

Isolation of Flavonoid Compound and Antioxidant Activity of *Salix tetrasperma* Roxb. Leaves

Ria Januarti^{1*}, Adlis Santoni¹, Mai Efdi¹

¹Department of Chemistry, Faculty of Mathematic and Natural Science, Andalas University, Limau Manis Padang, West Sumatera, Indonesia, 25163.

*Corresponding Author: riajanuarti@gmail.com

Abstract

Salix Tetrasperma Roxb. is a plant that found in Indonesia were used as traditional medicine such as diabetes and wound healing. In this study, a flavonoid compound of the ethyl acetate extract of *Salix tetrasperma* Roxb. leaves was isolated by chromatography technique and the antioxidant activity was determined by DPPH assay. The isolation led to obtain 5,7-dihydroxy-3'-methoxyflavone based on NMR spectra. The ethyl acetate extract exhibited the highest antioxidant activity with the IC₅₀ is 65.89 µg/mL. This study shows that the *Salix tetrasperma* Roxb. has good potential as source of antioxidant agent.

Keywords: *Salix tetrasperma* Roxb., flavonoid, antioxidant activity

Abstrak (Indonesian)

Salix Tetrasperma Roxb. adalah salah satu tumbuhan yang ditemukan di Indonesia yang digunakan sebagai obat tradisional seperti diabetes dan obat luka. Pada penelitian ini telah dilakukan isolasi senyawa metabolit sekunder dari ekstrak etil asetat daun *Salix tetrasperma* Roxb. dengan berbagai macam teknik kromatografi. Dari hasil pemurnian diperoleh senyawa 5,7-dihidroksi-3'-metoksiflavan dengan rumus molekul C₁₆H₁₂O₅. Ekstrak etil asetat tumbuhan ini memiliki aktivitas antioksidan yang tinggi dengan nilai IC₅₀ 65.89 µg/mL. Penelitian ini menunjukkan bahwa tumbuhan *Salix tetrasperma* Roxb. memiliki potensi sebagai sumber antioksidan yang baik.

Kata Kunci: *Salix tetrasperma* Roxb., flavonoid, aktivitas antioksidan.

INTRODUCTION

Indonesia is one of a country that has the largest forest area in the world, around 884.950 km², about 46.46% of Indonesia's territory is forest. Forests are an area which are filled with diversity of biological resources. The biodiversity of tropical forests is a storehouse of natural organic which compounds consisting of primary metabolites and secondary metabolites with various types of activity. Primary metabolite compounds including carbohydrates, proteins, fats and nucleic acid while secondary metabolites consist of alkaloids, terpenoids, pirones, acetogenins, lignans, flavonoids and polyketides [1].

Flavonoids are found in all parts of plants including roots, leaves, wood, skin pollen, flowers, fruit and seeds. Most of these flavonoids are in plants, except algae. However, there are also

flavonoids found in animals, for example in the beaver smell glands and bee secretions. The spread of flavonoids is scattered in plant groups, namely angiosperms, chlorophyta, fungi, bryophytes [2].

Flavonoids are polyphenol compounds which contain the skeleton of flavone C₆-C₃-C₆ and consist of flavones, flavonols, flavanone and flavanonols, which are present along with other secondary metabolites in plants. Flavonoids have important role to protect plants from pests and disease [3]. In addition, flavonoids have bioactivity potential as an antioxidant [4], antimicrobial [5], and anticancer [6].

The plants from *Salix* genus are known also contain flavonoids, such as 2',5-dihydroxy-4'-methoxyflavone-7-O-β-D-glucopyranoside that has been isolated from *Salix denticulate* [7].

Salix tetrasperma Roxb is one of the species from the *Salix* genus which belong to Salicaceae family that found in Sumatra. Several studies showed the good biological activities of *Salix tetrasperma* Roxb such as antibacterial, anti-fungal [8], anti-diabetic, antinociceptive, antipyretic [9], anti-inflammatory [10], hypoglycaemic [11], [12], insecticidal, in vivo cytotoxicity [12], laxative, diuretic [13], analgesic, and antischistosomal[14]. These biological activities were influenced by the secondary metabolites such as flavonoid [7] and phenolic compounds [15].

