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Isolation of flavonol rhamnosides from *Pometia pinnata* leaves and investigation of α-glucosidase inhibitory activity of flavonol derivatives

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ABSTRACT

Pometia pinnata belonging to the *Sapindaceae* family has been traditionally used as the therapeutic agent for burns and wounds in Indonesia. Based on the result of the experiment conducted, the ethyl acetate fraction of *P. pinnata* leaves showed strong α-glucosidase inhibitory activity. After two flavonol rhamnoside compounds were isolated from ethyl acetate fraction of *P. pinnata* leaves methanol extract using chromatography method, their structures were identified as kaempferol-3-*O*-rhamnoside (**1**) and quercetin-3-*O*-rhamnoside (**2**). The ultra-performance liquid chromatography-electrospray ionization time-of-flight mass spectrometry (UPLC-ESI-TOFMS) chromatogram showed compounds **1** and **2** were the major compounds of the ethyl acetate fraction. In this study, the structure–activity relationship among the kaempferol, quercetin, and their derivatives bearing sugar moiety were also evaluated. Among tested eight compounds, kaempferol **7** (percent inhibition = 80.10% ± 0.8) and quercetin **8** (percent inhibition = 82.93% ± 0.4) had stronger α-glucosidase inhibitory activity than that of other derivatives. Among kaempferol derivatives bearing sugar moiety, compound **1** showed the strongest activity. Moreover, compound **2** showed strong α-glucosidase inhibitory activity among quercetin derivatives. Therefore, it can be confirmed that the hydroxyl group at C-3 position is very important for α-glucosidase inhibitory activity of flavonol compounds.

INTRODUCTION

Pometia genus belongs to the *Sapindaceae* family. This genus comprises 10 species that are distpersed from Malaysia to India (Jayasinghe *et al*., 2000). *Pometia pinnata* is one of the interesting species among *Pometia* genus. This plant has been traditionally used as a therapeutic agent for burns and wounds in Indonesia (Lense, 2012). Previously, the biological activities of *P. pinnata* extracts were reported. The methanol extract of the bark revealed antioxidant and antifungal activities (Kawamura *et al*., 2010). The ethanol extract of the bark also was reported to have α-glucosidase and α-amylase inhibitory activities (Elya *et al*., 2015). Recently, the tyrosinase inhibitory activities from the ethanol extract of the bark and leaves were studied (Sauriasari *et al*., 2017). Furthermore, the ethanol extract of the leaves and proanthocyanidin A2 from *P. pinnata* leaves indicated strong anti-HIV activity (Suedee *et al*., 2013). Nevertheless, there are no reported flavonol glycosides isolated from *P. pinnata* leaves as α-glucosidase inhibition. The aims of this research are to isolate secondary metabolites of *P. pinnata* leaves and to evaluate the α-glucosidase inhibitory activity of flavonol derivatives.

MATERIAL AND METHODS

General

All solvents and reagents were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) and used without

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further purification. Silica gel for column chromatography (CC) was performed on silica gel N60 (Wakogel 60N, 38–100 µm) and TLC plates pre-coated with silica gel 70 F_{254} (60–200 mesh Wako gel) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The separation spots of CC were monitored under the UV lamp (254 and 365 nm) and also by spraying with 10% $CeSO_4$ followed by heating. IR spectra were measured on a PerkinElmer FT-IR/FIR Spectrometer 400. ^{1}H, ^{13}C, heteronuclear multiple-quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC), and ^{1}H-^{1}H-correlation spectroscopy (^{1}H-^{1}H-COSY) nuclear magnetic resonance (NMR) spectra were recorded with JEOL JNM-ECS 400 spectrometer using tetramethylsilane (TMS) as an internal standard. MS spectra were recorded using the Waters UPLC-ESI-TOFMS system (Aquity UPLC XevoQTof) (Waters Corporation, Milford).

Plant material

The fresh leaves of *P. pinnata* (12 kg) were collected from the environment of Limau Manis, Padang, West Sumatera, Indonesia in June 2017 and later identified at Herbarium of Universitas Andalas, Padang, West Sumatera, Indonesia.

Extraction and isolation of secondary metabolites

The dried leaves (4 kg) were extracted using MeOH (12 L) at room temperature for 3 days, after that filtered and evaporated under vacuum. Afterward, the crude MeOH extract was partitioned subsequently to yield *n*-hexane fraction (45.5 g), dichloromethane (DCM) fraction (25.4 g), and EtOAc fraction (48.7 g), respectively.

EtOAc fraction of *P. pinnata* leaves (40.0 g) was separated by SiO_2 CC eluting with mixtures of *n*-hexane/EtOAc (100:0-0:100 v/v), EtOAc/MeOH (100:0-0:100 v/v) to obtain 15 fractions (Fr. A to Fr. O). Fraction D (14.1 g) was performed on silica gel CC using a mixture of *n*-hexane/EtOAc (9:1 v/v) to yield five sub-fractions (D1–D5). Fraction D4 (1.1 g) was separated by CC using EtOAc to give three sub-fractions (D4.1–D4.3). Fraction D4.1 (361.2 mg) was purified by recrystallization to obtain compound **1** (211.7 mg). Fraction D4.2 (188.9 mg) was purified by CC using $CHCl_3$/MeOH (5:1 v/v) to yield compounds **2** (17.4 mg) and also **1** (39.0 mg).

Identification of secondary metabolites

According to the analysis of spectroscopic data, including IR, NMR (1D, 2D), and MS, the structures of compounds (**1**–**2**) were determined. The NMR spectroscopic data were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C using TMS as an internal standard.

