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Case Report Paper

Thin Layer Chromatography of Secondary Metabolite Profiles and Determination of Total Flavonoid Levels of the Sawi Langit Extract that Growing on Peatlands

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Abstract: Peatland is a type of soil with a high organic content, typically more than 65 percent organic matter, high acidity, and low nutrient content. Vernonia cinerea, also known as Sawi Langit (*Vernonia cinerea L.*) in Indonesia, is one of the nutritious plants. The community uses this herb to treat fevers, wounds, discomfort, and ulcers. The secondary metabolite concentration of plants in peatlands is affected by nutrients such as carbon, nitrogen, and pH. Find out the secondary metabolite content of Sawi Langit leaf extract and the total flavonoid levels that grown on peatlands. The Rf value was estimated after the descriptive observational approach was obtained from the Thin Layer Chromatography (TLC) profile of secondary metabolites with spray reagents, and the quantitative data was determined by UV-Vis spectrophotometry. Sawi Langit plants were found growing on peatlands with a pH of 3.77, N-total of 1.51%, and C-Organic of 4.16%. The best eluent for separating compounds is chloroform: methanol: water (2:5:3) for flavonoid compounds, ethyl acetate: methanol: water (16:1:2) for alkaloid compounds, chloroform: methanol (9:1) for steroid compounds, n-hexane: ethyl acetate (12:8) for terpenoid compounds, ethyl acetic: chloroform: acetic acid (15:5:2) for tannin compounds, and chloroform: ethanol: water (10:6:1) for saponin compounds. Sawi langit leaf extract has a total flavonoid concentration of 5.527 mgQE (Quersetin Equivalent)/g. Flavonoid, alkaloids, steroids, terpenoids, tannins, and saponins compounds were found in the TLC profile of Sawi Langit leaf extract, and the total flavonoid content of the leaf extract of Sawi Langit was 5.527 mgQE (Quersetin Equivalent)/g.

Keywords: Sawi Langit, Thin Layer Chromatography, Total Flavonoids, UV-Vis Spectrophotometry, Vernonia cinerea L.

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1. Introduction

Indonesia is a tropical country and has a variety of flora and fauna. Either, variations sample is the richness of soil types, such as peat soil. Peat soil is soil formed from imperfect decomposition of decaying plant remains with very high organic matter content and little pH [1] in [2]. Peat is a type of soil with high organic content which generally contains more than 65% organic matter with high acidity and nutrients-poor. Peat soil quality is highly dependent on vegetation that produces organic material forming peat soil, mineral material beneath it, environmental factors where peat soil is formed, soil formation process and management process [3].

Secondary metabolites are organic compounds produced by plant metabolism whose production is closely related to environmental factors where they grow. This compound is an indicator of interaction between plants and their environment [4]. Secondary metabolites production is influenced by several factors such as according to Discomo & Tower in [5] which states that light, pH, aeration and microorganisms will affect the production and secondary metabolite compounds.

Secondary metabolite compounds found in plants are bioactive substances related to chemical content in plants. Secondary metabolites are only found in specific organisms and they only produced under certain conditions. Peat lands have differences in nutrients, so it'll affect to secondary metabolites of a plant.

Indonesia is also an archipelagic located on the equator, which makes this country a tropical country that's a lot of biodiversity, especially the Kalimantan Island. One of the nutritious plants is Vernonia genus, including in Indonesia. It can be used as a traditional medicine to treat fever, sores, pain, and ulcers. One species of Vernonia genus is Vernonia cinerea L. or in Indonesia better known as Sawi Langit. Based on [6] this plant contains secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, glycosides, and saponins. In addition, Vernonia cinerea L. contains chemicals such as triterpenes, 24-hydroxytaraxer-14-ene, b-amyrin acetate, b-amyrin benzoate, lupeol and its acetate, beta-sitosterol, sigmasterol and aspinasterol. While, it seeds have fatty oil [7]. These secondary metabolites have pharmacological effects as antipyretic, analgesic, anti-inflammatory, antioxidant, and antibacterial.

