CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *Salix tetrasperma* ROXB

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ABSTRACT

Six (1-6) and two (7-8) compounds were obtained from ethyl acetate extract of *S. tetrasperma* Roxb bark and leaves, respectively. Structures of isolated compounds were determined by spectroscopic data, including ^1H NMR, ^{13}C NMR, and two dimensional NMR as β -sitosterol glucoside (1), 3,4-dihydroxybenzoic acid (2), 2-(hydroxymethyl)phenol (3), 1,3-dihydroxyphenol (4), 3-(hydroxymethyl)phenol (5), 4-methylbenzaldehyde (6), 1,2-dihydroxybenzene (7) and 3',4',5,7-tetrahydroxyflavone (8). Interestingly, compounds 2, 4, 5, 6, and 8 were reported for the first time isolated from this plant. The antioxidant activities of all crude extracts were evaluated by the diphenylpicrylhydrazyl (DPPH) method. Among all extracts, methanol extract of the bark has the highest antioxidant activity with an IC_{50} value of 6.85 $\mu\text{g/mL}$. The activity of ethyl acetate extract of the root (IC_{50} 19.53 $\mu\text{g/mL}$) was stronger than the bark (IC_{50} 22.69 $\mu\text{g/mL}$).

Keywords: *Salix tetrasperma* Roxb, NMR, Antioxidant Activity, DPPH Method.

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INTRODUCTION

Salix tetrasperma Roxb belongs to the *Salicaceae* family. This plant is commonly called “dalu-dalu” in West Sumatera, Indonesia and has been used as a traditional medicine to treat coughs and skin diseases as well as to reduce fever. The pharmacological activities of this plant showed that the bark extract of this plant possessed hypoglycemic activity¹, while the leaf extract showed antimicrobial², antipyretic³, analgesic, and anti-inflammatory activities.⁴ The previous phytochemical work on this genus revealed that most species contain numerous phenolic compounds. According to our previous study, the polyphenolic compounds showed good biological activities.⁵⁻⁶ Salicin glycosides from *S. glandulosa* showed anti-neuroinflammatory activity.⁷ Recently the salicin derivatives from *S. acmophylla* were reported as cytotoxic and anti-inflammatory.⁸ Simple phenolic compounds from *S. capensis* bark had the antibacterial activity to *P. aeruginosa*.⁹ Only a few phytochemical studies have been reported on *S. tetrasperma* Roxb.^{4,10-11} The phytochemical composition of *Salix* from different regions may vary, due to the environment of the growing plant. However, the information on chemical constituents from *S. tetrasperma* Roxb growing in Indonesia is still limited. Due to a large number of phenolic compounds that were reported from this genus, the authors are also interested to evaluate the antioxidant activity from various parts of this plant. Hence, this study aims to isolate secondary metabolite compounds from ethyl acetate extract of *S. tetrasperma* Roxb bark and leaves and to investigate the antioxidant activity of *n*-hexane, ethyl acetate, and methanol extracts various parts of *S. tetrasperma* Roxb.

EXPERIMENTAL

Material and Methods

All solvents were purchased from the suppliers with further purification before usage. Silica gel for column chromatography was performed on silica gel 60 (Merck). TLC plates pre-coated with silica gel 70 F₂₅₄ (60-200 mesh) were purchased from Wako Pure Chemical Industries, Japan. TLC spots were detected under the UV lamp (254 and 365 nm). ^1H , ^{13}C , HMQC, HMBC, and ^1H - ^1H COSY NMR spectra were recorded with JEOL JNM-ECS 400, 500, and 600 spectrometers using TMS as an internal standard. Two-dimensional ^1H - ^1H COSY, HMQC, and HMBC correlations were made by using standard pulse sequences.

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Plant Material

Leaves, root, and bark of *S. tetrasperma* Roxb were collected from the surrounding of Singkarak Lake, West Sumatera Province, Indonesia in May 2015. The plant was identified by a taxonomist at Herbarium of Andalas University (ANDA), Padang, Indonesia.