Kaempferol-3-O-rhamnoside (1)

Yellow amorphous powder. IR $_{\nu max}$ 3253, 2956, 1651, 1361, 1171, and 828 cm^{-1}. ^{1}H NMR (400 MHz, $(CD_3)_2CO$ (ppm): δ 7.84 (2H, dd, *J* = 8.7, 2.8 Hz, H-2′ and H-6′), 7.01 (2H, dd, *J* = 8.7, 2.8 Hz, H-3′ and H-5′), 6.45 (1H, d, *J* = 2.3 Hz, H-8), 6.25 (1H, d, *J* = 1.8 Hz, H-6), 5.52 (1H, d, *J* = 1.4 Hz, H-1″), 4.20 (1H, d, *J* = 1.4 Hz, H-2″), 3.70 (1H, dd, *J* = 3.7, 8.7 Hz, H-3″), 3.30 (2H, m, H-4″ and H-5″), and 0.90 (3H, d, *J* = 6.0 Hz, Me-6″). ^{13}C NMR (100 MHz, *$(CD_3)_2CO$,* ppm): δ 178.4 (C-4), 164.3 (C-7), 162.3 (C-5), 160.1 (C-9), 157.7 (C-4′), 157.1 (C-2), 134.9 (C-3), 130.9 (C-2′ and C-6′), 121.6 (C-1′), 115.5 (C-3′ and C-5′), 104.9 (C-10), 101.9 (C-1″), 98.8 (C-6), 93.8 (C-8), 72.0 (C-4″), 71.0 (C-2″), 70.7 (C-3″), 70.6 (C-5″), 17.0 (Me-6″). HRESITOFMS: *m/z* 455.0944 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_{10}Na$, 455.0954; error: 2.2 ppm).

Quercetin-3-O-rhamnoside (2)

Yellow amorphous powder. IR $_{\nu max}$ 3198, 2923, 1652, 1354, 1195, 808 cm^{-1}. ^{1}H NMR (400 MHz, *CD_3OD,* ppm): δ 7.33 (1H, d, *J* = 1.9 Hz, H-2′), 7.30 (1H, dd, *J* = 8.2, 2.3 Hz, H-6′), 6.91 (1H, d, *J* = 8.2 Hz, H-5′), 6.36 (1H, d, *J* = 1.8 Hz, H-8), 6.20 (1H, d, *J* = 1.8 Hz, H-6), 5.30 (1H, d, *J* = 1.4 Hz, H-1″), 4.20 (1H, dd, *J* = 3.2, 1.4 Hz, H-2″), 3.70 (1H, dd, *J* = 9.1, 3.7 Hz, H-3″), 3.40 (1H, dd, *J* = 5.9, 9.6 Hz, H-5″), 3.30 (1H, dd, *J* = 5.9, 9.6 Hz, H-4″), 0.90 (3H, d, *J* = 6.0 Hz, Me-6″). ^{13}C NMR (100 MHz, *CD_3OD,* ppm): δ 178.3 (C-4), 164.6 (C-7), 161.9 (C-5), 158.0 (C-9), 157.2 (C-2), 148,5 (C-4′), 145.1 (C-3′), 134.9 (C-3), 121.6 (C-1′), 121.5 (C-6′), 115.6 (C-5′), 115.0 (C-2′), 104.6 (C-10), 102.2 (C-1″), 98.4 (C-6), 93.4 (C-8), 71.9 (C-4″), 70.8 (C-2″), 70.7 (C-3″), 70.6 (C-5″), 16.3 (Me-6″) HRESITOFMS: *m/z* 471.0886 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_{11}Na$, 471.0903; error: 3.7 ppm).

Determination of α-glucosidase inhibitory activity

Chemical reagents

α-Glucosidase [(EC 3.2.1.20)] from *Saccharomyces cerevisiae, p*-nitrophenyl α-D-glucopyranoside (*p*NPG) and quercetin (**8**) were purchased from Sigma-Aldrich, Co. Dimethyl sulfoxide (DMSO) was purchased from Wako Pure Chemical Industries, Ltd (Japan). Three compounds, astragalin (**3**), isoquercetin (**4**), and nicotiflorin (**5**) were isolated from *Eleutherococcus sieboldianus* leaves (Nishina *et al*., 2017). Rutin (**6**) and kaempferol (**7**) were purchased from Tokyo Chemical Industry, Ltd (Tokyo, Japan). A 96-well microtiter plate was purchased from Corning Inc. (Corning Costar, Cambridge, MA).

α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity was evaluated according to the previous method (Dewi *et al*., 2015). α-Glucosidase (50 µl, 0.5 ~ Unit/mg) and 2 µl of various concentrations of the sample in DMSO were poured into 96-well microplate, and then pre-incubated at 37°C for 5 minutes. The reaction was started by the addition of 50 µl of *p*NPG (5 mM). The mixture was later incubated at 37°C for 5 minutes. The α-glucosidase activity was measured by using a microplate reader (E_{max} precision microplate reader, Molecular Devices Japan, Tokyo, Japan) at 405 nm. The % inhibition value expressed α-glucosidase inhibitory activity of each sample and calculated as follows (Eq. 1):

$$\%\ \text{Inhibition} = 100 - \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \right) \quad \text{(Eq. 1)}$$

where the control represents the assay in which the sample is replaced by the solvent used in its preparation.