Previously, this plant has been digging by [8] in India which has a soil pH in the range of 7.4-8.8 which contains antioxidants with an IC50 value of Vernonia cinerea L. Extract (VCE) against DPPH, superoxide, and nitric oxide free radicals were 429.94, 451.72, and 400.74 g/ml and total phenolic and flavonoid levels in Vernonia cinerea L. Extract (VCE) were found to be 112.41 ± 1.56 mgGAE/g and 13.61 ± 1.82 mgQE/g, but there has been no research on total flavonoid content of Vernonia cinerea L. secondary metabolite content that live on peat lands.

This study aims to determine secondary metabolite profile of Sawi Langit leaf extract (Vernonia cinerea L.) living on peat lands using TLC method and to determine total flavonoid content of Sawi Langit leaf extract (Vernonia cinerea L.) living on peatlands using UV-Vis Spectrophotometry method.

2. Literature Review

Flavonoids are one of a group of secondary metabolite compounds that most commonly found in plant tissues. Flavonoids are included in a group of phenolic compounds with a C6-C3-C6 chemical structure. The flavonoid framework consists of an aromatic ring A, an aromatic ring B, and a middle ring in the form of an oxygen-containing heterocyclic and oxidized form. This ring is used as the basis for the division of flavonoids into their sub-groups. The numbering system is used to distinguish the position of the carbon around the molecule.

The uses of flavonoids are as follows:

1. In plants
   Flavonoids provide protection against environmental stress, plant growth regulator. Protection against ultraviolet radiation and power attract insect pollinators, fungi, viruses, and bacteria, besides as a hormone controller and enzyme. Flavonoids involved in UV filtration, symbiotic fixation and flower pigmentation.
2. In humans
   Flavonoids in humans function as heart stimulants, diuretics, lowers blood sugar levels, and as an antifungal, has a function as a antibacterial, anti-inflammatory, antitumor, anti-allergic, and prevent osteoporosis. Flavonoids can prevent cardiovascular disease by reducing the rate

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of fat oxidation because of its role as an antioxidant. Some research results showed that flavonoids reduce hyperlipidemia in humans. Inhibition of LDL oxidation in cases of heart disease by flavonoids, can prevent the formation of foam cells and lipid breakdown [9]. Flavonoids are compounds with low molecular weight. The basic structure of C6C3C6 is that it consists of 2 benzene rings linked together with 3 carbons. Flavonoids have antioxidant activity in the body so that called bioflavonoids [10]. More than 4,000 flavonoids have been recognized, a lot happens in vegetables, fruits and beverages like tea, coffee and fruit drink.

3. Methodology

3.1. Tool

Tools used in this research are UV-Vis Spectrophotometry, silica GF254, UV light, chamber, rotary evaporator, water bath, glass jar, evaporation cup, volumetric flask, volume pipette, capillary tube, and cuvette.

3.2. Materials

Materials used were Sawi Langit leaves, quercetin, 96% ethanol, ethanol pa, 2% AlCl3, FeCl3, 5% and 10% acetic acid, aquadest, glacial acetic acid, butanol, n-hexane, ethyl acetate, acetone, chloroform, methanol, dragendorf reagent, Liebermann-Burchard reagent.

3.3. Peat Soil Inspection

The test is carried out by checking the content in the peat soil by taking samples of peat soil from several places where Sawi Langit leaves grow. Soil samples were checked at Banjarbaru Industrial Research and Standardization Center (Baristand).

3.4. Simplicity Management and Extraction

Simplicia management is processed starting from collection of raw materials, namely all parts of the leaves that have flowered from the Sawi Langit greens, then wet sorting, washing, chopping or changing shape, then drying at room temperature (20-25℃) and not exposed to direct sunlight. After drying, simplicia is made into powder. Sawi Langit leaf powder was weighed as much as 100 g then macerated with 750 mL 96% ethanol solvent and allowed to stand 3 × 24 hours with occasional stirring. Maceration results were then filtered using filter paper, then residue filtering was macerated again with a new 96% ethanol solvent. Do this until the solvent is almost clear. Afterwards, concentrate in a rotary evaporator with a temperature of 50℃.

3.5. Identification of Secondary Metabolites Using Thin Layer Chromatography (TLC) Method

Prepare GF254 silica TLC plate. Before TLC plate was used, it was activated in an oven at 100°C for 30 minutes. Prepare extract and mobile phase to be used. Prepared test or comparison material solution is smeared on plate (distance between the spots is about 1-1.5 cm) with a certain volume, a distance of 1.5 to 2 cm from the bottom plate edge.

