Isolation and Identification of Flavonoid Derivative Compounds from n-Butanol Fraction of Red Pakoba (*Syzygium* sp.)

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ABSTRACT

The red pakoba (*Syzygium* sp.) is a plant originating from North Sulawesi that is often used by local people as a basic ingredient of medicine. This research was conducted to isolate and identify flavonoid derivative compounds contained in the n-butanol fraction of red pakoba fruits (*Syzygium* sp.). The isolation process was carried out using the maceration method, for the separation and purification of compounds using Thin Layer Chromatography (TLC) and Gravity Column Chromatography and obtained F_{2.4} sub-fraction isolates with a sample weight of 4.5 mg. Identification of compound using the Proton Nuclear Magnetic Resonance (H-NMR) and Carbon Nuclear Magnetic Resonance (C-NMR) techniques. The results of identification of isolate F_{2.4} sub-fraction as flavonoid derivative compounds with the proposed name 3-O-methyl-butyrat-6,7,3',4' tetrahydroxy-5-methoxy-8-methyl flavan with molecular formula C₂₂H₂₅O₈.

ABSTRAK

Pakoba merah (*Syzygium* sp.) merupakan tumbuhan yang berasal dari Sulawesi Utara yang sering digunakan oleh masyarakat setempat sebagai bahan dasar obat. Penelitian ini dilakukan untuk mengisolasi dan mengidentifikasi senyawa turunan flavonoid yang terkandung dalam fraksi n-butanol buah pakoba merah (*Syzygium* sp.) Proses isolasi dilakukan menggunakan metode maserasi, untuk pemisahan dan pemurnian senyawa menggunakan metode Kromatografi Lapis Tipis (KLT) dan Kromatografi Kolom Gravitasi dan diperoleh isolat sub-fraksi F_{2.4} dengan berat sampel 4,5 mg. Identifikasi senyawa menggunakan teknik Resonansi Magnetik Inti Proton (H-RMI) dan Resonansi Magnetik Inti Karbon (C-RMI). Hasil identifikasi isolat sub-fraksi F_{2.4} sebagai senyawa turunan flavonoid dengan usulan nama 3-O-metil-butirat-6,7,3',4' tetrahidroksi-5-metoksi-8-metil flavan dengan rumus molekul C₂₂H₂₅O₈.

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Introduction

Red Pakoba an endemic plant from North Sulawesi Province, this plant belongs to the genus *Syzygium* which has similar properties to other plants that have similar Latin names such as *Syzygium aromaticum*, *Syzygium Cumini* (L), and *Syzygium luzonense*. The fruit of this plant is used by the local people as a basic ingredient for making drugs because it has pharmacological functions such as antioxidants and antidiabetic [1-10].

In general, the genus *Syzygium* contains secondary metabolites in the form of flavonoids, alkaloids, tannins and terpenoids

which are used in the world of medicine, mostly, for anti-inflammatory, pain relief and anti-fungal [11,12].

Some active compounds such as myricetin (3-O-(4"-O-acetyl-2"-O-gallolyl)-α-L-rhamnopyranoside) and mearnsetin (3-O-(4"-O-acetyl)-α-L-rhamnopyranoside) which has been isolated from the leaves of *Syzygium cumini* L. [13]. Flavonoid compounds are compounds that are commonly found in plants, no exception to Red Pakoba (*Syzygium* sp.) [14].

To get the compound contained in the red Pakoba fruit (*Syzygium* sp.), the isolation and identification process was carried out. The

isolation stage uses the extraction method, whereas for identification using the Proton Nuclear Magnetic Resonance (H-NMR) and Carbon Nuclear Magnetic Resonance (C-NMR) spectrometry.

Material and Method

Plant sample the Red Pakoba fruit (*Syzygium* sp.) obtained from Laikit Village, located in North Minahasa Regency, size 230-400 mesh silica gel, silica gel 60 GF₂₅₄, butanol, ethyl acetate, dichloromethane, methanol, distilled water, Thin Layer Chromatography (TLC), 10% H₂SO₄ solution in ethanol as stain emerger, and other materials such as Whatman 42 filter paper, aluminium foil, and label paper.