UPLC-ESI-TOFMS analysis

The EtOAc fraction of *P. pinnata* leaves was dissolved in DMSO/H_2O (1/1) at 20 mg/ml and filtered across 0.45 µm membrane filter (ADVANTEC®, Japan). An aliquot (5 µl) of the sample was injected in the Waters UPLC system (Aquity UPLC XevoQTof). Analysis was carried out by UPLC system using a UPLC BEH C_{18} analytical column (1.7 µm, ø 2.1 x 100 mm). The mobile phase contained solvent A (distilled water) and solvent B (MeOH). The condition of linear gradient system: 0–30 minutes (90% to 70% solvent A and 10% to 30% solvent B); was kept for 5 min; 35–45 minutes (70% to 50% solvent A and 30% to

50% solvent B). The column eluate was monitored at 260 nm UV absorbance. The negative mode was employed in ESI-TOFMS (Pardede and Koketsu, 2017).

RESULTS AND DISCUSSION

α-Glucosidase inhibitory activity of Pometia pinnata fractions

Fractions of *n*-hexane (45.5 g), DCM (25.4 g), and EtOAc (48.7 g) were obtained by successively fractionation of MeOH extract of *P. pinnata* leaves. As preliminary screening, we evaluated *in vitro* inhibitory activity of three fractions of *P. pinnata* leaves against α-glucosidase of *S. cerevisiae* with slight modification (Dewi et. al., 2015). In this study, the EtOAc fraction of *P. pinnata* leaves showed the strongest inhibitory activity against α-glucosidase (percent inhibition = 95.5% ± 0.7) at 100 µg/ml. DCM fraction had inhibitory activity (percent inhibition = 84.7% ± 0.1) at the same concentration, while *n*-hexane fraction showed poor inhibitory activity (percent inhibition = 38.7% ± 1.9) (Fig. 1). According to this result, the compounds from EtOAc fraction of *P. pinnata* leaves must be isolated.

Characterization of isolated compounds

To ascertain the potential compounds with respect to these properties, the EtOAc fraction of *P. pinnata* leaves was separated by CC on silica gel (SiO_2) and later purified by CC and recrystallization to obtain two flavonol rhamnoside compounds, namely, compound **1** (146.5 mg) and compound **2** (17.4 mg). The chemical structure of isolated compounds was elucidated based on spectroscopic data, including IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and HRESITOFMS. Compound **1** was obtained as a yellow amorphous powder. IR data exhibited the presence of hydroxyl and carbonyl functional groups at 3253 and 1651 cm^{-1}, C-H stretching at 2956 cm^{-1}, C=C olefin ring at 1361 cm^{-1}, asymmetric C-O-C stretching at 1171 cm^{-1}, and substituted benzene at 828 cm^{-1}. The ^{1}H NMR spectrum (see Supplementary Data) of **1** showed four aromatic hydrogen signals containing six protons (Table 1). Two protons at 7.84 ppm (2H, dd, J = 8.7, 2.8 Hz, H-2′ and H-6′) and two protons at 7.01 ppm (2H, dd, J = 8.7, 2.8 Hz, H-3′and H-5′) assigned symmetric pattern with substitution at 1 and 4 positions. ^{1}H-^{1}H COSY correlation showed two set of *ortho*-coupled aromatic protons at 7.84 ppm (H-2′/H-6′) and 7.01 ppm (H-3′/H-5′) with coupling constant as J = 8.7 Hz. Furthermore, the proton at H-2′ had *meta*-coupled with H-6′, and H-3′ with H-5′ (J = 2.8 Hz) of B ring of the flavone skeleton. A remaining two aromatic proton signals at 6.45 ppm (1H, d, J = 2.3 Hz) and 6.25 ppm (1H, d, J = 1.8 Hz) were assigned at H-8 and H-6 positions. Accordingly, this compound was predicted to have trihydroxyl substitutions at C-5 (162.3 ppm), C-7 (164.3 ppm), and C-4′ (157.7 ppm) in flavone skeleton. The ^{13}C NMR spectrum indicated 19 carbon signals. The correlation between proton and carbon in HMQC showed six methines aromatic at H-6 (6.25 ppm)/C-6 (98.8 ppm), H-8 (6.45 ppm)/C-8 (93.8 ppm), H-2′ or H-6′ (7.84 ppm)/C-2′ and or C-6′ (130.9 ppm), H-3′ or H-5′ (7.01 ppm)/C-3′ and or C-5′ (115.5 ppm). According to HMQC correlation, the remaining of annomeric proton located at 5.52 ppm (1H, d, J = 1.4 Hz), four oxygenated methines at 4.20 ppm (1H, d, J = 1.4 Hz, H-2″), 3.70 ppm (1H, dd, J = 3.7, 8.7 Hz, H-3″), 3.33 ppm (2H, m, H-4″ and H-5″), and one methyl at 0.90 ppm (3H, d, J = 5.9 Hz, Me-6″) indicated the occurrence of rhamnosyl unit in this compound. HMBC correlation of the annomeric proton at 5.52 ppm with the annomeric carbon at 134.9 ppm confirmed that sugar moiety was bound to the C-3 hydroxyl group. The HRESITOFMS data showed the molecular formula was established as $C_{21}H_{20}O_{10}$ for the peak *at m/z* 455.0944 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_{10}Na$, 455.0954; error 2.2 ppm). Compared the spectral data of the isolated compound with literature, we confirmed this isolated compound **1** as kaempferol-3-*O*-rhamnoside (afzelin) (Suedee *et al*., 2013).

