat Village, Aceh. A total of 1 kg of curry leaves is dried for 3 days and then ground using a blender. The resulting powder was soaked in ethanol in a ratio of 1:3 for 48 hours. The extract is filtered and evaporated in a water bath until the filtrate becomes concentrated.

Testing alkaloid levels

The curry leaf extract was weighed as much as 0.5 grams then 200 ml of 10% acetic acid in ethanol was added. The mixture was covered and left for 4 hours, then filtered. A concentrated ammonia solution is added to the filtrate after it has been heated in a water bath to one-fourth of its volume and allowed to settle. Filter the sediment then wash with a dilute ammonia solution. The precipitate is an alkaloid, dried and weighed.

Testing flavonoid levels

Carefully weigh 1 gram of curry leaf extract, put it in a measuring flask. Add 1.0 ml of 0.5% (w/v) hexamethylenetetramine solution, 20 ml of acetone and 2.0 ml of 25% (w/v) HCl, reflux for 2 hours from boiling. Strain the mixture using cotton wool into a 100 ml volumetric flask. Rinse the cotton with acetone, add acetone to 100 ml, shake until homogeneous. Put 20 ml of filtrate into a separating funnel, add 20 ml of water. Add 15 ml of ethyl acetate, shake 10 ml, let it separate to get the ethyl acetate phase. Continue extraction (3x) each with 10 ml of ethyl acetate. Combine the ethyl acetate phase, wash 2 times each with 50 ml of water. Put the extraction results into a 50 ml measuring flask. Add ethyl acetate to the mark line, shake until homogeneous

The procedure for determining flavonoid content is to put 10 ml of ethyl acetate fraction solution into a 25 ml volumetric flask. Add 1 ml of AlCl₃ solution (2 grams in 100 ml of glacial acetic acid – methanol (5+95)). Add glacial-methanol acetic acid (5+95) to the volume mark. Determine the blank solution, namely 10 ml of ethyl acetate fraction solution, put it in a 25 ml measuring flask. Add glacial-methanol acetic acid solution (5+95) to the volume mark. To measure flavonoid levels, leave the sample solution for 30 minutes, scan between 300-500 nm. Measure the absorbance of the solution at maximum γ (\pm 425 nm).

Testing saponin levels

Weigh 2 grams of curry leaf extract sample, put it in a measuring cup. Add 100 ml of 20% ethanol, heat in a water bath at 55°C for 4 hours while stirring. After the mixture was filtered, 200 ml of 20% ethanol was

used to extract the residue once again. In a water bath set at 90°C, the mixed extracts were reduced to 40 ml. After pouring the concentrate into a 250 ml separating funnel and adding 20 ml of ether, give it a good shake. Remove the ether layer, take the water phase, add 60 ml of butanol then shake. The water phase was discarded, the nbutanol phase was washed with 10 ml of 5% NaCl solution twice. Collect the butanol phase and put it in a container that has been weighed empty. Evaporate over a water bath until light, dry in the oven. Weigh until constant.

Testing polyphenol levels

The curry leaf extract was weighed 50 mg, dissolved in 50 ml H₂O. Dilution is carried out (5 ml to 20 ml). Pipette a 1.0 ml aliquot of the dilution result, put it in a vial. Then add 0.5 ml of Folin-Ciocalteu. Leave for 5 minutes, then add 2 ml of 10% sodium bicarbonate solution. Leave it for 10 minutes before measuring the absorbance (at γ = 770 nm).

A standard solution of gallic acid with a concentration of 5 to 25 ppm (H₂O solvent) is made. 1.0 ml of each level is pipetted into a vial. Then add 0.5 ml of Folin-Ciocalteu. After five minutes, add 2 ml of the 10% sodium carbonate solution. Leave it for 10 minutes before measuring the absorbance (at γ = 770 nm).

Tannin content testing

50 mg of curry leaf extract was weighed in a 25.0 ml volumetric flask, add water to the mark line. Dilution is pipetted 2 to 10 ml. Pipette a 1.0 ml aliquot, put it in the vial. Then add 0.5 ml of Folin-Ciocalteu. Add 2 ml of a 10% sodium carbonate solution after five minutes of waiting. Leave it for 10 minutes before measuring the absorbance (at γ = 760 nm).