Table 1. Mobile Phase Componen

No	Compound	Eluent	
		Mobile Phase Components	**Comparison**
1	Flavonoids	N-hexane: ethyl acetate	7:3
		Chloroform: methanol : water	2:5:3
		Dichloromethane: ethyl acetate	2:8
2	Alkaloids	Chloroform: methanol: water	2:5:3
		Ethyl acetate: methanol: water	16:1:2
		Chloroform: ethyl acetate	3:1
3	Steroids	Chloroform: methanol	9:1
		Ethyl acetate: n-hexane	8:2
		N-hexane: ethyl acetate: chloroform	5:3:1
4	Terpenoids	N-hexane: ethyl acetate	12:8

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		Ethyl acetate: methanol	16:4
5	Tannins	Methanol: water	6:4
		Ethyl acetate: chloroform: acetic acid 10%	15:5:2
		N-butanol: acetic acid: water	4:1:5
6	Saponins	Chloroform: methanol: water	13:7:2
		Chloroform: ethanol: water	10: 6: 1

3.6. Total Flavonoid Determination Levels

Total Flavonoid Determination Levels are follows:

1) Preparation of quercetin mother liquor
   Prepare 25 mg of quercetin mother liquor, dissolve in 25 ml of ethanol pa (1000 ppm) for 100 ppm in a 2.5 mL pipette and add to 25 mL with ethanol pa.
2) Determination of the maximum wavelength.
   The maximum wavelength was determined by making 60 ppm quercetin then 1 mL of the 60 ppm quercetin solution was reacted with 1 mL of 2% AlCl3 in a test tube. Then 8 mL of 5% acetic acid was added to the solution and the readings were taken at a wavelength range of 370-450 nm.
3) Determination of operating time (OT)
   The operating time was determined by taking 1 mL of 60 ppm quercetin solution in a test tube, then reacting it with 1 mL of 2% AlCl3, and adding 8 mL of 5% acetic acid to the solution. The absorbance of the solution was measured at the maximum wavelength that had been obtained with an interval of 2 minutes until a stable absorbance was obtained.
4) Standard curve creation
   Prepare a standard solution of 1000 ppm quercetin with a pipette and then put it into a 10 mL volumetric flask of 0.4 mL, 0.6 mL, 0.8 mL, 1 mL and 1.2 mL (40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm) then added ethanol pa up to 10 mL. Come by pipette 1 mL and add 2 mL of 2% AlCl3 and 8 mL of 5% acetic acid at each concentration, incubate for OT time. Standard curve was obtained from the measurement of the absorption of standard solution with levels of 40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm at maximum wavelength based on measurement results of standard solution obtained by regression equation.
5) Preparation of sample solution
   Take 50 mg of the extract and then dissolve it in 25 mL of ethanol pa to get 1000 ppm, for 100 ppm it is pipetted 2.5 mL and add to 25 mL with ethanol pa, then pipette 1 mL and add 2 mL of 2% AlCl3 and 8 mL of acetic acid 5%, incubate for OT time, then measure the absorbance at the maximum wavelength obtained and replicate 3 times.

4. Finding and Discussion

4.1. Soil Inspection Results

Soil examination results where the Sawi Langit (Vernonia cinerea L.) leaf grows is peat land. With a pH content of 3.77 which is already in the pH range of 3-5, total Nitrogen (N) is 1.51% which is in the very high category, and Carbon (C)-organic is 4.16% which is in the high category. Peat lands have very high carbon stocks and the availability of N for plants is relatively low because peat soil N is available in the organic N. This causes C/N ratio in peat lands to be relatively high when total N analysis is carried out [11].

Table 2. Soil Inspection Results

No	Test Parameters	Test Result			
		Snippet 1	Snippet 2	Snippet 3	Average
1	pH	3.78	3.79	3.75	3.77
2	Total Nitrogen (N)	1.53%	1.52%	1.50%	1.51%
3	Organic Carbon	4.16%	4.17%	4.15%	4.16%

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4.2. TLC Test

Plants used in this study were all parts of the leaves except flowers stems and roots taken from Sawi Langit (Vernonia cinerea L.) which was already flowering. Sawi Langit leaves are washed and cut into small pieces in order to speed up the drying process at room temperature (20-25℃) that is not exposed to direct sunlight. Dried leaves then made into powder which is then extracted.