Previous study reported the compounds of the *Salix tetrasperma* Roxb. such as salicin, friedelin, tremulacin, and β -sitosterol[16]. The aim of this study is to isolate the secondary metabolite and antioxidant activity of *Salix tetrasperma* Roxb.

MATERIALS AND METHODS

Materials

Plant material

The leaves of *Salix tetrasperma* Roxb. were collected from Tarusan, Pesisir Selatan and identified at Herbarium of Andalas University with a voucher specimen (SR-01, November).

Chemical material

The chemicals used in the research were hexane, ethyl acetate, methanol, silica gel 60 (230-400 mesh) from Merck, DPPH from sigma Aldrich, and CD₃OD.

Instruments

The general in organic laboratory glassware, Rotary evaporator (Heidolp WB 2000), oven, and melting point apparatus (Fisher John). FT-IR spectra were recorded on JASCO FT/IR-460. UV Spectrophotometer (Secoman S 1000, Thin Layer Chromatography (TLC) (DC-Alufolien Kiesegel 60 F₂₅₄ Merck), Column Chromatography, NMR 500 MHz.

Methods

Isolation

The dried powder of *Salix tetrasperma* Roxb leaf (5 Kg) was extracted by maceration technique with n-hexane, ethyl acetate and methanol solvent, respectively. The extracts were concentrated by the rotary evaporator.

30 g of ethyl acetate extract was separated by column chromatography to obtain 20 fractions (A-T). The J fraction was purified by the trituration method to obtain the pure compound (9 mg, yellow powder).

Antioxidant Activity

1 mL of 50 μ M DPPH solution was added into a test tube containing 3 mL extract in several concentrations (50, 100, 200, 400 μ g/mL). The mixture was allowed to stand for 30 min at room temperature.

The absorbance was measured at 517 nm. The ascorbic acid was used as positive control.

The inhibition percentage was calculated by this formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sampel}}{\text{Absorbance of control}} \times 100\%$$

RESULTS AND DISCUSSION

5,7-dihydroxy,3'-methoxy flavone

Pure compound was obtained solid form with the melting point of the compound is 231-232 °C. The result of the Shinoda test gives a red color which indicates the compound is flavonoid group[17].

The UV spectra data of isolated compound shows maximum absorption in a wavelength (λ_{max} , MeOH) 209; 268; 342 nm. This is a typical spectrum of flavone. The UV-Vis spectra of flavone exhibit two major absorption peaks: band I (usually 300–380 nm) and band II (usually 240–280 nm) [18]. The IR spectrum also showed the characteristic bands for 3337.97 cm^{-1} (V OH), 1494 cm^{-1} (V C=C aromatic), 2883.34 cm^{-1} (V C-H aliphatic), 1921 cm^{-1} (V C=O carbonyl) and 1269,43 cm^{-1} (V C-O alcohol/ether). Determination of structure by analysis of spectra data ¹H-NMR, ¹³C-NMR, DEPT-135, HMBC, HSQC, COSY. Based on the spectra data, the isolated compound was established as 5,7-dihydroxy,3'-methoxyflavone and the structure of isolated compound is shows in Figure 1.

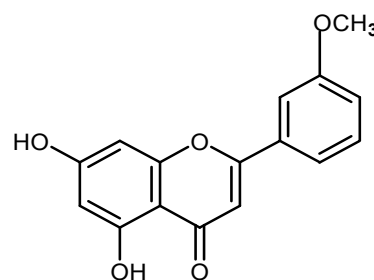


Figure 1. Structure of 5,7-dihydroxy,3'-methoxy flavone

Table 1. ^1H (500 MHz), ^{13}C (125 MHz) NMR data of isolated compound and ^{13}C -NMR data comparative by Gomes, et al [19].

