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NOTE

Antioxidative compounds from leaves of Tahongai (*Klienhovia hospita*)

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Abstract In our effort to find antioxidant agent, we focused on Tahongai (*Kleinhovia hospita*) which have been used traditionally in Indonesia as medicinal herbal to cure liver disease. Based on the biologically guided fractionation using DPPH radical scavenging assay, eleutherol and kaempferol 3-*O*-*β*-D-glucoside was isolated from the leaves of *K. hospita*. Kaempferol 3-*O*-*β*-D-glucoside (**1**) and eleutherol (**2**) scavenged the radical with IC_{50} of 71.4 and 491.8 μM, respectively. In addition, both of the compounds did not exhibit cytotoxicity on HepG2 cells.

Keywords Antioxidant · Kaempferol 3-*O*-*β*-D-glucoside · Eleutherol · Tahongai · *Kleinhovia hospita*

Introduction

In Indonesia, where herbal medicine has been popular, more than 1300 species are known as medicinal plants, called Jamu [1]. The uses of Jamu fall into four categories of medicine: health care, beauty care (cosmetics), tonics, and bodily protection [2]. The use of traditional medicines has increased in recent years, and provides an interesting, largely unexplored source for the development of potential new drugs.

The tree of *Kleinhovia hospita* is used as a traditional medicine in parts of Malaya, Indonesia and Papua New Guinea to treat scabies. The bark and leaves are used as hairwash for lice, while the juice of the leaves is used as an eyewash [3]. The leaves and bark of *K. hospita* contain cyanogenic compounds that are assumed to help to kill ectoparasites such as lice. Extracts of the leaves have shown anti-tumour activity against sarcoma in mice [4]. A number of fatty acids with a cyclopropenylic ring such as scopoletin, and flavonoids such as kaempferol, quercetin and rutin [4, 5] have been isolated from the leaves.

In Indonesia, the leaves of *K. hospita* are used traditionally for curing the liver disease by some tribes such as toraja, bugis, and makasar. In our previous study, we reported that ethyl acetate fraction showed antioxidant in DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay but not antiproliferation effect on HepG2 cells [6]. In this study, we isolated two compounds from ethyl acetate fraction and evaluated their antioxidant activities using DPPH radical scavenging assay and their antiproliferation effects on HepG2 cells.

Materials and methods

General

Ethanol (Merck, Darmstadt), DPPH (TCI, Tokyo), DMSO [dimethyl sulfoxide (Wako, Osaka)]. The DMEM (Dulbecco's modified eagle's medium) and fetal bovine

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serum were from Invitrogen (Tokyo). Sodium bicarbonate (Wako), streptomycin and penicillin and MTT [3-(4,5-dimethyl-2.thiazolyl)-2,5-diphenyltetrazolium bromide] were from Sigma (St. Louis). Other chemicals were high grade and commercial available.

The silica gel used for column chromatography was Wakogel C-200, 75–150 μm (Wako). Thin layer chromatography (TLC) aluminium sheets (Silica gel 60 F_{254}, 20 × 20 cm) were obtained from Merck. All other materials or solvents were of the highest purity or high-performance liquid chromatography (HPLC) grade. Melting point was measured with thermal analyzer on Shimadzu DTA-60/DSC-60. The $[\alpha]_D$ value was measured on JASCO DIP-370. Mass spectra were recorded on a Shimadzu GC–MS QP 5050A (Shimadzu Corp., Kyoto, Japan) at an electron energy of 70 eV (direct inlet); ^{1}H, ^{13}C, HMQC, and HMBC of NMR spectra were recorded on a JEOL JNM-400 (JEOL, Ltd., Tokyo).

Plant material

The leaves of *K. hospita* were collected in Samarinda, East Kalimantan, Indonesia on February 2007. The plant was identified in Laboratory of Dendrology and the voucher specimen (THG-CW-07) was deposited at Laboratory of Wood Chemistry of Forestry Faculty, Mulawarman University, Indonesia.

Extraction and isolation

1 kg of dried leaves of *K. hospita* was extracted with 15 l of ethanol at room temperature for 24 h, and then filtered using Whatman No. 2 filter paper. The filtrates were dried under vacuum using a rotary evaporator and resulted in 178.38 g of extract. A portion of the extract (90.95 g) was suspended in methanol–water (1:2) and partitioned with *n*-hexane, diethyl ether and ethyl acetate. The results were 41.97, 2.95, and 2.70 g, respectively, and 34.97 g for its residue.