Table 3. Secondary Metabolite Test Results of Sawi Langit Extract (Vernonia cinerea L.)

Secondary Metabolites	Mobile Phase	Light	Detection	Spray color	Rf value after spraying
Flavonoids	Chloroform : methanol : water (2:5:3)	Visible light	$AlCl_3$ reagent	Greenish yellow	0.66 0.83 0.98
		UV light 254			0.66 0.83
		UV light 366			0.08 0.16 0.33 0.66 0.83 0.98
Alkaloids	Ethyl acetate : methanol : water (16:1:2)	Visible light	Dragendorf reagent	Orange brown	0.96
		UV light 254			-
		UV light 366			0.96
Steroids	Chloroform : methanol (9:1)	Visible light	Liebermann-Burchard reagent	Bluish green	0.41 0.5 0.63
		UV light 254			0.5 0.56 0.63 0.86
		UV light 366			0.41 0.5 0.63
Terpenoids	N-hexane : ethyl acetate (12:8)	Visible light	Liebermann-Burchard reagent	Red-purple	0.05 0.1 0.16 0.95
		UV light 254			0.05 0.1 0.55 0.61 0.75 0.95
		UV light 366			0.43 0.55
Tannins	Ethyl acetate : chloroform : acetic acid (15:5:2)	Visible light	$FeCl_3$ reagent	Black	0.16 0.96
		UV light 254			0.08 0.21 0.63 0.93
		UV light 366			0.08 0.93

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Secondary Metabolites	Mobile Phase	Light	Detection	Spray color	Rf value after spraying
					0.96
Saponins	Chloroform : ethanol : water (10:6:1)	Visible light	Liebermann-Burchard reagent	Green	0.33
					0.96
		UV light 254			0.58
					0.75
					0.83
					0.96
		UV light 366			0.75
					0.83
					0.96

The extraction process was carried out by means of 100 grams of Sawi Langit leaf powder macerated using 750 mL of 96% ethanol solvent while occasionally stirring. Maceration process was carried out for 3 x 24 hours and the filtrate was macerated again with new 96% ethanol solvent until it was clear because it indicated secondary metabolite compounds contained extract maximally [12]. 96% ethanol is intended so Sawi Langit leaves chemical compounds completely extracted because ethanol is a polar solvent of alcohol group able to extract most of plants chemical content and ethanol also has several properties instance good absorption neutral non-toxic, and difficult to grow molds in ethanol 20% and above [13]. Sawi Langit thick leaves extract obtained was 6 grams with a yield of 6%. It's the weight ratio of viscous extract produced by simplicia weight. The higher of yield extract [14] and the higher of substances content that are attracted to a raw material and according to the yield value is related to the amount of bioactive content contained in plants [15].

Thin Layer Chromatography (TLC) is a method of separating a compound based on difference distribution of two phases, namely stationary phase (plate) and mobile phase (eluent). Determination of the best eluent was carried out using TLC method with eluent variations in secondary metabolites of flavonoids, alkaloids, steroids, terpenoids, tannins, and saponins. Separation by TLC uses a spray reagent or a fluorescent indicator to help fluorescent appearance spots (emitting light) on eluted layer when irradiated with light with wavelengths such as UV light at a length of 254 nm and 366 nm [16]. Eluent was chosen as the best if it had many separate spots and a color change reaction occurred after spraying.

4.3. Determination of total flavonoid levels

Determination of total flavonoid levels in Sawi Langit leaves in this study was based on the formation of a colored solution with the addition of specific reagents that only reacted with flavonoids. Thus the analytical method used can be selective for flavonoids. Quercetin is used as a standard for comparison because quercetin is a type of flavonoid that is commonly used as a standard in determining the flavonoids levels. Which are flavonoids of the flavonol group which have a keto group on the C-4 atom and a hydroxy group on the C-3 or C-4 atom which is neighboring from flavones and flavonols [17] and their glycosides are in the amount of about 60-70% of flavonoids [18].