C	Isolated Compound in CD_3OD					Comparative Compound (Acacetin in CDCl_3)
	HSQC	δC (ppm)	δH (ppm)	$J_{\text{H-H}}$ (Hz); multiplicity	HMBC	δC (ppm)
C-2	-C-	162.93	-	-	-	164.0
C-3	-C-H	103.97	6,6	(s)	104 (10)	103.6
C-4	-C-	185.35	-	-	-	182.3
C-5	-C-	163.38	-	-	-	161.4
C-6	-C-H	100.28	6,21	13.5;(brt)	104 (10)	99.13
C-7	-C-	166.24	-	-	-	163.9
C-8	-C-H	95.31	6,47	6.5;1.5;(dd)	104 (10), 159 (9)	94.1
C-9	-C-	159.58	-	-	-	157.8
C-10	-C-	104.33	-	-	-	104.4
C-1'	-C-	123.4	-	-	-	123.3
C-2'	-C-H	110.7	7,49	2.5;(d)	123 (1'), 149 (3')	127.9
C-3'	-C-	149.9	-	-	-	114.3
C-4'	-C-H	121.9	7,53	8;2;(dd)	149 (3')	162.5
C-5'	-C-H	117.9	6,9	8;8.5;(dd)	123 (1'), 149 (3')	114.3
C-6'	-C-H	129.6	7,85	7;2.5;(dd)	162 (2)	127.9
O- CH_3	O- CH_3	56.7	4,0	(s)	149 (3')	55.3

The ^1H -NMR spectrum data (500 MHz) showed one methyl proton signals at δ_{H} 4.0 ppm and seven methine proton signals at δ_{H} 6.6 ppm (H-3); 6.21 ppm (H-6); 6.47 ppm (H-8); 7.49 ppm (H-2'); 7.53 ppm (H-4'); 6.9 ppm (H-5'); 7.85 ppm (H-6').

The ^1H -NMR spectrum data showed doublets at δ_{H} 7.49 ppm (H-2'); δ_{H} 7.53 ppm (H-4'); δ_{H} 6.9 ppm (H-5'); and δ_{H} 7.85 ppm (H-6'), indicating a di-substituted aromatic ring. A singlets signal at δ_{H} 4.0 ppm correlated to C-3' (δ_{C} 149.9), indicating O- CH_3 -3'. The splitting proton in aromatic B ring consist of 4 proton groups was 2', 4', 5' and 6'. H-2' coupling pattern meta with H-4', because of appear doublet signal at H-2'. Then, H-4' coupling pattern ortho with H-5' and coupling pattern meta with H-2'. H-5' coupling pattern ortho with H-4' and H-6'. H-6' coupling pattern ortho with H-5' and coupling pattern meta with H-4', because at H-4', H-5' and H-6' was showed proton signal (dd).

^{13}C NMR spectrum data isolated compound (125 MHz) showed sixteen carbon signals. Through DEPT-135 analysis, it's known that from 16 carbon signals included one methyl carbon signal (ppm) δ_{C} 56.7 (C-1'), seven methine carbon signal (ppm) showed at δ_{C} 103.97 (C-3); δ_{C} 100.28 (C-6); δ_{C} 95.31 (C-8); δ_{C} 110.7 (C-2'); δ_{C} 121.9 (C-4'); δ_{C} 117.9 (C-

5'); δ_{C} 129.6 (C-6'), and eight quaterner carbon signals (ppm) at 162.93 (C-2); 185.35 (C-4); 163.38 (C-5); 166.24 (C-7); 159.58 (C-9); 104.33 (C-10); 123.4 (C-1'); 149.9 (C-3') was showed. The chemical shift 185.35 ppm shows the presence of carbonyl group and shift at 56.7 ppm indicates the presence of a methoxy group in the isolated compound.

Two hydroxyl group at A ring, bound to C-5 with chemical shift δ_{C} 163.38 ppm and C-7 with chemical shift at δ_{C} 166.24 ppm. At B ring showed one methoxy group bound to C-3' with chemical shift δ_{C} 56.7 ppm. Further, at C ring showed one carbonyl group bound to C-4 with chemical shift 185.35 ppm. This spectrum was also supported by data from ^1H - ^{13}C (HSQC and HMBC) isolated compound.

Based on (Figure 2) the chemical shift of isolated compound (a) was compared to acacetin(b) by gomes, et al (2011) showed difference at C-3'(a) and C-4'(b) chemical shift (Table 1). It caused the differences of the position of methoxy group on B ring. In acacetin, methoxy group bound to C-4' atom, where as in isolated compound, methoxy group was bound to C-3' atom.