A standard tannin solution with a content of 5 to 40 ppm (H₂O solvent) is made. Pipette 1.0 ml of each level into the vial. Then add 0.5 ml of Folin-Ciocalteu. Leave for 5 minutes, then add 2 ml of 10% sodium carbonate solution. Leave it for 10 minutes before measuring the absorbance.

RESULTS

In table 1, it can be seen that curry leaf extract contains flavonoid alkaloid compounds, saponins, polyphenols and tannins. In the alkaloid examination, a fairly high level of 23.73% was found, which was indicated by a positive alkaloid test with the formation of a white precipitate. When examining flavonoids, low levels were found, namely 1.24%, which was indicated by a positive test for flavonoids with the formation of a pink color. In the saponin examination, a level of 8.74% was found, which was indicated by a positive saponin test that formed consistent foam within \pm 1 minute. When examining polyphenols, a level of 4.4% was found, which was indicated by a positive phenolic test with the formation of a greenish black color. When examining tannin, a level of 5.2% was found, which was indicated by a positive test for tannin with the formation of a greenish orange color.

Absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 422 nm (Figure 1). Each flavonoid standard absorbance value has a different absorbance value, namely 1.00810 and 1.01230. The results obtained from standard curve measurements are that the

Table 1. Levels of each bioactive compound in curry leaf extract.

Sample	Tested compounds	Result	Levels (%)
Curry leaf extract	Alkaloid	Positive	23.73%
	Flavonoid	Positive	1.24%
	Saponin	Positive	8.74%
	Polifenol	Positive	4.4%
	Tanin	Positive	5.2%

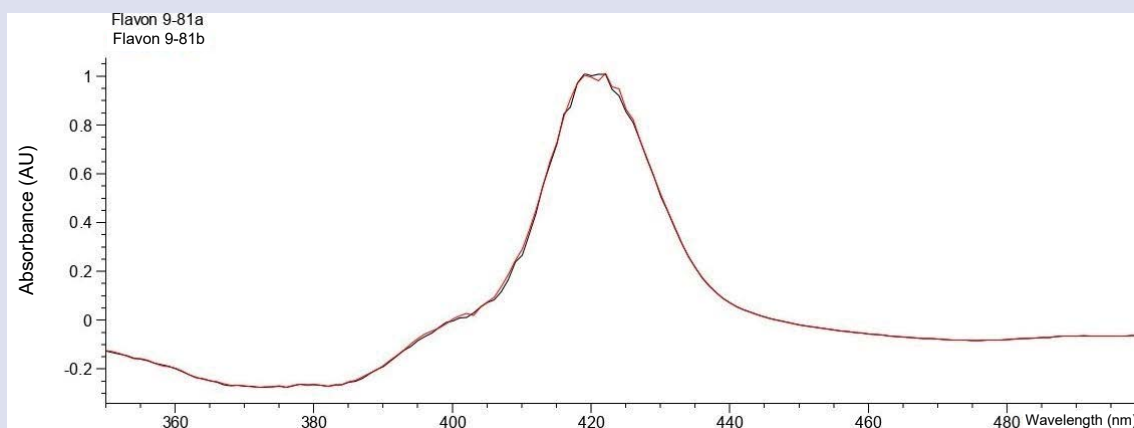


Figure 1. Flavonoid wavelength.

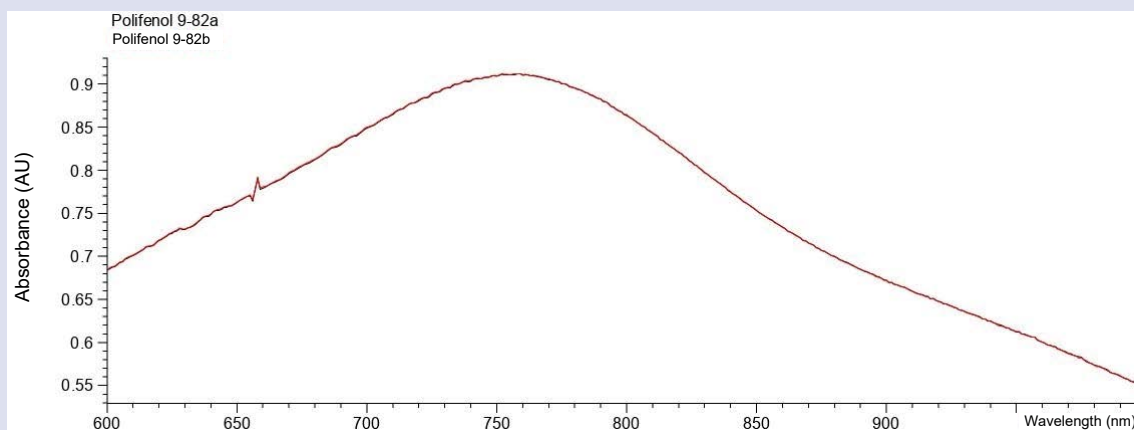


Figure 2. Polyphenol wavelength.

