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Metabolite Profiles and Bioactivity in Leaf Development Stages of Curry (*Murraya koenigii* (L.) Spreng)

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ABSTRACT

Murraya koenigii (L.) Spreng, often known as Curry or Kari, has long been used in traditional medicine as a multi-potential medicinal plant and as a food seasoning. Curry possesses bioactivity and is known to be active as an antitumor, antioxidant, antimutagenic, anti-inflammatory, antidiabetic, antidysenteric, stimulant, and antibacterial agent. This bioactivity depends on its metabolite profile, which changes during development. This study aimed to determine the metabolite profile and bioactivity of Curry leaves across developmental stages using GC-MS metabolomic profiling. The results of the research revealed a total of 35 with 19 classified as terpenoids, 6 as esters, 4 as organic acids, 1 to the fatty acid group, and 5 to the unknown class from compounds in young, mature, and old Curry leaves. *Caryophyllene* was the dominant compound in young, mature and old leaves, with anti-inflammatory, antibacterial, anticancer, and germ-fighting activities. In mature leaves, the dominant compound was *1-Methyl-pyrrolidine-2-carboxylic acid,* which functions as a moisturizing agent and humectant agent. Referring to the variety of compounds and bioactivities found in Temurai leaves, they have the opportunity to be developed as medicinal ingredients.

KEYWORDS: *Murraya koenigii* (L.) Spreng, metabolite profile, bioactivity, leaf development.

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I. INTRODUCTION

Murraya koenigii (L.) Spreng, commonly known as Curry or Curry, is a remarkable plant recognized for its extensive medicinal and culinary applications. In Indonesia, it is referred to as "Kari" in Aceh and "Salam koja" in South Sumatra, where it is highly esteemed as a flavoring agent that enhances traditional dishes. Research conducted by Chauhan *et al.* (2017) highlights that Curry has been an integral element of the Indian community for centuries, playing a significant role in Ayurvedic medicine and being recognized as a valuable multi-medicinal resource in ancient Siddha and Unani traditions.

As a medicinal plant, numerous studies have demonstrated that Temurai contains a variety of bioactive compounds. Traditionally, Curry leaves are used not only as a spice but also in herbal medicine to treat various ailments, such as dysentery and diabetes, and as a stimulant. Research indicates that Curry exhibits a wide range of bioactive properties, including antioxidant, anti-mutagenic, anti-tumor, anti-diabetic, anti-dysenteric, stimulant, anti-inflammatory, and antibacterial effects.

Previous research has identified a variety of metabolites in the ethanol extracts of Curry leaves, including flavonoids, alkaloids, saponins, tannins, and steroids. These compounds are well-known for their antioxidant and antibacterial properties, indicating potential applications in both the pharmaceutical and food industries (Aziman *et al*., 2012). Furthermore, Juswardi and Ulya (2023) reported that young Curry leaves have high levels of antioxidants, followed by mature leaves, and the lowest in mature leaves.

Sukma (2018) detected terpenoid compounds in methanol extracts of Curry leaves. Juswardi and Ulya (2023) found hat higher levels of terpenoid compounds were found in older leaves, followed by younger leaves, and the lowest in mature leaves.
It is crucial to recognize that plant metabolites are influenced by their growth and development stages. One plant organ that undergoes significant development is the leaf, which changes in shape and color as it grows. While most leaves are green, some show various colors. Setiawati (2016) notes that chlorophyll levels increase during growth but decline as leaves mature. The chemical composition of leaves also evolves continuously (Sumenda, 2011), impacting photosynthesis and metabolism.
Metabolism encompasses the chemical reactions in cells that convert substances into metabolites, produced via biosynthesis (Blanco and Blanco 2017). Metabolite profiling is used to analyze these compounds in plants. Understanding changes in metabolites during leaf development is essential for selecting leaves for medicinal or culinary uses. This study aims to examine the variations in metabolite profiles at different stages of Curry leaf development.