Compound **2** was obtained as a yellow amorphous powder. On the TLC, the spot of compound **2** was active under UV lamp 254 and 365 nm and came out yellow after spraying with 10% $CeSO_4$, which was characteristic of flavone compound. The IR data of compound **2** indicated to bear the similar functional groups as similar as to compound **1**, which were the vibrations of hydroxyl

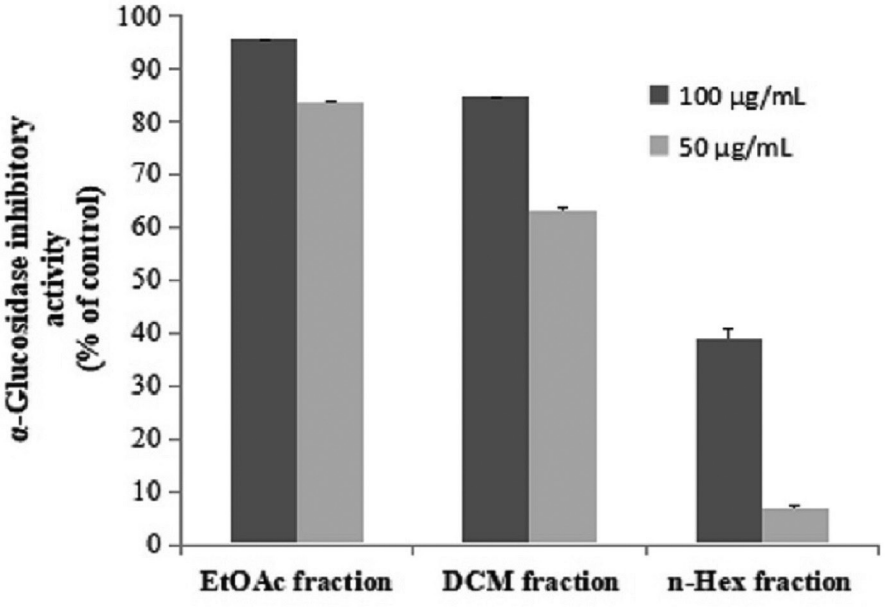

Figure 1. α-Glucosidase inhibitory activity of Pometia pinnata leaves fractions (mean ± SEM; n = 5).

group, C-H stretching, carbonyl functional group, C=C olefin ring, asymmetric C-O-C stretching, and substituted benzene at 3198, 3002, 1652, 1354, 1195, and 808 cm−1, respectively. The ^{1}H and ^{13}C NMR spectra of the compound **2** were also similar to the spectra of compound **1**. The ^{1}H NMR spectrum showed five aromatic proton signals at 7.33 ppm (1H, d, J = 1.9 Hz, H-2′), 7.30 (1H, dd, J = 8.2, 2.3 Hz, H-6′), 6.91 (1H, d, J = 8.2 Hz, H-5′), 6.36 (1H, d, J = 1.8 Hz, H-8), 6.20 (1H, d, J = 1.8 Hz, H-6). One set of *meta*-coupled aromatic protons appeared at 7.33 ppm (d, J = 1.9 Hz) and 7.30 ppm (dd, J = 2.3 Hz), in other than proton at 7.30 ppm (dd) also had *ortho* coupling with proton at 6.91 ppm (d) with coupling constant of J = 8.2 Hz. We confirmed the presence of three substitutions in B ring. Remaining two *meta*-coupled aromatic protons at 6.25 and 6.36 ppm (J = 1.8 Hz) indicated dihydroxyl substitutions in A ring of flavone skeleton. The ^{13}C NMR spectrum showed 21 signals, containing 6 rhamnosyl carbon signals (102.2, 70.8, 70.7, 71.9, 70.6, and16.3 ppm) in Table 1. The molecular formula was established as $C_{21}H_{20}O_{11}$ for the peak *at m/z* 471.0886 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_{11}Na$; error: 3.7 ppm) from HRESITOFMS. Comparing the spectral data of the isolated compound with related literature, we confirmed this isolated compound **2** as quercetin-3-*O*-rhamnoside (quercitrin) (Gopi *et al*., 2016).

UPLC-ESI-TOFMS analysis

The EtOAc fraction and isolated kaempferol-3-*O*-rhamnoside (**1**) and quercetin-3-*O*-rhamnoside (**2**) were analyzed by UPLC-ESI-TOFMS. The chromatogram of the EtOAc fraction revealed two major peaks at retention time 34.16 and 41.23 min (Fig. 2A). Figure 2B shows the peak of kaempferol-3-*O*-rhamnoside (**1**) which was detected at the retention time of 41.05 min, while the peak of quercetin-3-*O*-rhamnoside (**2**) revealed at the retention time 34.13 min (Fig 2C). Furthermore, a minor peak at retention time 28.92 min from EtOAc fraction of this plant also appeared (Fig 2A). Compared with the previously isolated compound, quercetin-3-*O*-glucoside (isoquercetin), appeared at retention time 28.54 min (Fig 2D). The minor peak at retention time 28.92 min in EtOAc fraction of *P. pinnata* leaves was confirmed to be quercetin-3-*O*-glucoside (Nishina *et al*., 2017).

α-Glucosidase inhibitory activity of isolated compounds

In this study, two flavonol compounds kaempferol-3-*O*-rhamnoside (**1**) and quercetin-3-*O*-rhamnoside (**2**) were isolated. The authors are interested in the structure–activity relationship of kaempferol derivatives and quercetin derivatives for α-glucosidase inhibitory activity. Four kaempferol derivatives and four quercetin derivatives were chosen, which include eight derivatives, including kaempferol (**7**) and quercetin (**8**) and their derivatives, bearing sugar moiety into the C-3 position, such as kaempferol-3-*O*-glucoside (**3**), quercetin-3-*O*-glucoside (**4**), kaempferol-3-*O*-glucosyl-(1→4)-rhamnoside (**5**), and quercetin-3-*O*-glucosyl-(1→4)-rhamnoside (**6**) for this assay (Fig. 3).