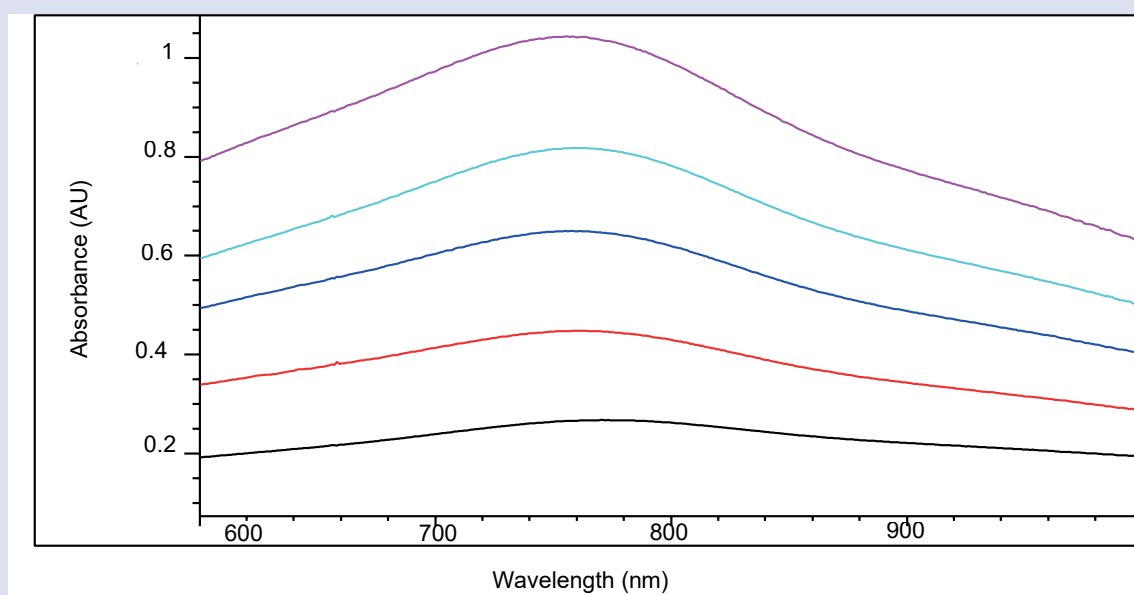


Figure 3. Proses Standard Spectra Polifenol.

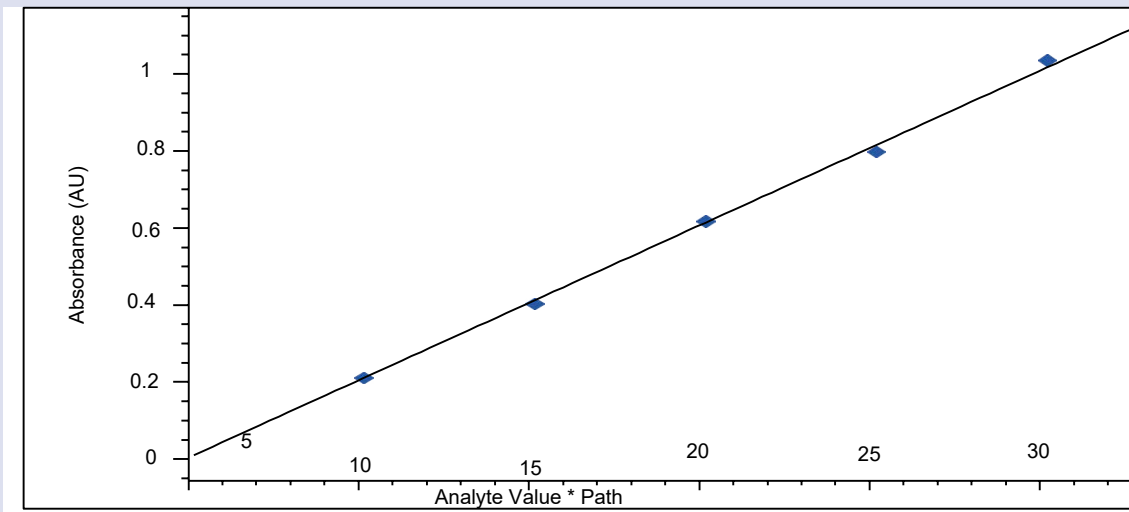


Figure 4. Calibration Curve of Polifenol.