Extraction and Isolation

Each of the powdered samples (5 kg of leaves, 200 g of the root, and 11 kg of bark) was macerated in *n*-hexane for 3 days at room temperature and the process was repeated 4 times. All *n*-hexane filtrates of each part were concentrated by a rotary evaporator. The residue was immersed in ethyl acetate (EtOAc) and lastly followed in methanol (MeOH) in the same aforementioned process.

The crude EtOAc extract of the bark (100 g) was fractionated on silica gel 60, eluted with increasing polarity of solvents (100% *n*-hexane, dichloromethane (DCM), EtOAc until 100% MeOH) to afford nineteen fractions (A-S). Fraction R (12.22 g) was recrystallized with MeOH to obtain compound **1** (50 mg) as a white powder that turned into purple after treatment with 10% H₂SO₄ on the TLC plate. Fraction K (0.787 g) was purified on CC, eluted with a mixture of *n*-hexane/DCM (1:0→0:1, v/v), DCM/EtOAc (1:0→0:1, v/v) to yield nine fractions (K1-K9). Compound **2** (8 mg) was attained from fraction K4 as a yellow crystal. Fraction G (0.455 g) was separated on CC (DCM/EtOAc, 1:0→1:1, v/v) to give 16 fractions (G1-G16). Fraction G7 (50 mg) was purified on CC to give compound **3** (6 mg) as an orange crystal and compound **4** (4 mg) was acquired from fraction G14 as a white crystal. Fraction D (5.2 g) was separated into SiO₂ to yield five sub-fractions (D1-D5). Fraction D1 (1.04 g) was separated on sephadex (DCM/MeOH, 1:1, v/v) to give three fractions (D11-D13). Fraction D13 (0.450 g) was purified using CC (*n*-Hex/EtOAc, 4:1, v/v) to give compound **5** (10 mg) as an orange crystal. Fraction E (8.30 g) was separated on CC to give eight fractions (E1-E8). Fraction E2 (4.76 g) was separated on CC (DCM/EtOAc, 1:0→0:1, v/v) to obtain nine fractions (E21-E29). Fraction E24 (30 mg) was purified on sephadex by using MeOH to get compound **6** (3 mg) and was attained as a brownish crystal. The dried EtOAc extract of the leaves (60 g) was separated on silica gel with *n*-hexane/CHCl₃ (1:1, v/v), CHCl₃/MeOH (10:0→0:10, v/v) to give seven fractions (L1-L7). Fraction L5 (3 g) was chromatographed on silica gel to yield four sub-fractions (L51-L54). The fraction of L52 (1 g) was purified using silica gel (CHCl₃/EtOAc) to obtain compound **7** (70 mg) as a colorless crystal. Fraction L6 (5 g) was separated on silica gel (CHCl₃/EtOAc, EtOAc/MeOH, 10:0→0:10, v/v) to yield ten sub-fractions (L61-L610). Compound **7** was also obtained from fraction L62. The recrystallization of fraction L66 obtained compound **8** (40 mg).

Spectral Data

β-Sitosterol Glucoside (**1**)

