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Metabolite Profiling of Kirinyuh Leaf (*Chromolaena Odorata* L.) Ethanol Extract using UPLC-MS

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ABSTRACT

Aphthous stomatitis has become one of the oral diseases causing pain in mucous tissue. The cure of aphthous stomatitis needs flavonoid compounds that have antioxidant, antibacterial, anticancer, and antiviral activities. Kirinyuh grows well in Bali province. Although plenty of plants have grown, few investigations have been conducted on kirinyuh leaves, especially for oral disease treatment. This paper aims to examine the compounds in kirinyuh leaves to find whether it has compounds for aphthous stomatitis requirement compounds. This study was performed by metabolite profile of Kirinyuh leaves which indicates the compounds produced by its metabolic process by using the UPLC-MS instrument with ethanol extract. The metabolite profile was begun by making simplisia of the leaves with the heating process in an oven at 50°C and sifted. The powder of kirinyuh then was macerated using ethanol with ration 1:5 for 2 days and sifted with Whatman No.1, evaporated until the ethanol extract was obtained. Afterwards, the metabolite profiling was performed by UPLC-MS separating the metabolite components. The results show that there are 14 compounds in the ethanol extract of Kirinyuh leaves. The major compound in the ethanol extract of Kirinyuh leaves is [(2,2-Dimethyl-6-oxo-3,4,6,7,8,9-hexahydro-2H-cyclopenta[c]pyrano[2,3-h]chromen-10-yl)oxy]acetate with an iFit percentage of 97.56%. This compound is a pyranoflavone turnover compound including flavonoids. Besides, it is also found phenol in kirinyuh leaves. Flavonoids in kirinyuh leaves can be used as herbal plants to treat mouth ulcers. In conclusion, it is found flavonoids in Kirinyuh leaves, a compound needed to cure aphthous stomatitis.
Keyword : kirinyuh; compounds; herbal medicine; UPLC-MS

Introduction

Traditional medicine in Indonesia has several origins, one of which is derived from plants. These herbal plants are widely used by the community as alternative medicine [1]. One of the potential plants as a medicine is the kirinyuh leaf (*Chromolaena odorata*), which is often considered a wild weed [2]. Although in fact, the contents of kirinyuh leaves have important ingredients such as tannins, phenols, flavonoids, saponins, alkaloids, and steroids, as well as essential oils which include α-pinene, cadinene, camphora, limonene, β-caryophyllene, and cadinol isomers [3].

Common diseases in Indonesia are oral diseases, one of them is aphthous stomatitis, an inflammatory oral disease in mucous tissue that causes pains. It happens in 10 – 40 years old people and is found in 10-25% of Indonesians [4]. It is mentioned by Panche, et al (2014) that anti-inflammatory, anticancer, antiallergic, and antiviral compounds need to cure aphthous stomatitis [5]. Many treatments are used to cure aphthous stomatitis such as using antibiotics, topical anesthetic, and steroid therapy, some as well as involving drugs and corticosteroid therapy [6], [7]. However, this therapy has significant side effects for the patients such as impaired wound healing,

skin thinning and atrophy, weight gain, fluid retention, and breathing difficulty [8], [9]. Besides, people nowadays prefer to herbal medicine as shown by the statistic that 70% of world's population of developing countries prefer to herbal treatment [10].

Some herbal medicine mentioned in Hidayat, et al (2021) of the leaves found to have flavonoid such as *Persea americana Mill* with antifungal activity, *Abrus precatorius Linn* and *Camellia sinensi (L.)* with antibacterial activity, *Aloe vera* with anti-inflammation activity. Besides, the fruit of *Psidium guanjava Linn*, *Citrus hystrix DC* also have anti-inflammation and antifungal activities respectively. The rhizome of the plant *Curcuma longa* has also antibacterial activity [11], [12], [13], [14]. However, the activities of the plant compounds are mentioned as incomplete to cure mucous tissue diseases which have anti-inflammatory, and antiviral compounds. Besides, some also don't have flavonoid compounds such as *Aloe vera*, *Curcuma longa* [15].