2. MATERIALS AND METHODS

Plant Materials

Leaf samples of Curry were collected from housing PT GPI, Sekayu, Musi Banyuasin, South Sumatra. The collection site had coordinates of 2°88'43" S and 103°86'10" E.

Instruments and Chemicals

The instruments used in this research were GC-MS Trace TM 1310 ISQ and a vacuum rotary evaporator. The materials needed for this research included distilled water and 96% ethanol.

Preparation of Simplicia

This research involved selecting three stages of Curry leaves based on their development: young leaves (shoots), mature leaves, and old leaves. The leaf samples were dried in the sun, away from direct sunlight, ground, and sieved to obtain a fine powder.

Extraction of Curry Leaves Metabolites

The leaf powder was weighed to 100 gs and extracted using a maceration method with 750 ml of 96% ethanol for three 24-hour periods. The resulting filtrate was evaporated using an evaporator to obtain the extract yield.

Metabolite Profile Analysis with GC-MS Instruments

GC-MS analysis was performed on the 96% ethanol extract of Curry leaves. To prepare for the analysis, 10 ml of 96% ethanol was added to the extract, and 1 µl of this solution was injected into the GC-MS according to the protocols for the GC-MS Trace™ 1310 ISQ.

Gas Chromatography Mass Spectrometry (GC-MS) Data Analysis

The GC-MS results were obtained in the form of chromatograms of the most dominant bioactive compounds, based on the criteria of the highest peak and molecular weight. The data were then compiled using the *PubChem*, *KEGG*, *Spectrabase PlantCye*, and *ChEBI libraries*.

3. RESULTS AND DISCUSSION

Identification of the metabolite profile of Curry leaf ethanol extract based on GC-MS chromatograms detected thirty-five compounds. The identified compounds, their classification, bioactivity, and abundance in developing leaves are presented in Table 1.

Table 1. Metabolite Profile of Curry (*Murraya koenigii* (L.) Spreng) Leaves at Leaf Development Stages (young, mature, and old) and Compound Bioactivities.

Compounds	Class	Leaf Develomment			Bioactivity	References
		young	mature	old		
Caryophyllene	Terpenoids	27.41	22.20	27.60	Anti-inflammatory, antibacterial, anti-cancer and germ-fighting	Maulidya *et al.* (2016)
Phytol	Terpenoids (Diterpenoids)	5.48	8.02	16.58	Antibacterial, antifeedant	Setyaningsih *et al.* (2014)
Hexadecanoic acid, methyl ester	Ester	2.39	-	-	Antibakterial	Karunia *et al.* (2017)