H NMR (500 MHz, DMSO-*d*₆) δ ppm: 0.65 (3H, s, H-18), 0.81 (3H, d, *J* = 8 Hz, H-27), 0.80 (3H, d, *J* = 8 Hz, H-26), 0.83 (3H, s, H-19), 0.89 (3H, d, *J* = 6.5 Hz, H-21), 0.95 (3H, d, *J* = 8 Hz, H-29), 3.12 (1H, m, H-3), 3.05 (1H, d, *J* = 5.2 Hz, H-2'), 3.46 (1H, m, H-4'), 3.64 (1H, dd, *J* = 5.2 Hz, H-5'), 4.21 (1H, d, *J* = 7.8 Hz, H-3'), 4.43 (1H, t, *J* = 5.2 Hz, H-1'), 4.87 (1H, t, *J* = 5.2 Hz, H-6'b), 4.90 (1H, dd, *J* = 4.5 Hz, H-6'a); and 5.38 (1H, brs, *J* = 4.5 Hz, H-6). C NMR (125 MHz, DMSO-*d*₆) δ ppm: 36.83 (C-1), 29.27 (C-2), 76.87 (C-3), 39.28 (C-4), 140.44 (C-5), 121.22 (C-6), 31.42 (C-7), 31.37 (C-8), 49.60 (C-9), 36.21 (C-10), 20.60 (C-11), 38.30 (C-12), 41.86 (C-13), 56.17 (C-14), 23.87 (C-15), 27.80 (C-16), 55.42 (C-17), 11.68 (C-18), 19.11 (C-19), 35.48 (C-20), 18.62 (C-21), 33.34 (C-22), 25.43 (C-23), 45.14 (C-24), 28.70 (C-25), 19.72 (C-26), 18.94 (C-27), 22.61 (C-28), 11.79 (C-29), 100.77 (C-1'), 73.47 (C-2'), 76.91 (C-3'), 70.11 (C-4'), 76.75 (C-5'), 61.10 (C-6').

3, 4-Dihydroxybenzoic Acid (**2**)

H NMR (500 MHz, DMSO-*d*₆) δ ppm: 12.28 (brs), 9.62 (brs), 9.30 (brs), 7.33 (d, 1H, *J* = 2.1 Hz, H-2), 7.28 (dd, 1H, *J* = 8.2; 2.1 Hz, H-6), 6.77 (d, 1H, *J* = 8.2 Hz, H-5). C NMR (125 MHz, DMSO-*d*₆) δ ppm: 168.24 (C-7), 150.60 (C-4), 145.41 (C-3), 122.73 (C-6), 122.20 (C-1), 117.08 (C-2), 115.811 (C-5).

2-(Hydroxymethyl) Phenol (**3**)

H NMR (400 MHz, Acetone-*d*₆) δ ppm: 7.21 (d, 1H, *J* = 7.32 Hz, H-3), 7.07 (t, 1H, *J* = 8.24 Hz, H-5), 6.78 (t, 1H, *J* = 7.32; 8.24 Hz, H-4), 6.77 (d, 1H, *J* = 8.24 Hz, H-6), 4.71 (s, 2H, H-7). C NMR (100 MHz,

Acetone- d_6) δ ppm: 155.36 (C-1), 127.99 (C-3), 127.53 (C-5), 127.45 (C-2), 119.32 (C-4), 115.17 (C-6), 61.02 (C-7).

1, 3-Dihydroxyphenol (4)

H NMR (400 MHz, Acetone- d_6) δ ppm: 7.88 (dd, 1H, $J = 2.28$; 1.84 Hz, H-2), 7.51 (td, 1H, $J = 7.76$; 1.84 Hz, H-5), 6.91 (dd, 2H, $J = 8$; 0.92 Hz, H-4 and H-6). C NMR (100 MHz, Acetone- d_6) δ ppm: 135.9 (C-1 and C-3), 130.45 (C-5), 119.00 (C-4 and C-6), 117.00 (C-2).

3-(Hydroxymethyl) Phenol (5)

H NMR (400 MHz, $CDCl_3$) δ ppm: 7.21 (td, 1H, $J = 8$; 0.92 Hz, H-5), 7.03 (dd, 1H, $J = 8$; 1.36 Hz, H-6), 6.87 (dd, 1H, $J = 1.36$; 0.92 Hz, H-4), 6.84 (dd, 1H, $J = 8$; 1.36 Hz, H-2), 4.85 (s, 2H, H-7). C NMR (100 MHz, $CDCl_3$) δ ppm: 156.14 (C-1), 129.61 (C-3), 127.92 (C-5), 124.73 (C-4), 120.18 (C-6), 116.62 (C-2), 64.74 (C-7).