Kirinyuh leaves have various health benefits, including their use in healing wounds, and anticancer, anti-inflammatory, antimicrobial, and antioxidant properties. Kirinyuh leaves can also accelerate angiogenesis by increasing the number of blood vessels [16]. Compounds obtained from kirinyuh leaves with ethanol extract are associated with anti-inflammatory, antibacterial, and antioxidant activities for its phytochemicals such as flavonoids, tannins, and saponins in terms of healing wounds [17]. In addition, kirinyuh leaves have been shown to have anti-inflammatory effects by inhibiting NO, NF-κβ, p38 MAPK, IL1β, TNF-α, as well as suppressing leukocyte cell migration, reducing edema, and functioning as an analgesic to relieve abdominal pain [18]. Diba et al. (2022) also found that kirinyuh leaves have an antifungal value of 2% - 10% due to the alkaloid content which can inhibit the growth of fungal cell walls [19].

Although kirinyuh leaves are widely found in Indonesia, especially in Bali Province, few investigations on it were performed especially phytochemical analysis. Phytochemical analysis is important to determine the content of chemical compounds and provide information on pharmacological effects and the potential for new drug discovery [20]. Besides as a wound healing medicine that has analgesic and anti-inflammatory properties, Kirinyuh leaves are also expected to be used to cure aphthous stomatitis, which is wounds on the oral mucosa that often occur in dental health problems [21]. The use of chemical drugs often causes side effects and high costs [22], [23], [24]. Therefore, exploration of secondary metabolite compounds in Kirinyuh leaves using the LC-MS method needs to be carried out to obtain information related to the molecules, structure, and identity of sample components.

This paper aims to examine the compounds in kirinyuh leaves to find whether it has compounds for aphthous stomatitis treatment requirements. Thus, if these results are known, it can strengthen the contribution of using Kirinyuh leaves as a traditional medicine for healing aphthous stomatitis.

Methods

The location of this research was at the Laboratory of the Faculty of Food Technology, Udayana, and the Agricultural Analysis Laboratory of the Faculty of Agriculture, Warmadewa University.

A. Population and Sample

Kirinyuh leaves growing in Bali Province became the population object. Meanwhile, research samples were taken from three areas in Bali, namely Jimbaran (Badung), Tabanan, and Bangli, based on the plant's geomorphology and ease of growth.

B. Tools and Material

The tools used in this study include a blender (Philips), stirring rod, 1000 mL glass beaker (Pirex), 200 mL Erlenmeyer flask (Pirex), 100 mL measuring cylinder (Pirex), incubator (Mammert), test tube, maceration container, rotary evaporator (Buchi), receiving flask (Buchi), vial bottle, and UPLC-MS. The materials used in this study were kirinyuh leaves, ethanol, methanol (hypergrade for LC-MS), formic acid (ultrapure for UPLC-MS), acetonitrile (hypergrade for LC-MS), and 0.05% water injection for UPLC-MS.

C. Research Procedure

a. Making simplicia

The kirinyuh leaves taken were mature leaves because the older the leaves affect the secondary metabolite content. Then the leaves are washed under running water. Next, the leaves are chopped into smaller pieces. An oven at 50°C for 24 hours is used in the drying process. After the leaves are dry, the kirinyuh leaf simplicia is made by blending the kirinyuh leaves. The blended kirinyuh leaves are sieved with a 60 mesh sieve.

b. Extract preparation and fractionation

Maceration of kirinyuh leaves in powder form was carried out with ethanol in a ratio of 1:5 for 2 days at room temperature (20–25°C). After that, the filtrate was obtained through Whatman No. 1

filtration. Maceration of the remaining drugs was carried out with 1000 mL of ethanol twice. After this process, the filtrate was obtained and then processed by evaporation with a vacuum rotary evaporator (Buchi, Sweden) at a temperature of 40°C. The evaporation results in a crude ethanol extract from kirinyuh leaves. The ethanol extract of kirinyuh leaves obtained was then tested using LC-MS.

c. Metabolite Profiling

The weighing process was carried out on a 10.00 mg extract sample carefully and dissolved in methanol in a 10 ml measuring flask. A total of 5 µl of the solution was taken with a micro syringe to be injected into the UPLC-MS column. This process was repeated 4 times. The next step was to convert the liquid sample into small drops using a needle that had been given a positive ESI (+) charge. The detector will produce ions which are then separated using a Q-ToF analyzer. The eluent used is a mixture of (A) formate water (99.9:0.1) and (B) acetonitrile formate (99.9:0.1) with a gradient elution system (Table 1), at a speed of 0.2 ml/min. The first appearance on the chromatogram is a polar compound, which is then followed by a low polar compound. Furthermore, these results will be analyzed using a Q-ToF-MS detector to produce a chromatogram peak. Interpretation of the chromatogram peak results was done through the Masslynx application.