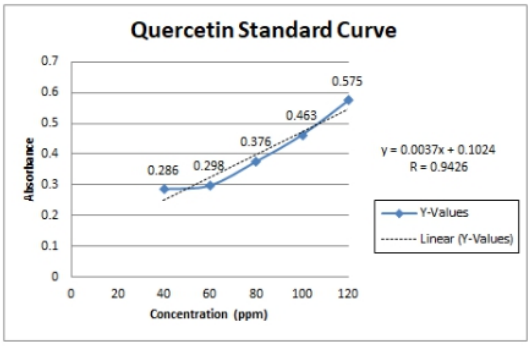

Figure 1.Comparison graph of flavonoid levels standard concentrations with absorbance values

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Total flavonoid determination levels using the method. The principle of the AlCl3 method is the formation of stable complexes with C-4 keto groups as well as at C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition. Aluminum chloride forms acid-stable complexes with orthohydroxyl groups on the A- or B- rings of flavonoid compounds. Total flavonoid range levels based on absorbance value ranges from 0.2-0.8. And the absorbance values obtained in the Sawi Langit leaves extract were 0.400. 0.400. and 0.400 respectively. Results obtained from Sawi Langit leaves extract contain high levels of flavonoids. To calculate it content. The absorbance of sample that has been replicated three times was first calculated and the average was calculated. It sample results that have been obtained are entered into the linear equation y = 0.0037x + 0.1024 with a correlation coefficient of 0.9426 so that the Sawi Langit leaves extract total flavonoid content (Vernonia cinerea L.) is 5.527 mgQE (Quersetin Equivalent)/g. From the determination results of total flavonoid levels from Sawi Langit leaves extract that live on peat lands. It obtained are higher than 5.527 mgQE (Quersetin Equivalent)/g. while research conducted by [8] in India. Total flavonoid content is 1.82 mgQE (Quercetin Equivalent)/g. The peat land in this study has a pH of 3.78 which means that the condition has a high level of acidity. This can affect the content of secondary metabolites especially flavonoids because if the place where the plant grows has a high level of acidity. The plant produces secondary metabolites because secondary metabolites function is one of them to defend themselves from unfavorable environmental conditions. This can be seen in the results of measuring Sawi Langit (Vernonia cinerea L.) the total flavonoid content higher than [8] study.

Table 4. Measurement results of Sawi Langit (Vernonia cinerea L.) Leaf Extract

Absorbance value	**Total flavonoid content (mgQE$^{(\text{Quercetin Equivalents})}$/g**
0.400	5.527

5. Conclusion

Based on research results. Sawi Langit (Vernonia cinerea L.) soil examination is located is peat land with pH. Total nitrogen organic C in accordance with peat lands. Identification results of secondary metabolites from Sawi Langit (Vernonia cinerea L.) leaves extract are secondary metabolites containing flavonoids, alkaloids, steroids, terpenoids, tannins, and saponins. Best eluent used to separate flavonoid compounds is chloroform: methanol: water (2:5:3). For alkaloid compounds it is ethyl acetate: methanol: water (16:1:2). For steroid compounds it is chloroform: methanol (9:1). For terpenoid compounds it is n-hexane: ethyl acetate (12:8). For tannin compounds it is ethyl acetate: chloroform: acetic acid (15:5:2) and for saponin compounds it is chloroform: ethanol: water (10:6:1). Total flavonoid content determination results of Sawi Langit leaves extract (Vernonia cinerea L.) were 5.527 mgQE (Quercetin Equivalent)/g.Based on research results. Sawi Langit (Vernonia cinerea L.) soil examination is located is peat land with pH. Total nitrogen organic C in accordance with peat lands. Identification results of secondary metabolites from Sawi Langit (Vernonia cinerea L.) leaves extract are secondary metabolites containing flavonoids, alkaloids, steroids. Terpenoids, tannins and saponins. Best eluent used to separate flavonoid compounds is chloroform: methanol: water (2:5:3). For alkaloid compounds it is ethyl acetate: methanol: water (16:1:2). For steroid compounds it is chloroform: methanol (9:1). For terpenoid compounds it is n-hexane: ethyl acetate (12:8) for tannin compounds it is ethyl acetate: chloroform: acetic acid (15:5:2) and for saponin compounds it is chloroform: ethanol: water (10:6:1). Total flavonoid content determination results of Sawi Langit leaves extract (Vernonia cinerea L.) were 5.527 mgQE (Quercetin Equivalent)/g.

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