4-Methylbenzaldehyde (6)

H NMR (400 MHz, Acetone- d_6) δ ppm: 9.13 (brs), 7.87 (dd, 2H, $J = 8$; 4 Hz, H-2 and H-6), 6.90 (dd, 2H, $J = 8$; 4 Hz, H-3 and H-5), 2.46 (s, 3H, Me). C NMR (100 MHz, Acetone- d_6) δ ppm: 195.73 (C-7), 161.86 (C-4), 130.79 (C-2 and C-6), 129.66 (C-1), 115.19 (C-3 and C-5), 28.90 (Me).

1, 2-Dihydroxybenzene (7)

H NMR (400 MHz, Acetone- d_6) δ ppm: 6.67 (m, 2H, H-3 and H-6), 6.82 (m, 2H, H-4 and H-5). C NMR (125 MHz, Acetone- d_6) δ ppm: 145.14 (C-1 and C-2), 119.97 (C-3 and C-6), 115.38 (C-4 and C-5).

3', 4', 5, 7-Tetrahydroxyflavone (8)

H NMR (600 MHz, Methanol- d_3) δ ppm: 7.38 (d, 1H, $J = 2.76$ Hz, H-2'), 7.37 (d, 1H, $J = 8.94$ Hz, H-6'), 6.90 (d, 1H, $J = 8.94$ Hz, H-5'), 6.53 (s, 1H, H-3), 6.43 (d, 1H, $J = 2.04$ Hz, H-8), 6.20 (d, 1H, $J = 2.04$ Hz, H-6). C NMR (150 MHz, Methanol- d_3) δ ppm: 182.52 (C-4), 165.00 (C-7), 164.65 (C-2), 161.87 (C-5), 158.06 (C-9), 149.64 (C-4'), 145.70 (C-3'), 122.33 (C-1'), 118.94 (C-6'), 115.42 (C-5'), 112.80 (C-2'), 103.96 (C-10), 102.51 (C-3), 98.75 (C-6), 93.63 (C-8).

Antioxidant Activity

The antioxidant activities of various *S. tetrasperma* Roxb extracts were determined by using DPPH radical scavenged method¹² with slight modification. 3 mL of each solution [in various concentrations] of tested samples (*n*-hexane, EtOAc, and MeOH extracts of the leaves, roots, and bark) was poured into 1 mL of DPPH solution [0.1 mmol/L]. The mixture was incubated for 30 minutes at room temperature in the dark place and then recorded by UV-spectrophotometer at 517 nm. As a comparison, the ascorbic acid and methanol were used as a standard and a blank, respectively. The antioxidant activity was calculated based on the percentage of DPPH radical scavenged (% Inhibition) using the following equation:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of control containing all reagents that exclude the sample, and A_{sample} is the absorbance of the sample. The measurements were taken in triplicate. The antioxidant results were expressed as the IC_{50} value $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Characterization of Isolated Compounds

After separation by chromatography technique, six (1-6) and two (7-8) compounds were isolated from EtOAc extract of *S. tetrasperma* Roxb bark and leaves, respectively. The structures of isolated compounds were displayed in Fig.-1.

Compound 1 was acquired as a white powder (50 mg). The H NMR spectrum of 1 showed the presence of a broad singlet signal at δ_H 5.38 ppm characterized as an olefinic proton (H-6). While one proton as a

multiplet at δ_H 3.12 ppm was assigned as an oxymethine proton (H-3). A steroidal nucleus was assigned by the presence of two protons at δ_H 3.12 (H-3) and 5.38 (H-6).

Fig.-1: Isolated Compounds from *S. tetrasperma* Roxb Bark; β -Sitosterol Glucoside (1), 3,4-Dihydroxybenzoic Acid (2), 2-(Hydroxymethyl) Phenol (3), 3-(Hydroxymethyl) Phenol (5), 4-Methylbenzaldehyde (6), and from the Leaves; 1,2-Dihydroxybenzene (7) and 3',4',5,7-Tetrahydroxyflavone (8).