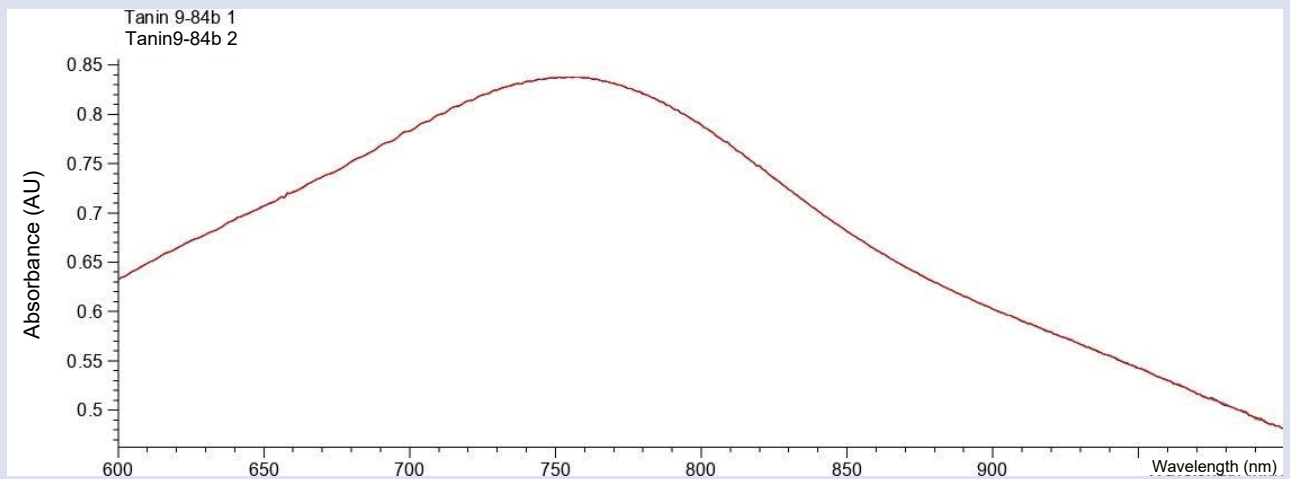


Figure 5. Tannin wavelength.

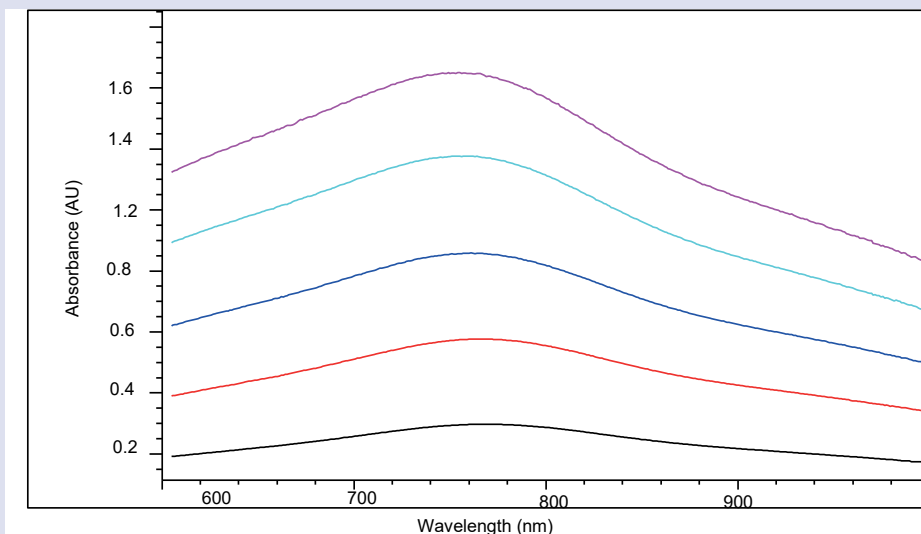


Figure 6. Spectra Standard Process on Tannin.

