Antibacterial Activity and Structure Elucidation of Salicin from Stem Bark of *Salix tetrasperma* ROXB.

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**Abstract**

*Salix tetrasperma* Roxb. (Family Salicaceae) is a plant that used as traditional medicine for anti-inflammatory, analgesic, reduces fever, and itching medicine. In this study was carried out extraction, isolation, structure elucidation of salicin from *Salix tetrasperma* Roxb. stem bark and it’s antibacterial activity. The extraction method was used the maceration method by *n*-hexane, ethyl acetate, and methanol solvents. Isolation of compound from ethyl acetate extract of *Salix tetrasperma* Roxb. stem bark using chromatography methods and obtained white solid (15 mg). The structure was elucidated using spectroscopic analysis, including Ultraviolet (UV), Infrared (IR), Nuclear Magnetic Resonance (NMR) and comparative literature, identified as salicin compound with molecule formula C$_{13}$H$_{18}$O$_{7}$. Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* bacteria using disk diffusion method. This compound has a great antibacterial activity against *Staphylococcus aureus* bacteria with clear zone diameter of 10.2 ± 0.3 mm. This shows that the *Salix tetrasperma* Roxb. stem bark has great potential as a source of antibacterial compound.

**Keywords**: *Salix tetrasperma* Roxb., salicin, antibacterial activity

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**INTRODUCTION**

*Salix tetrasperma* Roxb. plant (Family; Salicaceae) commonly called Indian Willow [1]. In Indonesia, it's known as dalu- dalu used as traditional medicine. The phenolic glycosides contained in *Salix tetrasperma* Roxb. reported as...
an anti-inflammatory, analgesic, reduce fever and reduce rheumatic infection, headache, the cronic pain syndrome[2], cough, scorpion sting, bug bite, wounds, warts[3], and dysmenorrhea in women[1].

Various bioactivities have been report of *Salix tetrasperma* Roxb. plant including anti-inflammatory, analgesic [4], antioxidant [4,5], antiprotozoa [6], hypoglycemic [7], diuretic, laxative [8], cytotoxic [9,10], antischistosoma [11], insecticidal [12], antifungal [13], and antibacterial activities [9].

Phytochemical investigation from the bark extract of this plant has been reported steroid, sterol, triterpene, tannin, phenolic compounds, saponin, and flavonoid [7, 8]. Research conducted at Zagazig University, Egypt has been reported that the *Salix tetrasperma* Roxb. plant generated some pure compound, including β-sitosterol acetate, friedelin, 3β-friedelinol, β- amyrin, β-sitosterol, β-sitosterol-O-glucoside, and palmitic acid, which has been isolated from the methanol extract of *Salix tetrasperma* Roxb. from dichloromethane fraction of this leaf has been isolated catechol and tremulacin. Salicin and its derivatives tremuloidin and 2′-O-β-(E)-coumaroyl salicin were isolated from the ethyl acetate fraction of the leaf [4].

This paper report the salicin isolated compound from ethyl acetate extract of *Salix tetrasperma* Roxb. stem bark. Structure of the salicin compound was elucidated using spectroscopic analysis, including ultraviolet visible spectrophotometer, FT-IR spectrophotometer and Nuclear Magnetic Resonance spectrometer. Futhure, Antibacterial activity from the salicin compound, *n*-hexane, ethyl acetate, and methanol extracts of Salicin were evaluated using disk diffusion method against *Escherichia coli* and *Staphylococcus aureus* bacteria.

**MATERIALS AND METHODS**

**Materials**

**Plant material**

The stem bark of *Salix tetrasperma* Roxb. was collected from Pesisir Selatan Regency, West Sumatera, Indonesia. The sample has been identified in the Herbarium of Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University with the specimen code 237/K-ID/ANDA/V/2018 and collection number SR-01.

**Media and Chemical material**

The media and chemicals used in the research were Mueller-Hinton agar (Hi-media), dimethyl Sulfoxide (DMSO), NaCl 0.9%, distilled methanol, acetone, ethyl acetate, dichloromethane, *n*-hexane (Brataco) as the solvents, H₂SO₄ 2N, silica gel 60 (Merck, 0.063-0.200 mm), TLC plate (Merck, DC-Alufolien Kiesegel 60 F₂₅).

**Instrument**

Distillation apparatus, macerator, Rotary Evaporator (Heidolph VV 2000), melting point apparatus (Fisher-Johns), oven, vacuum desiccator, scales, UV lamps GL-58 (λ 254 and 365 nm), ultraviolet visible spectrophotometer (Thermo Scientific, Genesys 10 s UV- Vis), FT-IR spectrophotometer (Perkin Elmer, Frontier), Nuclear Magnetic Resonance (NMR) spectrometer (JEOL JNM-ECZ500R), column chromatography, petri dish, disc paper, laminar flow and commonly used glassware in laboratories.