Table 1. Gradient elution solvent proportions

Time (Minute)	Mixture A (%)	Mixture B (%)
0.00	95.0	5.00
2.00	75.0	25.0
3.00	75.0	25.0
14.00	0.00	100.0
15.00	0.00	100.0
19.00	95.0	5.0
23.00	95.0	5.0

The results found then were tabulated to show the details of the compounds as well as to see whether the treatment requirement for aphthous stomatitis was found.

Results and Discussion

Metabolite profile analysis of ethanol extract of kirinyuh leaves was carried out using UPLC-MS. UPLC is a more modern and efficient liquid chromatography technique than HPLC, with the ability to separate mixture components down to the molecular level of two-micron analyte particles. The advantages of the UPLC method are that it can reduce the mobile phase by up to 80% and provide results in about 1.5 minutes faster than HPLC. UPLC-MS in this study used an ESI (+) ion source MS detector and a Q-ToF MS analyzer, which offer advantages in terms of selectivity, sensitivity, high resolution, and shorter analysis time [13]. The analysis process begins by injecting the sample into the column, where the separation of metabolite components occurs. The C18 or octadecyl silica column is used as the stationary phase, which effectively separates compounds with varying degrees of polarity from low, medium, and high [14].

The chromatogram of the metabolite profile analysis results was then processed with the Masslynx 4.1 application to determine and predict the molecular formula of each compound. Figure 1 shows a chromatogram from the metabolite profile analysis of ethanol extract of Kirinyuh leaves.

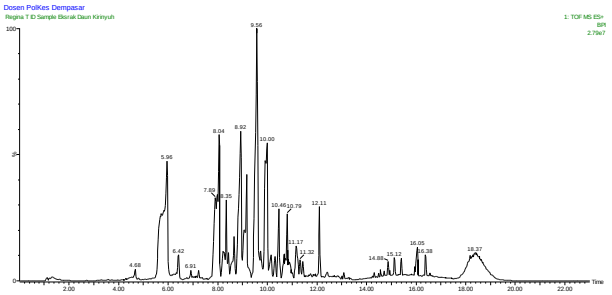

Figure 1. UPLC-MS chromatogram of ethanol extract of kirinyuh leaves

Each peak in Figure 1 indicates the chromatogram represents one compound as shown in Table 2. The axis x shows the retention time and the axis y shows the percentage of the compound or mass response. Retention time shows the amount of time a compound spends in the C18 column after it has been injected. Each of the peaks produced a measured mass such as peak 1 with a retention time of 4.68 produced a mass of 223.0604. Peak 2 with a retention time 5.96 produced a measured mass of 237.0759. By comparing the measured mass value and the calculated mass in the spectra, the molecular formula of the compound can be obtained. It is important to reduce the mass of one hydrogen atom (1.0078) because, during the separation process with the column, there is an addition of hydrogen atoms from the ESI (+) ion bombardment. The predicted molecular formula that has a difference between the measured mass and the calculated mass of ± 0.0005 will be selected. Information from the molecular formula was searched and obtained from the website www.chemspider.com.

Compound content analysis using UPLC-QToFMS showed the presence of 14 compounds in the ethanol extract of Kirinyuh leaves (Table 2). The major compounds were identified with a higher area percentage than other compounds. The major compound in the ethanol extract of kirinyuh leaves is [(2,2-Dimethyl-6-oxo-3,4,6,7,8,9-hexahydro-2H cyclopenta[c]pyrano[2,3-h]chromen-10yl)oxy]acetate with an iFit percentage of 97.56%. This compound is a pyranoflavone derivative and is included in the flavonoid group [25], [26].

This research is in line with Elshamy, et al (2020) that examined the *Cyperus conglomeratus* with ethanol extract with UPLC-qTOF-MS phytochemical profile for gastric ulcers [27]. It shows that the results of UPLC-qTOF-MS resulted in some peaks with retention time in minutes and mass response. The results show that some metabolites are obtained including phenolic acids, flavonoids, stilbenes, aurones, quinones, terpenes, and steroids. The highlight is on phenol and flavonoids which can mediate the cytoprotective effects to reduce the ulcer. It functions as an anti-inflammation in the ulcer. This study also resulted in phenol and flavonoids that confirm the potential to be used as a natural remedy to mucous ulcers of aphthous stomatitis. Another research by El-Din, et al (2022) also shows the results of phytochemical profiling of *Lantana camara L* and *Lantana montevidensis* found flavonoids and phenolic acids which has anticancer activity [28]. This research shows that herbal plants that contain flavonoids is the potential as anticancer agents which also becomes a requirement in ulcer diseases. The kirinyuh leaves with methanol extract have also been examined to reduce calcium in kidney stones. The LCMS/MS shows that it contains tannins, flavonoids, steroids, and tannins. The highest peak has the same result as the study is flavonoids with a retention time of 8.96 [29].

