

p-Hydroxybenzoic Acid and Kaempferol from *Desmodium triquetrum*

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Abstract

Desmodium triquetrum (Fabaceae) is widely distributed in Indonesia and used as herbal medicines for diuretic, hemorrhoids, tonics, and anti-inflammatory. Two known compounds, *p*-hydroxybenzoic acid (**1**) and kaempferol (**2**), were isolated from the MeOH extract of the leaves of *Desmodium triquetrum*. The structures of isolated compounds were determined based on ¹H and ¹³C NMR data. The MeOH extract showed very active with 6,5 µg/mL in addition, compound (**1**) and (**2**) showed weak cytotoxicity against murine cell leukemia P-388 with IC₅₀ 55,0 and 24,7 µg/mL, respectively. Compound (**1**) was the first reported in this species.

Keywords: *Desmodium triquetrum*, kaempferol, *p*-hydroxybenzoic acid

1 Introduction

Desmodium triquetrum or known as the synonym *Tadehagi triquetrum*, is a plant native to China, India, Sri Lanka, Australia and Southeast Asian countries such as Indonesia [1]. *D. triquetrum* produced significant anti-inflammatory and antioxidant activity, for example the leaves have potent hepatoprotective and antioxidant activities against CCl₄-induced liver toxicity [2], besides the chloroform and alcoholic extracts of *D. triquetrum* (L.) was reported have their antibacterial properties [3]. The phytochemistry of the plants has revealed that this species produces diverse secondary metabolites, such as the leaves of this plant contains tannins, alkaloids, flavonoids and the fruits of this plants contains saponin and flavonoids, while the roots contain alkaloids, saponins, flavonoids, tannins [2,4,5].

Some of these compounds from *D. triquetrum* showed various bioactivities, such as flavonoid derivatives, baicalein, naringin and neohesperidin as antioxidant [1] or lignin derivative, adehaginosin reported to have hypoglycemic activity on Hep G2 cells [6]. As a continuation of phytochemical studies, in this paper, we report the isolation of secondary metabolites and preliminary cytotoxic evaluation of the compounds from the leaves of *D. triquetrum*.

2 Material and Method

2.1 General

¹H and ¹³C NMR spectra were measured at JEOL ECA500 at 500 MHz (¹H) and 125 MHz (¹³C). Chemical shifts are given on sigma (ppm) scale with acetone-d₆ solvent. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were carried out using Merck silica gel 60 GF₂₅₄ art. 7731 and 7749, respectively. For column chromatography, were used silica Sephadex LH-20. Solvents such as MeOH, acetone, EtOAc, and *n*-hexane, for extraction, fractionation, and purification were of technical grades, which were distilled before used, while CHCl₃ used in the purification was a pro-analysis grade. For thin layer chromatography (TLC) analysis, precoated silica gel plates (Merck Kieselgel60 GF₂₅₄ 0.25 mm thickness) were used, and spots were detected by UV irradiation and spraying with cerium sulfate followed by heating.

2.2 Plant material dan isolation

The dried leaves powdered of *D. triquetrum* (850 g) (collected from Solo, central java in 2005) were macerated with methanol three times (each 24 h) at room temperature. The extract was then evaporated under vacuum to give acetone extract (81 g). The MeOH extract was applied to a silica gel vacuum liquid chromatography (VLC) and eluted with *n*-hexane–EtOAc (10:0–0:10, MeOH)

to give five major fractions (Frs. A–E). Fraction B (0.7 g) was purified on radial chromatography eluted with CHCl_3 –MeOH = 39:1) to give compound (1) (12 mg). Fraction E (0.7 g) was purified on column chromatography by Sephadex LH-20 eluted methanol to give compound (2) (10 mg).

p-Hydroxybenzoic acid (1): white powder, ^1H NMR (acetone- d_6 , 500 MHz) δ_{H} ppm: 7.90 (2H, *d*, $J = 7.6$ Hz, H-2 and H-6), 6.91 (2H, *d*, $J = 7.6$ Hz, H-3 and H-5). ^{13}C NMR (acetone- d_6 125 MHz) δ_{C} ppm: 122.6 (C-1), 132.7 (C-2 dan C-6), 115.9 (C-3 dan C-5), 162.5 (C-4), 167.4 (-COOH).