**Methods**

**Extraction**

The stem bark of *Salix tetrasperma* Roxb. was dried in the shade and finally ground to a powder. The dry powder stem bark of *Salix tetrasperma* Roxb (6 kg) was extracted by maceration method using *n*-hexane, ethyl acetate, and methanol solvents at room temperature. It was obtained crude extracts of *n*-hexane (20 g), ethyl acetate (33 g) and methanol (250 g).

**Isolation and Purification**

The ethyl acetate extract (20 g) was applied on to silica gel of vacuum liquid chromatography, packed with *n*-hexane. The polarity of the eluent was increased gradually using *n*-hexane- ethyl acetate (8:0 to 2:10) and ethyl acetate:methanol (10:0 to 0:10). Each fraction in TLC and fractions the same spot
Further, the melting point of the isolated compound was measured and characterization of the isolated compound using UV, IR, NMR spectroscopy.

**Test bacteria and Inoculum Preparation**

The *Escherichia coli* and *Staphylococcus aureus* bacteria were cultured in tilted Nutrient Agar media for 24 hours and incubated at 37 °C, then taken loopful and suspended in a tube containing 200 µL of physiologic salt solution (NaCl 0.9%). Sterile liquid Mueller-Hinton agar was added to 20 ml of each petri dish and allowed to solidify at room temperature. The media was dripped with 200 µL of the bacterial suspension tested and flattened with an L stem, then left to dry for 15 minutes in a laminar flow [14].

**Antibacterial activity**

Sterile disc paper with a diameter of 6 mm added *n*-hexane, ethyl acetate, methanol solvents and salicin compound (20 µL). The concentrations of each extract are 1000, 500 and 250 µg / mL. Further, the paper discs placed on MHA media and incubated at room temperature for 24 hours. Positive control used Amoxicillin 250 µg / mL and negative control used DMSO 100% and methanol. The clear zone around the disc showed the area of bacterial resistance. The diameter of the clear zone measured horizontally and vertically using a scale ruler [15].

**RESULTS AND DISCUSSION**

**Isolation and Purification**

Phytochemical profile study of ethyl acetate extract of *Salix tetrasperma* Roxb. showed that the stem bark of this plant containing phenolic group, flavonoid, terpenoid, steroid, and saponin.

Phytochemical profile study of ethyl acetate extract of *Salix tetrasperma* Roxb. showed that the stem bark of this plant containing phenolic group, flavonoid, terpenoid, steroid, and saponin. Purification using chromatography method obtained 15 mg of white solid. The TLC result showed single pink spot after added H2SO4 2N. The melting point of the isolated compound was 189-190 °C. The ultraviolet spectrum in methanol showed absorption at 223 nm and 271 nm with the presence of a phenolic moiety. Infrared spectrum showed absorption at (cm⁻¹) 3317.09 (OH); 2921.39 (CH); 1593.52 (C=C aromatic); and 1021.65 (C-O) cm⁻¹. ¹H-NMR (CD3OD), 500 MHz (ppm) spectra data salicin compound showed the value of chemical shift at 7.34 (1H); 7.26 (1H); 7.22 (1H); 7.03 (1H); 4.87 (1H); 4.79 (1H); 4.56 (1H); 3.91 (1H); 3.71 (1H); 3.51 (1H); 3.46 (1H); 3.42 (1H) and 3.39 (1H). ¹³C-NMR (CD3OD), 125 MHz (ppm); spectra data Salicin compound showed the value of chemical shift at 61.0; 62.6; 71.4; 75.1; 78.0; 78.3; 103.4; 117.1; 123.7; 129.9; 130.0;132.2 (ppm). ¹H-NMR and ¹³C-NMR of isolated compound result showed thirteen minimum C atoms supported by HSQC spectrum data. Which appear as two secondary C atoms, nine tertiary C atoms and two quaternary C atoms. This spectrum showed the relationship between ¹H-¹³C. Proton H-3, H-4, H-5 and H-6 were aromatic ring protons bound to C-3, C-4, C-5 and C-6 atoms which were located in the chemical shift C=C.

HBMC correlation, shown in Figure 1, correlation between H-7and H-7b with atoms C-1, C-2, and C-3; H-1’with atom C-1; H-2’ with atom C-3’; H-3’ with atom C-5’; H-4’ with atom C-3’; H-5’ with atom C-4’; and H-6’ with atom C-5’. This proved there was a correlation between protons and carbons.
Table 1. \(^1\)H(CD\(_3\)OD; 500 MHz), \(^{13}\)C(CD\(_3\)OD; 125 MHz) NMR data of the isolated compound and NMR data comparative of salicin compound in D\(_2\)O:CD\(_3\)OD (4:1) by Dias [16].