Naphthalene,1,2,3,4,4a ,5,6,8a-octahydro- 4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2à,4aà,8aá)]- or (*a-selinene*)	Terpenoids (Sesquiterpenoids)	13.05	6.45	12.03	Na	-
1-Methyl-pyrrolidine-2-carboxylic acid	Organic acids	4.42	32.10	7.72	Antifungal, anti bacterial	Ojinnaka, *et al.* (2016)
Globulol	Lipids	2.60	1.21	1.25	Antimicrobial	Tan *et al*. (2008)
Isopropyl myristate	Terpenoids	4.69	2.47	4.41	Cosmetic, skin Moisturizer	Pakki, *et al*. (2019)
Benzoic acid, ethyl Ester	Ester	3.16	-	2.51	Antibakterial	Widyasari (2008)
á-Phellandrene	Terpenoids	4.46	5.45	2.62	Anticancer, anti-inflammatory	Gaich *et al*. (2012)
Cyclooctaneacetic acid, 2-oxo-	Organic acids	-	-	1.51	The sensory aspects, Cosmetic	Kalasariya *et al.* (2023)
Propanoic acid, chloro- 2-hydroxy-	Organic acids	-	-	0.89	Antibakterial	Wang, *et al.* (2014)
1,3,7-Octatriene, 3,7-dimethyl-	-	-	-	1.27	Na	-
2-Furanone, 3,4-dihydroxytetrahydro	Ester	-	-	1.09	Anticancer	Sumantri, *et al.* (2014)
1H- Cycloprop[e]azulene, decahydro-1,1,7- trimethyl-4-methylen e-	Terpenoids (Sesquiterpenes)	3.14	-	2.44	Na	-
1,4,7,- Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-	Sesquiterpenes	4.54	4.52	4.62	na -Na	-
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimeth yl-7-(1-methylethenyl)-,[1R-(1à,3aá,4à,7á)]-	Terpenoids	-	-	1.26	Anti-inflammatory, skin moisturizer Anticandida	Hameed *et al.* (2016)
Naphthalene, decahydro-4a-methyl- 1-methylene-7-(1 -methylethenyl)-, [4aR-(4aà,7à,8aá)]-	Terpenoids	7.06	2.54	5.99	na -	-
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7- dimethyl-1 -(1-methylethyl)-, (1S- cis)-	Terpenoids	-	0.52	0.84	na -	-
1-Naphthalenol, decahydro-1,4a- dimethyl-7-(1-methyle thylidene)-, [1R-(1à,4aá,8aà)]-	Terpenoids	3.29	-	2.56	na -	-
Cyclopenta[g]-2- benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	Terpenoids	1.94	0.98	1.74	Parfume, cosmetics	Api and Ford (2019)
Phthalic acid, di(2-propylpentyl) ester	Ester	-	-	1.06	Na	-
2-Trifluoroacetoxydodecane	-	-	0.73	-	Na	-
3-Carene	Terpenoids (Monoterpenes)	-	1.98	-	Anti-inflammatory	Soleh and Margantara, (2019)
Bicyclo[3.1.1]hept-2- ene, 3,6,6-trimethyl-	Terpenoids (Seskuiterpen)	1.24	1.31	-	Insecticides, fungicides	Lunggela, *et al.* (2022)

Cyclohexene, 4-methylene-1-(1- methylethyl)-	Terpenoids (Monoterpenes)	-	1.77	-	Antibakterial	Pratiwi dan Salimah, (2020)
á-Ocimene	Terpenoids (Monoterpenes)	-	1.19	-	Parfume, flavor	Yunez. (2021)
Cyclohexane, 1-ethenyl-1-methyl- 2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	Terpenoids (Monoterpenes)	-	3.46	-	Antibakterial	Pratiwi dan Salimah, (2020)
Bicyclo[7.2.0]undec-4- ene, 4,11,11-trimethyl- 8-methylene-	Terpenoids (Seskuiterpenes)	-	1.00	-	Insecticides, fungicides	Lunggela, *et al.* (2022)
Guaia-1(10),11-diene	Terpenoids	-	1.34	-	Na	-
n-Hexadecanoic acid	Organic acids	-	0.77	-	Antifungal	Warsinah, *et al.* (2011)
1-Methoxymethoxy-hexa-2,4-diene	Alkoxy	1.68	-	-	Na	-
1-Butanol, 3-methyl-, formate	-	3.44	-	-	Na	-
2-Isopropenyl-4a,8- dimethyl-1,2,3,4,4a,5,6,7 octahydronaphthalene	-	1.54	-	-	Na	-
Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	Ester	1.11	-	-	Anti-cancer, anti-diabetic, anti-inflammatory	Maharini and Fernandes (2021)
trans-13-Octadecenoic acid, methyl ester	Ester	3.35	-	-	Antikanker, antidiabetes	Maharini and Fernandes. (2021)

Table 1 presents 35 compounds identified across three stages of Curry leaf development: young, mature, and old. These compounds belong to various metabolite classes, with 19 classified as terpenoids, 6 as esters, 4 as organic acids, 1 to the fatty acid group, and 5 to the unknown class.