The *α*-glucosidase inhibitory activities all of the compounds (**1**–**8**) were evaluated according to the previous method with slight modification (Dewi *et al*., 2015). The % inhibitions of compounds **1**–**8** are summarized in Table 2. The results showed that quercetin **8** had the strongest α-glucosidase inhibitory activity among all the tested eight compounds (**1**–**8**). The % inhibition of compounds **1** and **2** at final concentration 50 μM were 45.06% ± 0.22 and 34.83% ± 0.59 against α-glucosidase, respectively, which were lower than that of kaempferol (**7**) and quercetin (**8**) with percent inhibition 82.93% ± 0.37 and 80.10% ± 0.76, respectively. We confirmed that the introduction of sugar moiety at the C-3 position in the flavonol structure reduced α-glucosidase inhibitory activity and that the hydroxyl group at the C-3 position was an essential requirement for the activity. Similar results have been reported, the hydroxyl group at the C-3 and C-5 positions on the A and C rings of

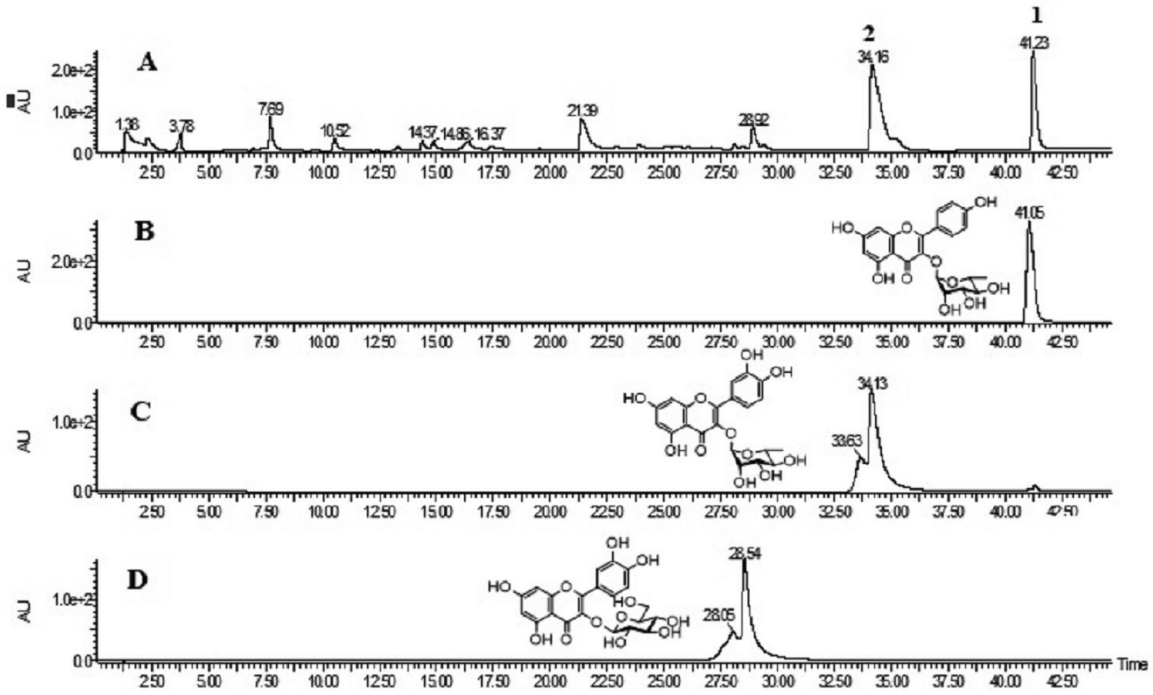

Figure 2. UPLC-ESI-TOFMS analyses of (A) EtOAc fraction of P. pinnata leaves, (B) Kaempferol-3-O-rhamnoside, (C) Quercetin-3-O-rhamnoside, and (D) Quercetin-3-O-glucoside.

Table 1. ^{1}H and ^{13}C NMR data of isolated compounds.

Position	Compound (1)[a]		Compound (2)[b]	
	δ C	δ H (mult., J (Hz))	δ C	δ H (mult., J (Hz))
2	157.1	-	157.2	-
3	134.9	-	134.9	-
4	178.4	-	178.3	-
5	162.3	-	161.9	-
6	98.8	6.25 (1H, d, J = 1.8)	98.4	6.25 (1H, d, J = 1.8)
7	164.3	-	164.6	-
8	93.6	6.45 (1H, d, J = 2.3)	93.4	6.36 (1H, d, J = 1.8)
9	160.1	-	158.0	-
10	104.9	-	104.5	-
1′	121.6	-	121.6	-
2′	130.9	7.84 (1H, dd, J = 8.7, 2.8)	115.0	7.33 (1H, d, J = 1.8)
3′	115.5	7.01 (1H, dd, J = 8.7, 2.8)	145.1	-
4′	157.7	-	148.5	-
5′	115.5	7.01 (1H, dd, J = 8.7, 2.8)	115.6	6.91 (1H, d, J = 8.3)
6′	130.9	7.84 (1H, dd, J = 8.7, 2.8)	121.5	7.30 (1H, dd, J = 8.3, 2.3)
1″	101.9	5.52 (1H, d, J = 1.4)	102.2	5.30 (1H, d, J = 1.4)
2″	71.0	4.20 (1H, d, J = 1.4)	70.8	4.20 (1H, d, J = 1.4)
3″	70.7	3.70 (1H, dd, J = 3.7, 8.7)	70.7	3.70 (1H, dd, J = 3.7, 9.1)
4″	72.0	3.33 (2H, m)	71.9	3.34 (1H, dd, J = 6.0, 9.6)
5″	70.6	3.33 (2H, m)	70.6	3.43 (1H, dd, J = 6.0, 9.6)
6″	17.0	0.90 (3H, d, J = 6.0)	16.3	0.90 (3H, d, J = 6.0)

[a] Measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $(CD_3)_2CO$.