The equipment used was Beaker glass, measuring cup, Erlenmeyer, funnel, pipette, chamber, chromatographic column, vial bottle. A set of re-distillation apparatus, a set of rotary vaporizers (Rotavapor Buchi), Buchner funnel, analytical scale, oven, hotplate, spatula, tweezers, scissors, ruler and pencil. Compound analysis were conducted using JEOL 500 MHz Proton Nuclear Magnetic Resonance (H-NMR) and JEOL 125 MHz Carbon Nuclear Magnetic Resonance (C-NMR) spectroscopy.

Extraction stage

Red pakoba fruit (*Syzygium* sp.) was mashed using a blender, then macerated 7x24 h with ethanol solvent whose level is 70%, allowed to stand for 24 hours until the filtrate is obtained. The filtrate obtained was then evaporated.

Separation and Purification of Compounds

A thick ethanol extract weighed 50 g was added with silica gel weighed 50 g and crushed to obtain powder. The powder was then eluted with 1 L of butanol solvent using a Buchner funnel and vacuum until a filtrate was obtained and evaporated.

The butanol extract obtained was dissolved in a 10 mL methanol solvent and 300 mL petroleum ether then filtered using filter paper yielded petroleum ether filtrate and butanol crude. The butanol krud was chromatographed with columns in a gradient with several volume ratio of ethyl acetate: Methanol. The results of TLC were each

sprayed with 10% H₂SO₄ as a stain emerger. Combination of solvents that produce a good separation pattern on TLC is used as an eluent in gravity column chromatography. The results obtained from column chromatography (eluate) are collected in vials and analysed by TLC. The TLC results obtained (in a vial bottle) are selected which have the same R_f number then combined. Eluate which still has two or more stains on TLC, then will have to be going under column chromatography again. This stage is continued until a pure compound is obtained. The pure compound was then further analyzed using the Proton Nuclear Magnetic Resonance (H-NMR) and Carbon Nuclear Magnetic Resonance (C-NMR) spectrometry.

Result and Discussion

Compound Extraction

2.92 kg of red pakoba was macerated using 70% ethanol for 7 x 24 hours and produced 10.927 litre of filtrate. The filtrate was then evaporated then yield 258.61 g thick ethanol extract of red pakoba (*Syzygium* sp.).

Separation and Purification of Compounds

The first step is weighing 50 g of red pakoba ethanol extract and 50 g of silica gel and crushed evenly until it becomes powder. The powder obtained was then eluted using butanol, yielding filtrate. The butanol filtrate was then evaporated and yielding 15,32 g of butanol extract of red pakoba in the form of solids with a hard texture. Red pakoba butanol extract of 15.3191 g was put in a beaker glass and dissolved in 10 mL of methanol, added with excess Petroleum Ether. The addition of petroleum ether here aims to clean the butanol extract which is coated by a layer of fat so that it has a hard texture. The filtered extract produced filtrate and residue in the form of soft solids, because the fat layer has been successfully removed. The residue obtained was evaporated in a fume hood and a net residual weight of 5.72 g was obtained.

The second step taken was the gradient chromatography column. 5.72 g of butanol Crude was crushed with silica gel until it turned into powder. Soaking silica gel as much as 73.9 g for 24 hours to be used as a stationary

phase. Column process were carried out by ratio of ethyl acetate: methanol eluents which eluted the solvent 7 times in a row. The results of the column are contained in vials of 10 mL each, resulted 37 vials. The eluate obtained was analysed by TLC using volume ratio of the ethyl acetate: methanol (7: 3) eluent. Based on the stain pattern on TLC eluate which have the same stains combined, 7 eluate groups were obtained. The results of TLC on 7 groups of eluate showed that eluate 2 and 3 had the same R_f value, so were eluate 4 and 5, therefore eluate 2 and 3 were combined, and also eluate 4 and 5 were combined so that the number of fractions obtained was 5 fractions (F .1 - F.5). F.2 fraction was chosen for the re-chromatography step because it has a good spot pattern which has 1 stain. F.2 fraction of 0.566 g was dissolved in dichloromethane: methanol (9: 1) sufficiently, column chromatography was performed using the same eluent. The stationary phase used is 74,01 g of silica gel which has been immersed in eluent for 24 hours. Eluate were collected in vials of 5 mL each with 185 vials obtained. The 100-150 vial bottle was re-tested for TLC because it had a stain pattern with similar R_f spacing to find out which fraction had good separation in the presence of one stain, which indicated that the sample was relatively pure and already contained one compound.