Table 2. Interpretation of metabolite profiling of kirinyuh ethanol extract

Time	Measured	Calculated	Formula	Compound
4.68	223.0604	223.0606	C11H11O5	(2E)-3-(4-Hydroxy-3,5-dimethoxyphenyl)acrylate (hydrocarbon compounds derived from hydroxycinnamic acid, cinnamic acid is a phenol compound)
5.96	237.0759	237.0763	C12H13O5	3,4,5-Trimethoxycinnamate (phenol derivative compound)
6.42	317.1031	317.1025	C17H17O6	[(4,8,8-Trimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-5-yl)oxy]acetate (flavone derivative compounds include flavonoids)
6.91	347.1127	347.1131	C18H19O7	{3,5-Dihydroxy-4-[3-(3-hydroxy-4-methoxyphenyl)propyl]phenoxy}acetate (phenol derivative compound)
7.89	331.1186	331.1182	C18H19O6	[(4-Ethyl-8,8-dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-5-yl)oxy]acetate (flavone derivative compounds include flavonoids)
8.04	331.1179	331.1182	C18H19O6	[(4-Ethyl-8,8-dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-5-yl)oxy]acetate (flavone derivative compounds include flavonoids)
8.92	373.1287	373.1287	C20H21O7	4-[(2,4-Dimethoxy-3,6-dimethylbenzoyl)oxy]-2-hydroxy-3,6-dimethylbenzoate (benzoyl derivative compound)
9.56	343.1182	343.1182	C19H19O6	[(2,2-Dimethyl-6-oxo-3,4,6,7,8,9-hexahydro-2H-cyclopenta[c]pyrano[2,3-h]chromen-10-yl)oxy]acetate (flavone derivative compounds include flavonoids)

10.00	343.1334	343.1338	C19H21O6	(1R,2R,5S,8S,9S,10R,11S,12S)-5,12-Dihydroxy-11-methyl-6-methylene-16-oxo-15-oxapentacyclo[9.3.2.1^{5,8}.0^{1,10}.0^{2,8}]heptadec-13-ene-9-carboxylate (carboxylic acid derivative compound)
10.46	359.1133	359.1131	C19H19O7	2-Hydroxy-4-[(2-hydroxy-4-methoxy-3,6-dimethylbenzoyl)oxy]-3,6-dimethylbenzoate (benzoyl derivative compound)
10.79	293.2105	293.2117	C18H29O3	8-{(1R,2R)-3-Oxo-2-[(2Z)-2-penten-1-yl]cyclopentyl}octanoate (caprylic acid derivative compound)
11.17	275.2025	275.2011	C18H27O2	(9E,11E,13E,15E)-9,11,13,15-Octadecatetraenoate (fatty acid derivative compounds)
11.32	275.2018	275.2011	C18H27O2	(9E,11E,13E,15E)-9,11,13,15-Octadecatetraenoate (fatty acid derivative compounds)
12.11	345.1338	345.1338	C19H21O6	(1R,2R,5S,8S,9S,10R,11S,12S)-5,12-Dihydroxy-11-methyl-6-methylene-16-oxo-15-oxapentacyclo [9.3.2.1^{5,8}.0^{1,10}.0^{2,8}] heptadec-13-ene-9-carboxylate (carboxylic acid derivative compound)
14.88	609.2726	609.2700	C34H41O10	Unknown
15.12	609.2716	609.2713	C35H37N4O6	Unknown
16.05	623.2875	623.2883	C37H35N8O2	Unknown
16.38	607.2909	607.2920	C36H39N4O5	Unknown
18.37	896.7675	896.7680	C53H98N7O4	Unknown

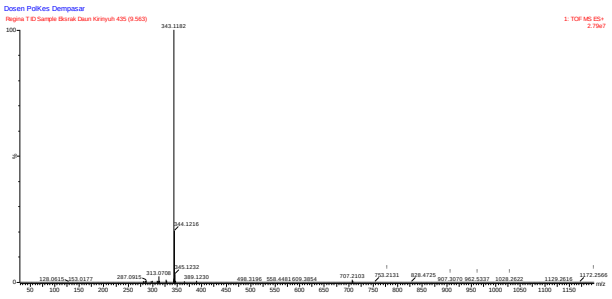

Figure 2. Spectra and chemical structure of major compounds in ethanol extract of kirinyuh leaves

Figure 2 shows the spectra and chemical structures of the major compounds. Secondary metabolite compounds have been proven to be contained in the ethanol extract of kirinyuh leaves, which have health benefits. Testing using UPLC-MS showed that this extract contains several secondary metabolite compounds, including phenol and flavonoid groups. The presence of flavonoids in this plant extract offers potential as a solution in the health sector [30], [31], [32].