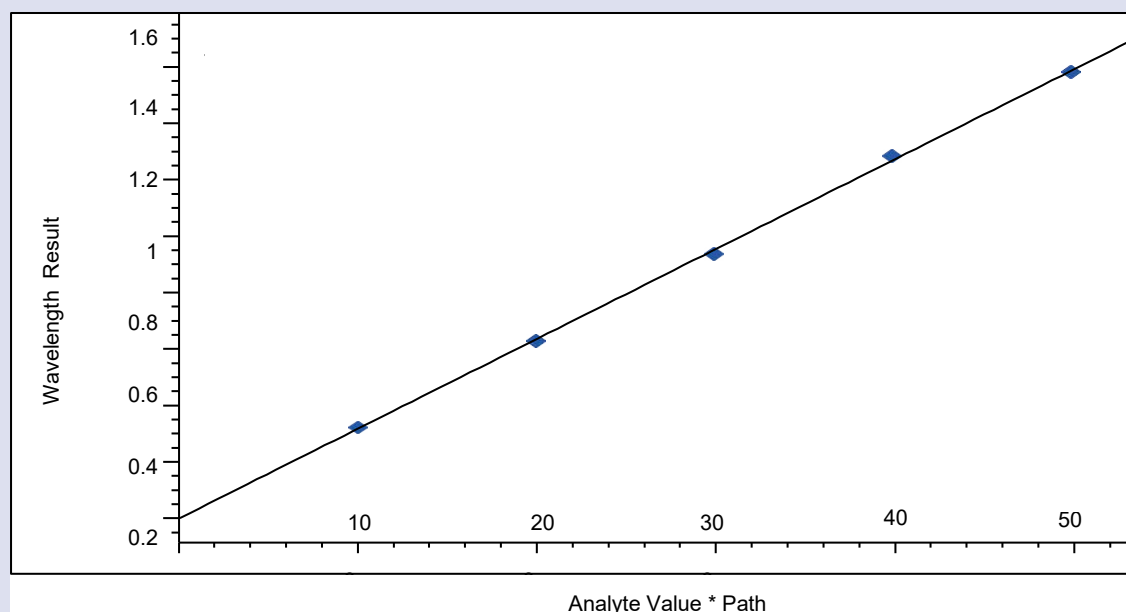


Figure 7. Calibration Curves on Tannins.

higher the concentration value, the higher the absorbance value. The range of total flavonoid levels based on the absorbance value is around 1.24%.

Absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 770 nm. Each polyphenol standard absorbance value has a different absorbance value, namely 0.90513 and 0.90586 (Figure 2). The results obtained from standard curve measurements are that the higher the concentration value, the higher the absorbance value. The range of total polyphenol content based on the absorbance value is around 4.4%.

Absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 760 nm (Figure 5). Each tannin standard absorbance value has a different absorbance value, namely 0.83670 and 0.83662. The results obtained from standard curve measurements are that the higher the concentration value, the higher the absorbance value. The range of total tannin content based on the absorbance value is around 5.2%.

DISCUSSION

Curry leaves (*Murraya koenigii*) are a type of leaf spice with authentic characteristics in Asian Indian cuisine and are used in small quantities to add aroma and to extend the shelf life of food.¹² Curry leaves are a popular plant among the people of Aceh and are often found in Aceh. These leaves are used as a seasoning for various typical Acehnese dishes because they give a delicious aroma and delicious taste to the food. Curry leaves contain water (66.3%), minerals (4.2%), carbohydrates (16%), fiber (6.4%), protein (1%), and fat (1%).¹³ The main mineral content per 100 g of leaves is phosphorus (600 mg), iron (2.1 mg), and calcium (810 mg).¹⁴ The vitamin content is nicotinic acid (2.3 mg), carotene (12,600 i.u.), and vitamin C (4 mg).¹⁵ Meanwhile, it is reported that there are 34 types of essential oil components found in curry leaves, including α -pinene (51.7%), α -humulene, β -pinene (9.8%), β -phellandrene (24.4%), β caryophyllene (5.5%), γ - terpinene (1.2%), sabinene (10.5%), bornyl acetate (1.8%), limonene (5.4%), terpinen-4-ol (1.3%), and contains alkaloids.¹⁶