The 100-109 vial bottle was selected and then combined and evaporated because it already has a stain separation pattern which indicates that the sample is relatively pure and contains at least one compound.

Analysis of Isolate Spectrum

F2.4 sub-fraction was elucidated using the Proton Nuclear Magnetic Resonance (H-NMR) and Carbon Nuclear Magnetic Resonance (C-NMR) spectrometry. Sample F2.4 weighing 4.5 mg was dissolved in chloroform-D (CDCl₃) solvent. The spectrum was recorded on a 500 MHz JEOL spectrometer for Protons and 125 MHz for Carbon.

Based on spectrum data fraction 2.4 has a framework of flavonoid compound with the chemical shift H-2 (4.56, d, J = 6.5 Hz), H-3 (4.1, m), H-4 (2.94, d, J = 4 Hz & 2.93, d, J = 4 Hz)[15]. The glycosidic bond is shown by the

chemical shift of oxygenated carbon at position C-3 (δ C 69.0) which attached to a methylbutyric acid and was supported by the chemical shift δ H 2.04 (H-2'''), 1.58 (H-3'''), 1.29 (H-4''') and 0.87 (H-5''') which is the chemical shifting regions for methylene, methylene and methyl compounds [16]. The position of methyl butyric acid is bound to C-3, also indicated by the presence of anomeric proton signal H-3 at δ H 4.1 (m). Isolate F2.4 on ring A has proton chemical shifts δ H 7.0 and 6.49 on H-6 and H-8 because it attached to hydroxy (OH) and methyl (CH₃) groups respectively, supported by the presence of NMR-¹³C carbon data on chemical shifts δ C 128.1, 127.3, 125.6. Ring A also has a methoxy group δ H 3.75 (OCH₃) on carbon C-5 supported by NMR-¹³C carbon data on the chemical shift δ C 52.6 [17]. Data tabulation is shown in [table 1](#).

Table 1. Spectrum tabulation H-NMR & C-NMR

Position	Fraction 2.4	
	δ H	δ C
2	4.56 (d, J = 6.5 Hz)	82.7
3	4.1 (m)	69.0
4	2.94 (d, J = 4 Hz) 2.93 (d, J = 4 Hz)	29.8
5	-	145.9
6	-	128.1
7	-	127.3
8	-	125.6
9	-	148.6
10	-	102.7
1'	-	131.5
2'	6.56 (m)	115.2
3'	-	142.4
4'	-	142.4
5'	6.49 (m)	116.1
6'	7.0 (m)	121.0
5-OMe	3.75 (s)	52.6
Position	Methyl-butyrates	
	δ H	δ C
1''	-	173.1
2''	2.04 (m)	44.4
3''	1.58 (m)	25.8
4''	1.29 (t)	22.6
5''	0.87 (s)	22.6

The proposed structure for isolate F2.4 is a flavonoid derivative compound which has a molecular formula ($C_{22}H_{25}O_8$) with a flavonoid which consist of flavan type framework and the proposed structure name is 3-O-methyl-butyrate-6,7,3', 4'-tetrahydroxy-5 -methoxy-8-methyl flavan ([Picture 1](#)).

Picture 1. 3-O-methyl-butyrate-6,7,3', 4'-tetrahydroxy-5 -methoxy-8-methyl flavan

Conclusion

Flavonoid derivatives compound have been isolated and identified from F2.4 isolate sample of n-butanol fraction of red pakoba fruit (*Syzygium* sp) with the proposed structure and name of compound 3-O-methyl-butyrate-6,7,3',4'-tetrahydroxy-5-methoxy-8-methyl flavan by molecular formula ($C_{22}H_{25}O_8$).

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