Furthermore, six methyl protons appeared in the 1H NMR spectrum as singlet protons at δ_H 0.65 (H-18) and 0.83 (H-19), doublet protons at δ_H 0.80 (H-26), 0.81 (H-27), 0.89 (H-21), and 0.95 (H-29) respectively. On the other hand, the sugar moiety protons resonated at δ_H 3.05-4.90 ppm, while the proton at δ_H 4.43 ppm indicated an anomeric proton (H-1'). The ^{13}C NMR spectrum of **1** appeared 35 carbon signals including 29 signals of a steroidal compound and 6 signals of glucose moiety in its structure. The signals at δ_C 140.44 and 121.22 ppm showed the occurrence of olefinic carbons at C-5 and C-6, respectively. Meanwhile, the signal at δ_C 76.87 ppm indicated oxymethine carbon at C-3. Moreover, six methyl carbon signals appeared at δ_C 11.68 (C-18), 11.79 (C-29), 18.62 (C-21), 18.94 (C-27), 19.11 (C-19), and 19.72 (C-26). The remaining six signals at δ_C 100.77 (C-1'), 73.47 (C-2'), 76.91 (C-3'), 70.11 (C-4'), 76.75 (C-5') and 61.10 (C-6') were assigned for sugar moiety. Based on 1H and ^{13}C NMR spectral data, and compared to related literature, compound **1** was identified as β -sitosterol glucoside.¹³⁻¹⁵

Compound **2** was obtained as a yellow crystal (8 mg). The 1H NMR spectrum of **2** showed three aromatic protons at δ_H 7.33 (d, 1H, J = 2.1 Hz, H-2), 7.28 (dd, 1H, J = 8.2; 2.1 Hz, H-6), and 6.77 (d, 1H, J = 8.2 Hz, H-5), while six aromatic signals at δ_C 115.81, 150.60, 145.41, 122.73, 122.20, 117.08 ppm, and one carbonyl signal at δ_C 168.24 ppm appeared in ^{13}C NMR spectrum. In 1H - 1H COSY spectrum, the proton at δ_H 7.28 ppm exhibited *meta*-coupling with the proton at δ_H 7.33 ppm (J = 2.1 Hz) and it also had *ortho*-coupling with the proton at δ_H 6.77 ppm (J = 8.2 Hz), that assigned trisubstitution at 1, 3 and 4 positions in the aromatic ring. A broad singlet signal in the most downfield region (δ_H 12.28 ppm) assigned the occurrence of carboxylic acid bound to the aromatic ring of **2**. By 1H and ^{13}C NMR analyses and comparison with the previously reported data, the chemical structure of **2** was determined as 3,4-dihydroxybenzoic acid or protocatechuic acid.¹⁶⁻¹⁸

Compound **3** was isolated as an orange crystal (6 mg). The 1H NMR of **3** displayed four aromatic protons and an oxidized methylene proton (δ_H 4.71 ppm). The most downfield proton at δ_H 7.21 ppm revealed *ortho*-coupling with the proton at δ_H 6.78 ppm (J = 7.32 Hz), while the proton at δ_H 7.07 ppm as a triplet

also showed *ortho*-coupling with the protons at δ_H 6.78 and 6.77 ppm ($J = 8.24$ Hz). The C NMR indicated seven carbon signals including six aromatic carbons at δ_C 155.36 (C-1), 127.99 (C-3), 127.53 (C-5), 127.45 (C-2), 119.32 (C-4), 115.17 ppm (C-6) and one oxidized methylene carbon (δ_C 61.02 ppm) bound to aromatic carbon, respectively. Based on the comparison of H and C NMR data with the literature, the structure of compound **3** was assigned as 2-(hydroxymethyl)phenol or salicyl alcohol.¹⁹