| No | \(\delta C\) (ppm) | \(\delta H\) (ppm) | \(J_{H-H}\) (Hz); multiplicity | HMBC | \(\delta C\) (ppm) | \(\delta H\) (ppm) |
|----|-----------------|-----------------|-----------------|------|-----------------|-----------------|
| 1  | 157.2           | -               | -               |      |      | 157.6           |
| 2  | 132.2           | -               | -               |      |      | 133.9           |
| 3  | 130.0           | 7.26            | 8.2; 1.4; (td)  |      | C\(_1\); C\(_5\) | 132.1           |
| 4  | 123.7           | 7.03            | 8.2; 0.9; (td)  |      | C\(_2\); C\(_6\) | 126.0           |
| 5  | 129.9           | 7.34            | 7.6 (d)         |      | C\(_1\); C\(_3\) | 132.3           |
| 6  | 117.1           | 7.22            | 7.9 (d)         |      | C\(_1\); C\(_2\); C\(_4\) | 118.0           |
| 7  | 61.0            | 4.56(1H)        | 12.9 (d)        |      | C\(_1\); C\(_2\); C\(_3\) | 62.0           |
|    |                 | 4.79(1H)        | 12.9 (d)        |      | C\(_1\); C\(_2\); C\(_3\) | 4.73 (d)       |
| 1' | 103.4           | 4.87            | 7.8 (d)         |      | C\(_1\) | 103.4           |
| 2' | 75.1            | 3.42            | overlapped      |      | C\(_3\)' | 75.7           |
| 3' | 78.0            | 3.51            | overlapped      |      | C\(_5\)' | 78.6           |
| 4' | 71.4            | 3.39            | (m)             |      | C\(_3\)' | 72.1           |
| 5' | 78.3            | 3.46            | (m)             |      | C\(_4\)' | 78.6           |
| 6' | 62.6            | 3.71 (1H)       | 11.9; 5.3; (dd) |      | C\(_5\)' | 63.3           |
|    |                 | 3.91 (1H)       | 11.9; 1.8 (dd)  |      | C\(_5\)' | 3.91 (d)       |

Based on \(^{13}\)C-NMR, \(^1\)H-NMR, HSQC, HMBC spectroscopy data and chemical shift data of isolated of compounds with the salicin compound reported data by Dias showed high suitability, Table 1 [16]. It can be concluded that isolated compound was salicin with molecule formula C\(_{13}\)H\(_{18}\)O\(_7\) as shown in Figure 2.

Antibacterial Activity

Antibacterial activity of *Salix tetrasperma* Roxb is listed in Table 2. The Table showed the ethyl acetate extract has the largest clear zone diameter compared with the other extracts for *Staphylococcus aureus* bacteria (14.5 ± 0.5 mm), followed by methanol extract (12.7 ± 0.6) and salicin compound (10.2 ± 0.3) for *Escherichia coli* bacteria, the largest clear zone diameter was found in *n*-hexane extract (11.7 ± 0.8 mm).

Difference of active extract against each bacteria was caused by differences in secondary metabolites that dominant content in each extract. The ethyl acetate extracts are dominant contains phenolic and flavonoid compounds. Phenol and polyphenol compounds are largest groups of secondary metabolites, has hydroxyl groups attached at aromatic phenol group [17]. Phenol can be to change the permeability of bacterial cells, caused them lost the macromolecule of the cells. The compound also disrupts membrane function and affect membrane protein caused that change in structure and function. At low concentration, phenolic compound affects enzyme activity and at high concentration, causes protein denaturation [18]. While for *Escherichia coli* bacteria, the extract that has higher inhibitory was *n*-hexane extract. In *n*-hexane extract,
secondary metabolites the most dominant of a compound terpenoid. Terpenoid was an organic compound that disrupts the formation of membranes by the lipophilic compound. The ability of terpenoid damage the cell membrane, deactivate enzyme and protein denaturation causes that permeability of bacterial cell walls to decrease, and cell walls are damage [19].

**Table 2.** Diameter of clear zone bark extract of *Salix tetrasperma* Roxb. and salicin compound against bacterial growth of *Escherichia coli* and *Staphylococcus aureus* bacteria

| Extract     | Concentrations (ppm) | Diameter of suppression zone (mm) |
|-------------|----------------------|-----------------------------------|
|             |                      | *Escherichiacoli*                  |
|             |                      | *Staphylococcus aureus*            |
| Methanol    | 250                  | 7.8 ± 0.3                          |
|             | 500                  | 8.3 ± 0.3                          |
|             | 1000                 | 8.8 ± 0.3                          |
| Ethyl acetate| 250                  | 7.8 ± 0.8                          |
|             | 500                  | 8.5 ± 0.5                          |
|             | 1000                 | 9.3 ± 1.2                          |
| *n*-hexane  | 250                  | 9.3 ± 1.0                          |
|             | 500                  | 10.0 ± 0.8                         |
|             | 1000                 | 11.7 ± 0.8                         |
| Salicin     | 250                  | 7.7 ± 0.3                          |
|             | 500                  | 8.2 ± 0.5                          |
|             | 1000                 | 8.5 ± 1.2                          |
| Control (+) | Amoxylin             | 16.7 ± 0.0                         | 27.7 ± 0.6

**CONCLUSION**

Salicin has been isolated from the ethyl acetate extract of *Salix tetrasperma* Roxb. stem bark and has great potential as a source of the antibacterial compound.

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