Terpenoids

Terpenoid compounds are a group of organic hydrocarbon compounds that are the main components of essential oils. Essential oils are widely used in perfumes and aromatherapy (Julianto, 2019). Table 1 shows that *Caryophyllene* has the largest total relative area percentage (%) at >27%, and is highest in mature leaves. In Erindyah's (2002) research, pine essential oil contains *Caryophyllene*, a compound from the terpenoid (sequiterpene) group. It has anti-inflammatory, antibacterial, and antimicrobial properties, in addition to being known to have a local anesthetic effect. Research by Fadila *et al*. (2020) revealed that the essential oil contained in Curry leaves is yellow. In general, sesquiterpenoids, monoterpenoids, and aromatic compounds are the main components of plant essential oils.

In addition to *caryophyllene*, several compounds belong to the terpenoid (sesquiterpene) group. One such compound is *3,6,6-Trimethyl-bicyclo[3.1.1]hept-2-ene*, which has a notable abundance of 1.31% in mature leaves. Another compound is *4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene*, found exclusively in mature leaves with an abundance of 1.98%. Both of these compounds exhibit biological activities, acting as insecticides and fungicides. According to Bawa and Perbhawa (2020), several terpenoid compounds, particularly sesquiterpenes, possess antifungal properties. Notably, sesquiterpenes such as *ricin, lubimin*, and *solavetivone* are essential phytoalexins that help control fungal infections in potato tubers (Kúc, 1995).

Curry leaves contain phytol compounds, which are particularly abundant in mature leaves (16.58%). *Phytol* compounds are part of the diterpenoid group and serve as precursors in the formation of vitamins E and K1. Research by Silva *et al*. (2014) demonstrated that *Phytol* compounds have anti-inflammatory effects. Additionally, a study by Mahardika *et al*. (2014) suggests that *Phytol* compounds may function as antifeedants, as they are the dominant component in pangi leaves. *Isopropyl myristate* is another terpenoid compound with notable abundance in young leaves (4.69%). According to Pakki *et al*. (2019), *Isopropyl myristate* derived from kasumba turate (*Carthamus tinctorius* L.) seeds is utilized as a penetration enhancer, which can increase the absorption of drugs through the skin in various studies.

α-Phellandrene is a terpenoid compound found in high abundance in mature leaves, with a concentration of 5.45%. Another terpenoid, *Azulene,* is also present in mature leaves, albeit at a lower abundance of 1.26%. *Cyclopenta[g]-2-benzopyran*, a compound belonging to the terpenoid group, is found predominantly in young leaves, with an abundance of 1.94%. These compounds exhibit various pharmacological activities, including anti-inflammatory and anticancer properties. According to Thangaleela *et al*. (2022), α-*Phellandrene* has been reported to have several biological activities, acting as an antimicrobial, antioxidant, antinociceptive, antitumor, and anti-inflammatory agent.

In addition to the sesquiterpene group, there are several monoterpenes present. One of these, *3-Carene*, is found exclusively in mature leaves, with an abundance of 1.98%. Another compound, *Cyclohexene-4-methylene-1-(1-methylethyl)-,* is also present only in mature leaves at a concentration of 1.77%. Additionally, *α-Ocimene* is found in mature leaves with an abundance of 1.19%. *Cyclohexane-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)*-, in the *[1S-(1α,2α,4α)]* configuration, is found in mature leaves with a higher abundance of 3.46%. Heliawati (2018) notes that monoterpenoids are terpenoids biosynthetically derived from precursors of two bonded isoprenes. Several monoterpenoid compounds are commonly found in small amounts in the essential secretions of many plants.