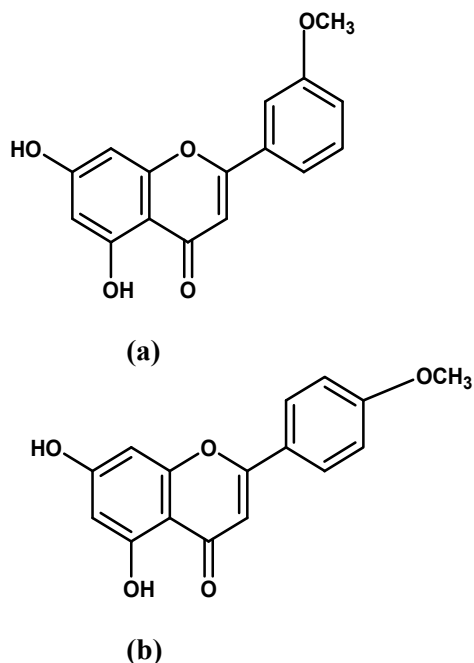


Figure 2. (a) Isolated compound (5,7-dihydroxy,3'-methoxy flavones)
(b) Acacetin (5,7-dihydroxy,4'-methoxy flavones)

HMBC correlation showed correlation between H-3 and H-6' with C-2, H-2'; H-4'; H-5' and H-1'' with C-3', H-2' and H-5' with C-1', H-8 with C-9 and H-3; H-6, and H-8 with C-10. This proven, correlation presence between proton and carbon, showed that methoxy group located at C-3' atom, shown in Figure 3.

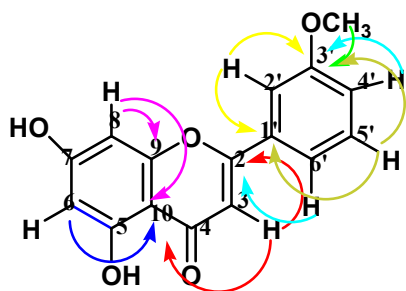


Figure 3. ^1H - ^{13}C -NMR HMBC correlation of 5,7-dihydroxy,3'-methoxy flavones

Further, ^1H - ^1H Correlated Spectroscopy (COSY) data showed correlation between proton to proton. COSY correlation shown in Figure 4.

COSY spectrum result showed correlation H-2' with H-4'; H-4' with H-2' and H-5'; H-5' with H-4' and H-6'; H-6' with H-5' and H-4'.

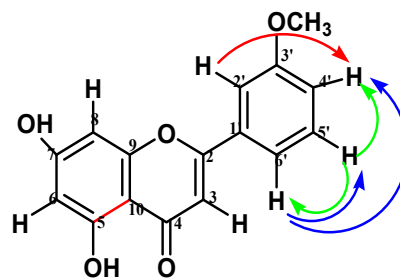


Figure 4. ^1H - ^1H COSY correlation of 5,7-dihydroxy, 3'-methoxy flavone

Antioxidant activity

The antioxidant activity of n-hexane, ethyl acetate, and methanol extract of *Salix tetrasperma* Roxb. were evaluated by DPPH method (2,2-diphenyl-1-picrylhydrazyl).

Table 1. Value of IC_{50} in n-Hexane, Ethyl Acetate, and Methanol fractions.

No.	Fraction	IC_{50} ($\mu\text{g/mL}$)
1.	n-Hexana	12555.25
2.	Ethyl acetate	65.89
3.	Methanol	170.75
4.	Ascorbic acid	10.65

The principal of DPPH method was based on the changes of colour from purple to yellow caused by the donor of hydrogen atom from the antioxidant compound.

The Table 3 showed that the ethyl acetate extract had the highest antioxidant activity [20]. It caused by the presence of flavonoid compound. Flavonoid is known to have a significant role in antioxidant activity [21].

CONCLUSION

5,7-dihydroxy, 3'-methoxy flavone has been isolated from the ethyl acetate extract of *Salix tetrasperma* Roxb. This plant has the great potential as an antioxidant.

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