In this study, the alkaloid content in curry leaf extract was 23.73%. Alkaloids are one of the secondary metabolic compounds produced by plants.¹⁷ This compound is found in many plant organs such as leaves, bark, twigs and seeds. Alkaloids have benefits in the health sector, including being able to increase blood pressure, as a trigger for

the nervous system, reducing pain, as an antimicrobial compound, as a sedative, as a medicine for heart disease and various other benefits.¹⁸ Alkaloids in plants have a role as a defense against biotic and abiotic disorders. Alkaloids are toxic to pathogens and predators. Alkaloids also play a role in helping plants survive stressful conditions due to drought stress, water stress, extreme temperatures, etc. In addition, certain types of alkaloids can attract pollinators, one of which is because small amounts of toxic alkaloids can kill pathogens in pollinators' bodies.¹⁹

In this study, the flavonoid content in curry leaf extract was 1.24%. There are many groups of compounds, including phenolic compounds contained in various plants. This group of phenolic compounds are flavonoids which have purple, blue, red and yellow dyes. The various benefits of flavonoids include anti-mutagenic, anti-inflammatory, antioxidant and anti-carcinogenic.²⁰ Then flavonoids are also able to modulate the function of key cellular enzymes. Flavonoids in plants have various important roles, such as providing color and aroma to flowers and fruit, as signaling molecules, and as detoxification agents.²¹ Apart from that, flavonoids also play a role in protecting plants from biotic and abiotic stress, as well as filtering UV rays, causing Flavonoids are found in many leaf organs of plants such as curry plants.

In this study, the saponin content in curry leaf extract was 8.74%. Saponin is a glycoside that is found in many parts of plants, where its chemical structure consists of glycones and aglycones.²² The aglycone part is sapogenin while the glycon part consists of glucose, fructose and other types of sugar. Saponins have various benefits in the health sector, including being able to reduce cholesterol concentrations in the blood, having high antioxidant activity, and functioning as anti-stress and anti-aging compounds.²³ In curry plants, saponins can be found in various organs and tissues. Many types of plants store saponin in the roots, but saponin can also be found in the leaves. Saponins have a function in plants as protective compounds from abiotic and biotic stresses such as attacks by microbes, insects and herbivorous animals.²⁴

In this study, the polyphenol content in curry leaf extract was 4.4%. One of the secondary metabolic chemicals produced by the metabolism of glucose is polyphenols.²⁵ The hydroxyl group on the benzene ring of this class of chemicals serves as an antioxidant. Polyphenolic compounds have good antioxidant power because this group can provide electrons to neutralize free radical electrons formed in the

body.²⁶ The mechanism of phenolic compounds as antioxidants occurs through the electron donor ability of phenol to pair with free radical electrons in the body. Unpaired free radical electrons will rob or bind body cell electrons as their partners, causing cancer. Antioxidant molecules are naturally found in the body, but the body's ability to produce antioxidants decreases with age.^{27,28}

In this study, the tannin content in curry leaf extract was 5.2%. Tannins are complex organic compounds found in plants as secondary metabolite products.^{29,30} Tannins are composed of phenolic compounds which are difficult to crystallize and separate and contain protein which is difficult to precipitate. Tannins also consist of polyphenolic compounds which have antibacterial, antioxidant and astringent activities.^{31,32,33} In plants, tannins can be found in various organs, such as leaves, stems, roots, fruit and bark. Tannin is stored in cell vacuoles in tissue. According to Margońska et al. (2021), tannin tends to be mostly stored in idioblast cells located in epidermal tissue, the outermost part of the organ. This is because tannin has a defensive function.^{34,35}

The results of the analysis regarding the content of secondary metabolite compounds in curry leaf extract play an important role in the development of future medicines and need to be carried out to provide knowledge to the public. This study can be a basis for bioactive content for further research to expand the use of medicinal plants in the future, especially curry plants.

CONCLUSION

Curry leaf extract contains alkaloids, flavonoids, saponins, polyphenols, and tannins as secondary metabolite chemicals. This makes the curry plant a potential candidate for traditional medicinal ingredients.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

This study used curry leaves as the research object so ethical approval was not required.

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.

AUTHORS' CONTRIBUTIONS

ARK and OSW formulated the study question and developed study design. SRA and SG collected data. MAA and RG analyzed data. WPL and IPH drafted the manuscript. ML interpretation of the results and revised the manuscript. SHW primary responsibility for final content. All authors read and approved the final manuscript.

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