The results revealed that water and ethanol extracts of Kirinyuh leaves, obtained from Badung, Tabanan, and Bangli, have active substances such as phenols, and flavonoids. Phenols and flavonoids are semipolar compounds that are soluble in semipolar solvents such as ethyl acetate. The choice of solvent for extraction is an important factor because it affects the type of active components that can be extracted because solvents have different selectivity abilities to dissolve active compounds [33], [34], [35].

Alt hough flavonoids are polar compounds, they are more abundant in ethyl acetate fraction which is a semipolar solvent [36]. Aquadest is a polar compound that can only dissolve polar compounds so phenol and polar flavonoid components tend to be more soluble in ethanol compared to water, which takes longer to dissolve [37], [38].

Ap hthous stomatitis is known as an ulcerative disease in the oral mucosa. It appears with painful shallow ulcers, rounded shape with erythematous haloes as well as yellow or grey pseudomembranous floors. Aphthous stomatitis affects labial, and buccal mucosa as well as the tongue. It creates uncomfortable conditions for those who suffer from

it. Aphthous stomatitis is typically treated using topical and systemic therapies to improve the function and the life quality of the patients by reducing the pain also the size of the ulcer, besides also reducing the recurrence frequency [39], [40].

To reduce the pain of mucous tissue aphthous stomatitis, it is needed such as anti-inflammatory and antiviral compounds. Anti-inflammatory reduces redness, swelling, and pain. Meanwhile, antiviral functions to ease the symptoms and shorten the viral infection. The results of the study found flavonoids the most and phenol besides. Flavonoids functions as an anti-inflammatory, and phenol functions as an antiviral in aphthous stomatitis. Some flavonoids' mechanism as anti-inflammation is to reduce edema [15], and inhibit the prostaglandins that affect the pain [41]. The use of the plant can be extracted and processed into a gel and applied to the mucous ulcer.

Th is research has found Kirinyuh leaves in Bali contain 14 compounds including phenol and flavonoid where flavonoid is the most compound found. Flavonoids and phenol have the functions of anti-inflammatory and antiviral for the treatment of mucous ulcers i.e. aphthous stomatitis. Therefore, this study confirms from the phytochemical investigation that kirinyuh leaves that has the potential as the herbal medicine. Kirinyuh leaves contain phenols, flavonoids, tannins, alkaloids, and saponins which can be used as antimicrobial compounds [42], [43]. It has the potential as an antiinflammation, antioxidant, and antibacterial as well as to cure the wound, not to mention is also potentially used to cure aphthous stomatitis. It is the research of Sari, et al (2020) that a plant containing compounds of flavonoids, alkaloids, terpenoids, and saponins can cure minor aphthous stomatitis, in this case, Bihanong leaves [44]. So as Kirinyuh leaves with those compounds can also be the alternative to cure minor aphthous stomatitis. However, people rarely use Kirinyuh leaves to cure wounds even though it has the potential to wound cure because this plant is often considered a wild plant [45].

Th e growth of Kirinyuh leaves is influenced by the sunlight. Sunlight can influence the production of compounds in the plant. The following research reveals that the production of secondary metabolites is influenced by exposure to sunlight if excessive exposure to sunlight can cause a decrease in the production of secondary metabolites [43]. Exploring the results of compounds in Kirinyuh leaves is expected to maintain Kirinyuh leaves so that they can be an alternative to herbal medicine for dental disease.

Conclusion

Kirinyuh leaves (*Chromolaena odorata*) are leaves that grow plenty in the province of Bali, Indonesia. These leaves have the potential to be used as a wound healing medicine. The results of the interpretation of compound content analysis using UPLC-QToFMS showed that the ethanol extract of Kirinyuh leaves contained 14 compounds most of which contain flavonoids and phenol which function as anti-inflammatory and antiviral for mucous oral disease such as aphthous stomatitis.

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