Silica gel column chromatography of ethyl acetate-soluble portion (2.7 g, 84% of radical scavenged activity at 100 μg/ml) with elution of the gradient of *n*-hexane–EtOAc (10:0–0:10) gave eight fractions (F1–F8). The active fraction, F6 (94.3 mg, 93% of radical scavenged activity at 100 μg/ml) was subjected to preparative HPLC with eluting with MeOH/H_2O (0.1% trifluoroacetic acid, TFA), 60:40, 8 ml/min by column (Inertsil Prep-ODS:20 mm i.d. × 250 mm), yielded compound **1** (5 mg, yield 0.00099%, Fig. 1) named kaempferol 3-*O*-*β*-D-glucoside or astragalin which was identical with reported data [6].

The other active fraction, F7 (455 mg, 82% of radical scavenged activity at 100 μg/ml) was repeated silica gel column chromatography with elution of the gradient of *n*-hexane–EtOAc (10:0–0:10) and gave eight fractions (F7-1 to F7-8). Fraction F7-5 (68.5 mg) was subjected to recrystallization with acetone and ethanol, then led to the isolation of compound **2** (6.3 mg, yield 0.0012%, Fig. 2).

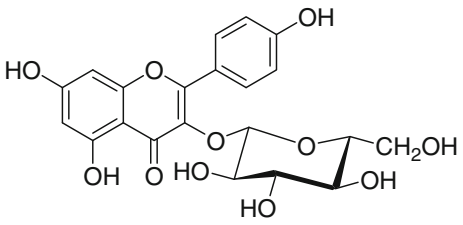
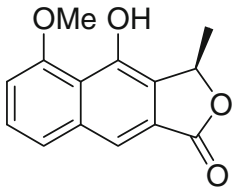


Fig. 1 Structure of compound **1**

Fig. 2 Structure of compound **2**



Compound 1 Kaempferol 3-*O*-*β*-D-glucoside Yellow amorphous powder. ^{1}H-NMR and ^{13}C-NMR (in DMSO-d_6) spectral data were coincided to that of published report [6]. The ^{1}H-NMR (400 MHz, DMSO-d_6) δ3.05 (2H, br), 3.21 (2H, m), 3.35 (1H, br d, J = 11.8 Hz), 3.55 (1H, br d, J = 11.8 Hz), 4.24 (1H, br), 4.88 (1H, br, OH), 5.04 (1H, br, OH), 5.27 (1H, br, OH), 5.44 (1H, d, J = 7.3 Hz), 6.19 (1H, d, J = 1.9 Hz), 6.42 (1H, d, J = 1.9 Hz), 6.87 (2H, d, J = 9.0 Hz), 8.03 (2H, d, J = 9.0 Hz). ^{13}C-NMR (400 MHz, DMSO-d_6): δ177.4 (C-4), 164.1 (C-7), 161.2 (C-5), 159.9 (C-4′), 156.4 (C-9), 156.2 (C-2), 133.1 (C-3), 130.9 (C-2′ and C-6′), 120.9 (C-1′), 115.1 (C-3′ and C-5′), 104.0 (C-10), 100.9 (C-2″), 98.7 (C-6), 93.7 (C-8), 77.5 (C-3″), 76.4 (C-5″), 74.2 (C-2″), 69.8 (C-4″), 60.8 (C-6″).

Compound 2 Eleutherol Colorless needles, mp 204–205°C, $[\alpha]_D^{25}$ + 91° ($CHCl_3$). ^{1}H-NMR and ^{13}C-NMR (in DMSO-d_6) spectral data were coincided to that of published report [7, 8]. The ^{1}H-NMR (400 MHz, DMSO-d_6) δ1.64 (3H, dd, J = 1.2 Hz), 2.49 (1H, d, J = 1.7 Hz), 4.05 (1H, s, OMe), 5.77 (1H, m), 7.12 (1H, d, J = 7.8 Hz), 7.48 (1H, dd, J = 7.8 and 8.3 Hz), 7.69 (1H, d, J = 8.3 Hz), 7.93 (1H, s). ^{13}C-NMR (400 MHz, DMSO-d_6): 170.1 (C-1), 77.2 (C-3), 149.1 (C-4), 156.7 (C-5), 107.3 (C-6), 127.7 (C-7), 123.2 (C-8), 116.2 (C-9), 125.5 (C-10), 128.1 (C-11), 117.5 (C-12), 137.3 (C-13), 56.85 (OCH_3), 19.37 (CH_3).