[b] Measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD_3OD.

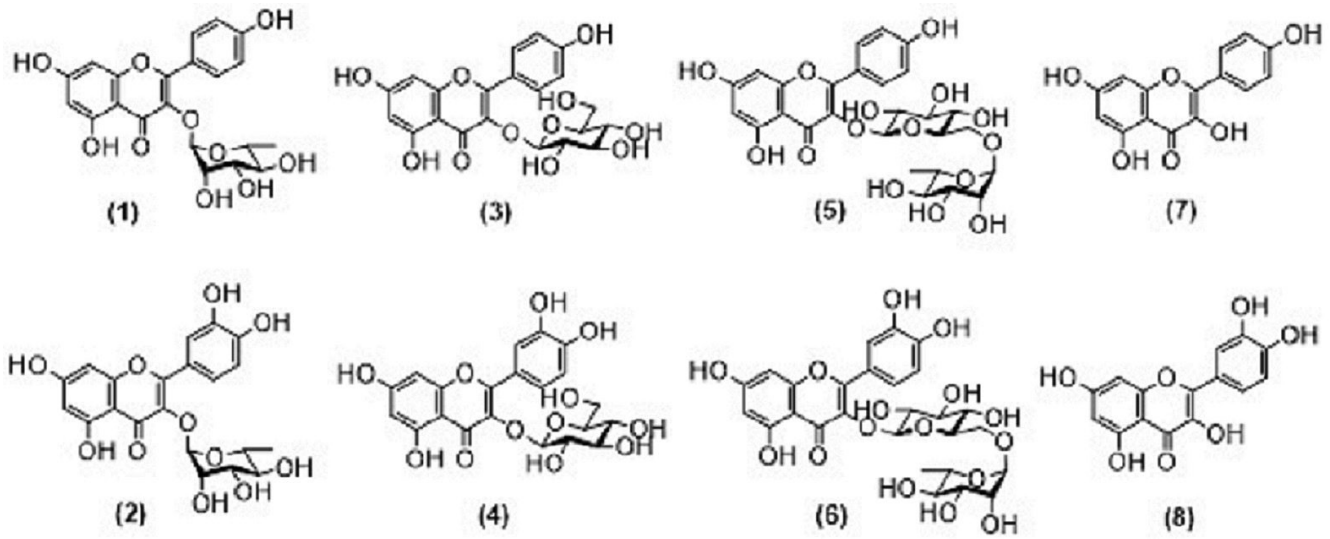

Figure 3. Chemical structures of flavonols and their derivatives compounds: Kaempferol-3-O-rhamnoside (afzelin) (1), Quercetin-3-O-rhamnoside (quercitrin) (2), Kaempferol-3-O-glucoside (astragalin) (3), Quercetin-3-O-glucoside (isoquercetin), (4), Kaempferol-3-O-glucosyl-(1→4)-rhamnoside (nicotiflorin) (5), Quercetin-3-O-glucosyl-(1→4)-rhamnoside (rutin) (6), Kaempferol (7), and Quercetin (8).

flavones enhancing the α-glucosidase inhibitory activity (Tadera *et al*., 2006). Compounds **1**, **3**, and **5** possessed the same aglycone as kaempferol. Compound **1** had a stronger inhibitory activity against α-glucosidase than that against of compounds **3** (percent inhibition = 37.18% ± 0.62) and **5** (% I = 26.38% ± 0.63). Quercetin-3-*O*-rhamnoside (**2**) had a stronger inhibitory effect among quercetin derivatives (**2, 4,** and **6**). The flavonol rhamnosides (**1** and **2**) were stronger than flavonol glucosides (**3** and **4**) and flavonol rutinosides (**5** and **6**). According to the literature data, kaempferol and quercetin showed stronger α-glucosidase inhibitory activity than that positive control (acarbose) (Abbasi *et al*., 2014; Proenca *et al*., 2017; Sheng *et al*., 2017). Kaempferol and quercetin from *C. asiatica* showed

Table 2. α-Glucosidase inhibitory activity of flavonols and their derivatives at the final concentration of 50 μM.