Compound **4** was acquired as a white pale crystal (4 mg). The H NMR spectrum showed three aromatic protons. While, four signal aromatic carbons (δ_C 135.9, 130.45, 119, and 117 ppm) appeared in the C NMR spectrum. The proton at δ_H 7.51 ppm (td, 1H) had *ortho*-coupled with two protons at δ_H 6.91 ppm (dd, $J = 8$ Hz) and also had *para*-coupling with a proton at δ_H 7.87 ppm (dd, $J = 1.84$ Hz). It is predicted that the aromatic ring had two hydroxyl groups at 1, 3-position. Based on the comparing spectral data of **4** with literature, it was identified as 1, 3-dihydroxyphenol or resorcinol.²⁰

Compound **5** was obtained as an orange crystal (10 mg). The H NMR signal of **5** showed the presence of four aromatic protons at δ_H 7.21 ppm (td, 1H, $J = 8$; 0.92 Hz, H-5), 7.03 ppm (dd, 1H, $J = 8$; 1.36 Hz, H-6), 6.87 ppm (dd, 1H, $J = 1.36$; 0.92 Hz, H-4), and 6.84 ppm (dd, 1H, $J = 8$; 1.36 Hz, H-2), respectively and one oxidized methylene proton at δ_H 4.71 ppm (s, 2H, H-7). This compound assigned two substituents bound to the aromatic ring. It was suggested that the hydroxyl and hydroxymethyl groups were separately attached to carbon at δ_C 156.14 (C-1) and δ_C 129.61 (C-3). Compared to the spectral data of **5** with the literature, the chemical structure of **5** was determined as 3-(hydroxymethyl) phenol.²⁰⁻²¹

Compound **6** was isolated as a brownish crystal (3 mg). Its H NMR showed two set double-doublet protons at δ_H 7.87 ppm (dd, 2H, $J = 8$; 4 Hz, H-2 and H-6) and δ_H 6.90 ppm (dd, 2H, $J = 8$; 4 Hz, H-3 and H-5) assigned symmetric pattern of the aromatic ring with substitution at 1 and 4 positions. A broad singlet signal at δ_H 9.13 ppm indicated the presence of carbonyl group, while the remaining of three protons at δ_H 2.46 ppm (s, 3H) assigned the presence of methyl group bound to the aromatic ring. The C NMR spectrum displayed six-carbon signals, consist of four aromatic carbons at δ_C 161.86, 130.78, 129.66, and 115.18 ppm, one carbonyl carbon at δ_C 195.72 ppm, and one methyl carbon at δ_C 28.90 ppm, respectively. After comparing the spectral data of **6** with literature, it was assigned as 4-methylbenzaldehyde.²²⁻²³

Compound **7** was obtained as a colorless crystal (70 mg). H and C NMR spectrum displayed only two aromatic proton signals as multiplet separately at δ_H 6.67 ppm (H-3 and H-6) and δ_H 6.82 ppm (H-4 and H-5), and three aromatic carbon signals (δ_C 115.38, 119.97, 145.14 ppm). Compared to the spectral data of **7** with literature, it was identified as 1, 2-dihydroxyphenol or catechol.⁴

Compound **8** was acquired as a yellow amorphous powder (40 mg). The H NMR spectrum of **8** contained six aromatic proton signals. Two aromatic protons signal at δ_H 6.43 ppm (1 H, d, $J = 2.04$ Hz) and δ_H 6.20 ppm (1H, d, $J = 2.04$ Hz) showed *meta*-coupling proton and suggested that two protons are attached at C-8 and C-6 of the A ring of the flavone skeleton. Furthermore, the presence of aromatic proton signals at δ_H 7.38 ppm (1H, d) and δ_H 6.90 ppm (1H, d) indicated an *ortho*-coupling proton of B ring ($J = 8.94$ Hz). The addition of aromatic proton at δ_H 7.37 ppm resonated *meta*-coupled with the proton at δ_H 7.38 ppm ($J = 2.74$ Hz). The C NMR and DEPT 135 spectra comparison showed **8** contained six methines and nine quaternary carbons. In HMBC spectrum, two *ortho*-coupling protons of B ring (δ_H 7.38 and 6.90 ppm) showed long-range correlations to C-2 (δ_C 164.65 ppm), C-4' (δ_C 149.64 ppm), C-3' (δ_C 145.70 ppm), and C-1' (δ_C 122.33 ppm) suggested that two hydroxyl groups are attached at C-3' and C-4'. A remaining proton as a singlet at δ_H 6.53 ppm exhibited long-range correlations to C-10 (δ_C 103.96 ppm), and C-1' (δ_C 122.33 ppm), indicated that proton is attached to the C-3 position of C ring. According to the HMBC spectrum, a hydroxyl group was suggested bound at C-5, C-7, C-3', and C-4' positions of **8**. In comparison, the NMR data of **8** with the related literature data,²⁴⁻²⁵ compound **8** was confirmed as 3',4',5,7-tetrahydroxyflavone or luteolin.