Antioxidant assay

The sample was first dissolved in DMSO and used for the actual experiment at 30 times dilution. The assay with

DPPH radical scavenging method was performed as previously described by Arung et al. [9].

Cytotoxicity on HepG2 cells

HepG2 cells were grown and maintained in DMEM with L-glutamine supplemented with 10% (v/v) fetal bovine serum, sodium bicarbonate, 100 μg/ml streptomycin and 100 U/ml penicillin at 37°C in humidified 5% CO_2 atmosphere. To determine cells viability, MTT assay was used as described by Arung et al. [6] with minor modification.

Results and discussion

In our previous study, we have reported ethanol extract of leaves from *K. hospita* possessed antioxidant ability and showed cytotoxicity in HepG2 cells [10]. Based on these results and the biological activity guided-fractionation, we isolated the active compounds. The active compounds were namely kaempferol 3-*O*-*β*-D-glucoside (**1**) and eleutherol (**2**) as seen in Figs. 1 and 2, respectively.

The isolated compounds were examined their antioxidant activities using DPPH radical scavenging assay as shown in Table 1. In this table, compounds **1** and **2** exhibited that the IC_{50} value were 71.4 and 491.8 μM, respectively. The trolox, as positive control have IC_{50} value with 6.0 μM. These results also implied that compound **1** was stronger than **2**. Several studies have reported that compound **1** has antioxidant ability which have been isolated from some plants [11–14]. As mentioned above that compound **2** exhibited lower antioxidant but to our knowledge, this is the first time that antioxidant of compound **2** was reported. It has been reported that the compound **2** has several biological activities such as inhibitor of Wnt/*β*-catenin signal [15], antistaphylococcal [16] and antifungal [17].

Table 1 Effect of isolated compounds on radical scavenging activity and on growth of HepG2 cells

Compounds	IC_{50} (μM)[a]	
	DPPH radical scavenging	HepG2 cells
Kaempferol 3-*O*-*β*-D-glucoside (**1**)	71.4	>223.2[b]
Eleutherol (**2**)	491.8	>409.8[c]
Trolox	6.0	–
5-Fluorourasil	–	423.0

[a] The IC_{50} was interpolated from graphed concentrations and determined graphically with statistical software

[b] 0% inhibition of cell proliferation at 223.2 μM

[c] 5% inhibition of cell proliferation at 409.8 μM

In our previous report, the ethanol extract showed cytotoxicity on HepG2 cells dose dependently [10]. These results led us to examine the effect of the isolated antioxidative compounds on HepG2 cells. As shown in Table 1, the IC_{50} value of compound **1** was above 223.2 μM with no cytotoxic effect at 223.2 μM. There is no report on the effect of compound **1** on the growth on HepG2 cells but Kinjo et al. [18] reported that kaempferol had cytotoxic effect on HepG2 cells with IC_{50} value of 72 μM. The diminishing cytotoxic effect of compound **1** may relate to the attachment of glucoside moiety on the parent compound, kaempferol. Lin et al. [19] reported that luteolin has cytotoxic effect 41% on HepG2 cells while luteolin-5-*O*-*β*-D-glucoside has 10%.

On the other hand, the compound **2** demonstrated IC_{50} value above 409.8 μM with only 5% growth suppression at 409.8 μM. Furthermore, Gan et al. [20] isolated several compounds that belongs to cycloartane triterpenoids such as 21*S*,23*R*-21/23,23/27-diepoxy-21-methoxycycloartan-1,24-diene-3,27-dione; 21*S*,23*R*-21/23,23/27-diepoxy-21-hydroxycycloartan-1,24-diene-3,27-dione; 21*R*,23*R*-21/23,23/27-diepoxy-21-hydroxycycloartan-1,24-diene-3,27-dione and cycloartan-1,24-diene-3,23-dione with IC_{50} values of 123.1, 37.9, 109.1 and 66.4 μM, respectively, on HepG2 cells.

In summary, the result above depicted the *K. hospita* extract act as antioxidant and active principles are kaempferol 3-*O*-*β*-D-glucoside (**1**) and eleutherol (**2**). It should be noted that these active compounds did not show the cytotoxic effect on HepG2. The cytotoxic effect of this extract on HepG2 might be related with cycloartane triterpenoids as mentioned above. Taking the consideration with all these results, the extract of *K. hospita* pronounces a good candidate for future as antioxidant and liver curing disease as experienced by some tribes in Indonesia.

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