Kaempferol (2): yellow pale powder, ^1H NMR (acetone- d_6 , 500 MHz) δ_{H} (ppm): 6.26 (1H, *d*, $J = 1.7$ Hz, H-6), 6.53 (1H, *d*, $J = 1.7$ Hz, H-8), 8.15 (2H, *d*, $J = 8.8$ Hz, H-2' and 6') and 7.01 (2H, *d*, $J = 8.8$ Hz, H-3' and 5').

2.3 Cytotoxic assay

The cytotoxicity assay was conducted according to the method described previously. The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis were run in triplicate, averaged, and the results are shown in table 1.

3 Results and Discussion

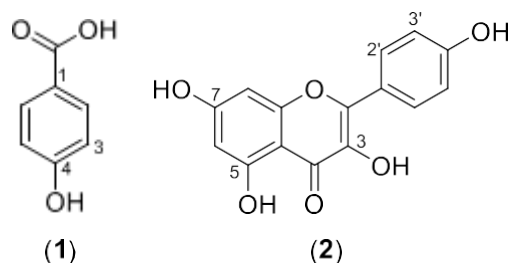


Figure 1. Structures of *p*-hydroxybenzoic acid (1) and kaempferol (2) from the leaves of *D. triquetrum*

Compound (1) has a pair of doublet δ_{H} 7.90 and 6.91 ppm (2H) in ^1H NMR, suggest as *p*-substitution of aromatic. Also, in ^{13}C NMR the presence of 167.4 ppm as carboxylic acid and four aromatic part (122.6; 132.7; 115.9 and 162.5) showed a comparison of the NMR data with those reported confirmed the structure compound (1) as *p*-Hydroxybenzoic acid [7]. Compound (1) is a part of hydroxybenzoic acid derivatives (HBAs), which are phenolic compounds with a general structure $\text{C}_6\text{-C}_1$. Four HBAs, including vanillic acid (3-methoxy-4-hydroxy), syringic acid (3,5-

dimethoxy-4-hydroxy), protocatechuic acid (3,4-dihydroxy) and *p*-hydroxybenzoic acid, are constituents of lignin. In addition, the previous investigation reported the *D. triquetrum* contains a lot of lignin and some other extra complexes such as tannin [1]. Compound (1) can be found from other plant such as *Daucus carota*, *Vitis vinifera*, *Xanthophyllum rubescens*, *Pterocarpus santalinus* [8] and *Camellia oleifera* [7]. This compound showed a variety of activities such as antimicrobe [9] nematocidal, antiviral and anti-inflammatory [10,11].

Compound (2) has a pair of doublet δ_{H} 6.26 and 6.53 ppm as meta coupling with $J = 1.7$ Hz. Also, *p*-substitution of aromatic δ_{H} 8.15 and 7.01 ppm. Comparison of the NMR data [12], compound (2) confirmed as kaempferol. Compound (2) was reported before as compound from *D. triquetrum* by fingerprint analysis using Ultra Performance Liquid Chromatography (UPLC) with photodiode array detector combined with chemometrics methods [13]. It's derivative of compound (2), kaempferol-3-O- β -D-rutinoside was determined for antihyperlipidemic effect but showed insignificant effect [14].

In preliminary cytotoxic evaluation against murine cell leukemia P-388, the MeOH extract showed very active with 6,5 $\mu\text{g/mL}$, after fractionation and purification resulted in compound (1) and (2), have been shown weak cytotoxicity.

Table 1. Preliminary cytotoxicity against murine cell leukemia P-388 of compound (1), (2) and MeOH extract of *D. triquetrum*

Compound	IC_{50} ($\mu\text{g/mL}$)
1	55,0
2	24,7
MeOH extract of <i>D. triquetrum</i>	6,5

4 Conclusion

Two compounds had been isolated from methanol extract of *D. triquetrum*, *p*-hydroxybenzoic acid (1) and kaempferol (2). Compound (1) was the first reported in this species. Even though both compounds exhibited weak cytotoxicity against murine cell leukemia P-388 in preliminary cytotoxic evaluation, but the MeOH extract showed very active with 6,5 $\mu\text{g/mL}$. This result suggested that *D. triquetrum* still has many potentials since this species reported contained a



lot of flavonoid and alkaloids derivatives, which commonly possess a broad spectrum of biological activity.

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