Compounds	α-Glucosidase inhibitory activity (% of control)
Kaempferol-3-O-rhamnoside (afzelin) (1)	45.06 ± 0.22
Quercetin-3-O-rhamnoside (quercitrin) (2)	34.83 ± 0.59
Kaempferol-3-O-glucoside (astragalin) (3)	37.18 ± 0.62
Quercetin-3-O-glucoside (isoquercetin) (4)	31.26 ± 0.46
Kaempferol-3-O-glucosyl-(1→4)-rhamnoside (nicotiflorin) (5)	26.38 ± 0.63
Quercetin-3-O- glucosyl-(1→4)-rhamnoside (rutin) (6)	26.67 ± 0.88
Kaempferol (7)	80.10 ± 0.76
Quercetin (8)	82.93 ± 0.37

Mean ± SEM, n = 5.

strong α-glucosidase inhibitory with IC_{50} 16~22 μg/ml (Dewi and Faiza, 2015). Both compounds also were reported to inhibit the rat small intestinal α-glucosidase (Tadera *et al*., 2006). On the other hand, the inhibitory activity of flavonoid glycosides is usually lower than that the aglycones (Sarian *et al*., 2017). The mechanism of kaempferol and quercetin as an antidiabetic treatment showed that they were competitive inhibitors for inhibitory α-glucosidase (Proenca *et al*., 2017; Sheng *et al*., 2017).

CONCLUSION

The EtOAc fraction from *P. pinnata* leaves contained the highest α-glucosidase inhibitory activity. This result was obtained after the isolation of two flavonol rhamnosides, such as kaempferol-3-*O*-rhamnoside (**1**) and quercetin-3-*O*-rhamnoside (**2**), from the EtOAc fraction of *P. pinnata* leaves. Based on the structure–activity relationship among tested compounds (**1–2** from *P. pinnata* leaves, **3–5** from *Eleutherococcus sieboldianus* leaves, and **6–8** from commercial available), the quercetin (**8**) showed the highest α-glucosidase inhibitory activity among eight flavonol derivatives. Compound **1** had stronger inhibitory activity than that of compound **2**. In the flavonol structure, the occurrence of sugar moiety at C-3 position reduced the α-glucosidase inhibitory activity. Among flavonol derivatives, the rhamnoside moiety bound to flavonol structure (**1** and **2**) was stronger than flavonol glucoside (**3** and **4**) and flavonol rutinoside (**5** and **6**) against α-glucosidase.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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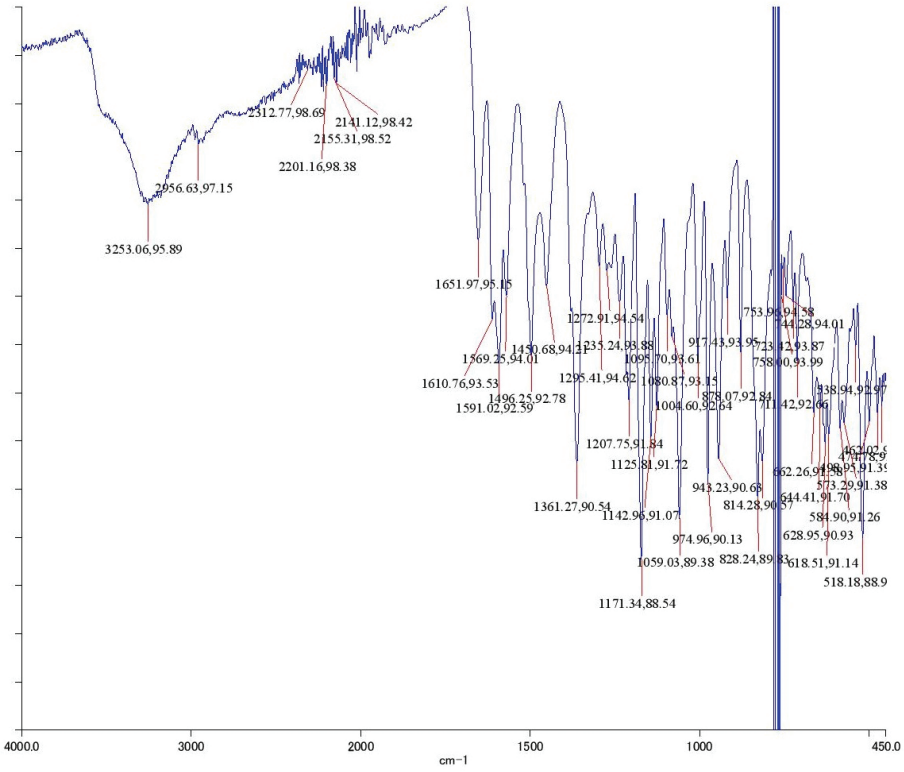
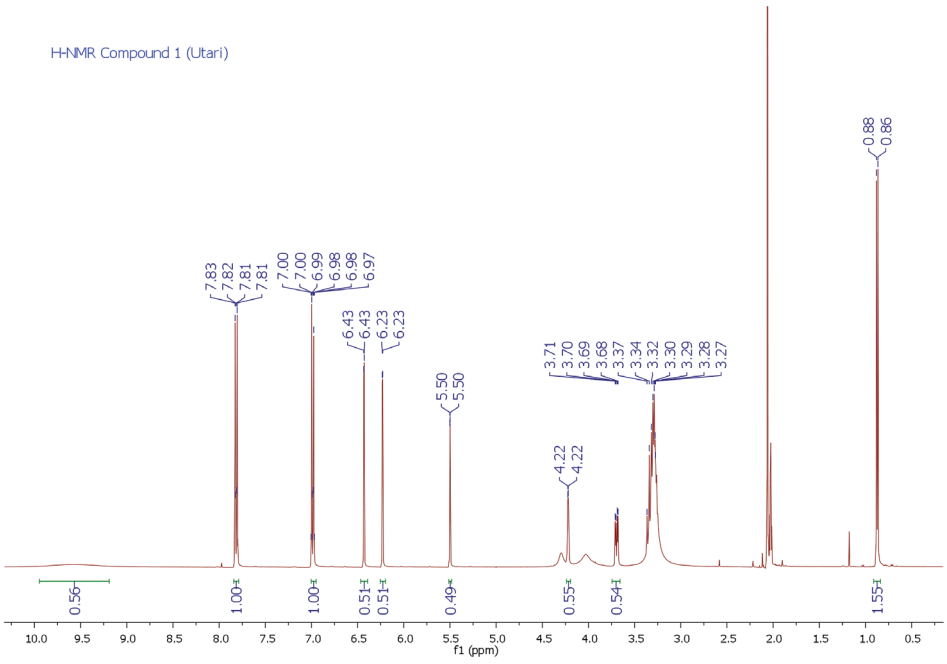
SUPPLEMENTARY DATA