Finally, there are several terpenoid compounds whose pharmacological activities are still unknown, including *Naphthalene-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-[2R-(2α,4aα,8aβ)]-*, *guaia-1(10),11-diene*, *Naphthalene-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S-cis)-*, *1H-cycloprop[e]azulene-decahydro-1,1,7-trimethyl-4-methylene, 1-Naphthalenol-decahydro-1,4a-dimethyl-7-(1-methyl ethylidene)-[1R-(1α,4aβ,8aα)]*-, and *Naphthalene-decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4aR-(4aα,7α,8aβ)].*

Ester Compounds

One of the most abundant ester compounds is *trans-13-Octadecenoic acid methyl ester*, which is found in young leaves at a concentration of 3.35%. According to Maharani and Fernandes (2021), this compound acts as an antioxidant and antimicrobial and is believed to possess antidiabetic properties.

Another notable ester is *Hexadecanoic acid methyl ester*, commonly known as *Methyl palmitate*. This compound is present in young leaves at a concentration of 2.39%. Padmini *et al*. (2010) reported that *Methyl palmitate* is a fatty acid compound with antibacterial properties. It disrupts cell wall and membrane structures through synergistic mechanisms with various active ingredients, enhancing its antibacterial effects.

Benzoic acid ethyl ester, another ester compound, is found solely in young leaves at a concentration of 3.16%. Additionally, *2-Furanone, 3,4-dihydroxytetrahydro*, which belongs to the ester group, is present only in old leaves at a concentration of 1.09%. The compound *Phthalic acid, di(2-propylpentyl) ester* is also an ester found exclusively in old leaves, with an abundance of 1.06%. Finally, *Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester* is included in the ester group and is found only in young leaves at a concentration of 1.11%.

Organic Acids

The most abundant organic acid compound is *1-Methyl-pyrrolidine-2-carboxylic acid*, found in mature leaves at 32.10%. This compound is used as a moisturizing agent, humectant, and as an important base compound in the manufacture of medicines and cosmetics. Then there is *n-Hexadecanoic acid*, an organic acid found only in mature leaves, with an abundance of 0.77%. According to Maharani and Fernandes (2021), *Hexadecanoic acid* can increase anti-inflammatory and insulin activity in the body, enabling it to act as an anti-diabetic.

Cyclooctaneacetic acid, 2-oxo-, is an organic acid found only in mature leaves, with an abundance of 1.51%. Then there is *Propanoic acid, chloro-2-hydroxy-*, an organic acid found only in mature leaves, with an abundance of 0.89%. Organic acids are organic compounds that contain carboxyl groups (Theron and Lues, 2011). Organic acids can be classified based on the type of carbon chain (aliphatic, alicyclic, aromatic, or heterocyclic), saturation, substitution, and number of functional groups.

Fatty acids

Globulol, the most abundant lipid compound, was found in young leaves at 2.60%. According to Tan *et al*. (2008), *Globulol* exhibited strong antifungal activity against the tested fungi *Alternaria solani*, *Fusarium graminearum*, *Rhizoctonia solani*, and *V. pirina*. Furthermore, *1-Methoxymethoxy-hexa-2,4-diene* was found only in young leaves at 1.68%.

Unknown Group

The five compounds of unknown class are *1-Butanol, 3-methyl-, formate*, *2-Trifluoroacetoxydodecane, 2-2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7 octahydronaphthalene, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-*, and *1,3,7-Octatriene, 3,7-dimethyl-.*

4. CONCLUSION

The metabolite profile study of Curry leaves at different developmental stages revealed a total of 35 compounds identified in young, mature, and old leaves. Among these, 19 are terpenoids, 6 are esters, and 4 are organic compounds. *Caryophyllene* is the predominant compound found in both young, mature and old Curry leaves. This compound is known for its numerous health benefits, including anti-inflammatory properties, antimicrobial effects, and potential anticancer capabilities. In mature leaves, *1-Methyl-pyrrolidine-2-*

carboxylic acid emerges as the main compound, accounting for 32.01% of the profile. This compound is beneficial for moisturizing and moisture retention.

Given the diverse health benefits associated with these metabolites, it is essential to consider the stage of leaf development when selecting them for medicinal or culinary purposes.

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