Antioxidant Activity

The DPPH radical scavenging results of *S. tetrasperma* Roxb barks, root, and leaves extracts were assayed as described in Materials and Methods. The highest antioxidant scavenging among the nine different extracts was in the MeOH extract of the bark, while the minimum was found in the *n*-hexane extract of the root (Table-1). All MeOH extracts possessed strong antioxidant activity with IC₅₀ value of bark, root, and leaves as 6.85 ± 0.05 ; 16.93 ± 0.12 ; and 27.43 ± 0.44 $\mu\text{g/mL}$, respectively. The activity of

EtOAc extract of the root (IC_{50} 19.53 ± 0.22 $\mu\text{g/mL}$) was stronger than that of the bark (IC_{50} 22.69 ± 0.29 $\mu\text{g/mL}$), whereas the EtOAc extract of the leaves was not active (IC_{50} 142.18 ± 1.29 $\mu\text{g/mL}$) and also all parts of *n*-hexane extracts. Compared to the positive control, all tested samples showed lower scavenging activity than ascorbic acid (IC_{50} 2.19 ± 0.04 $\mu\text{g/mL}$).

Table-1: Antioxidant Activity of *S. tetrasperma* Roxb Extracts (mean $\pm$ SD), $n = 3$

Extract	Part	IC_{50} ($\mu\text{g/mL}$) $\pm$ SD
<i>n</i> -Hexane	Root	916.35 ± 13.15
	Bark	487.36 ± 2.78
Ethyl acetate	Root	19.53 ± 0.22
	Bark	22.69 ± 0.29
	Leaves	142.18 ± 1.29
Methanol	Root	16.93 ± 0.12
	Bark	6.85 ± 0.05
	Leaves	27.43 ± 0.44
Ascorbic acid	-	2.19 ± 0.04

The number of electrons taken up is very important for antioxidant scavenging activity. This is due to the ability of hydrogen donors from polyphenol compounds and also the correlation with the total flavonoid content contained in the extract.²⁶⁻²⁷ Our phytochemical investigation showed that the presence of phenolic and flavonoid in MeOH and EtOAc extract of the bark and leaves of *S. tetrasperma* Roxb might contribute to the high antioxidant activity. Thus, this plant can be considered as a good natural antioxidant source.

CONCLUSION

The chemical constituents of *S. tetrasperma* Roxb were evaluated by isolating six compounds from EtOAc extract the bark such as β -sitosterol glucoside (1), 3,4-dihydroxybenzoic acid (2), 2-(hydroxymethyl)phenol (3), 1,3-dihydroxyphenol (4), 3-(hydroxymethyl)phenol (5), 4-methylbenzaldehyde (6) and two compounds from EtOAc extract of *S. tetrasperma* Roxb leaves as 1,2-dihydroxybenzene (7) and 3',4',5,7-tetrahydroxyflavone (8). MeOH extract from the bark, root, and leaves of *S. tetrasperma* Roxb had a strong antioxidant activity with IC_{50} value of 6.85 ± 0.05 ; 16.93 ± 0.12 ; and 27.43 ± 0.44 $\mu\text{g/mL}$, respectively.

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