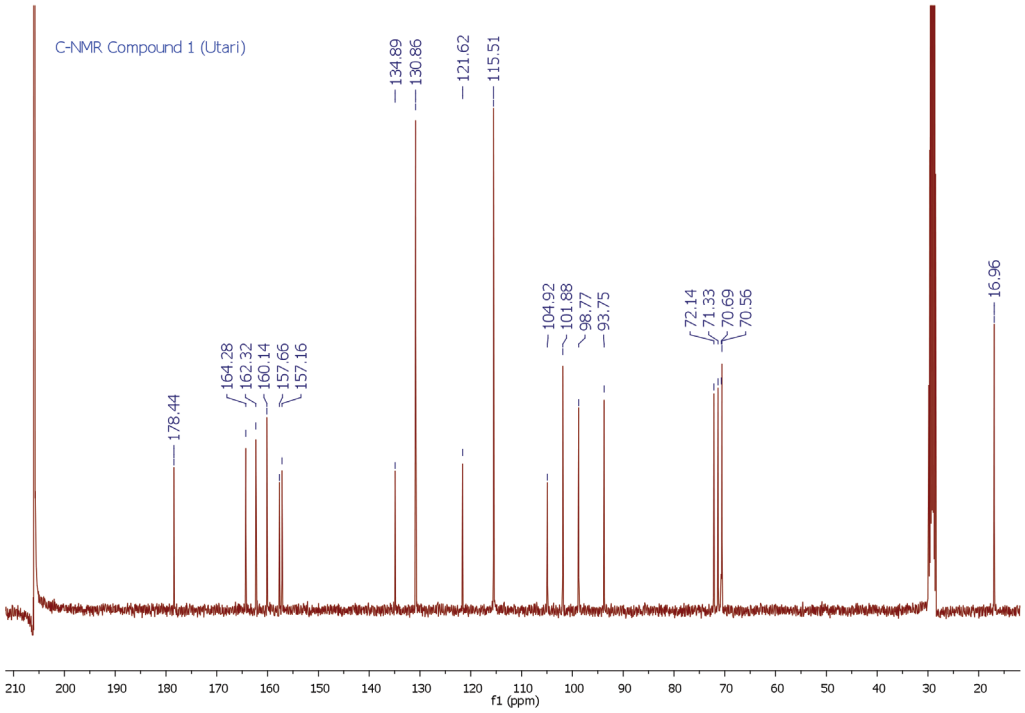
Compound 1



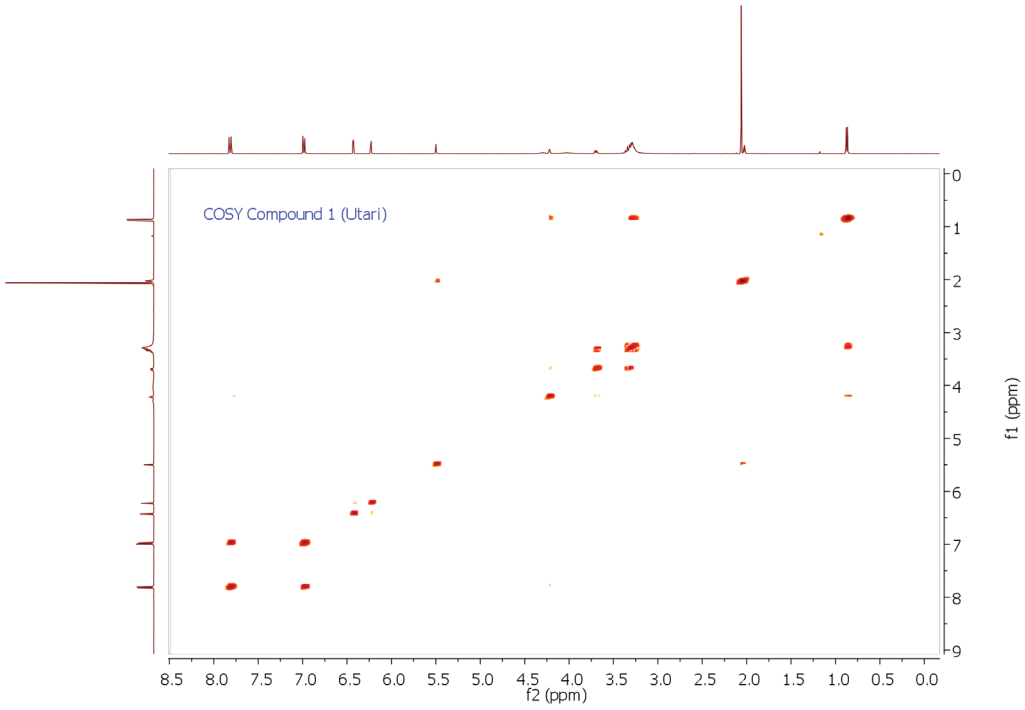
IR Spectrum of Compound 1



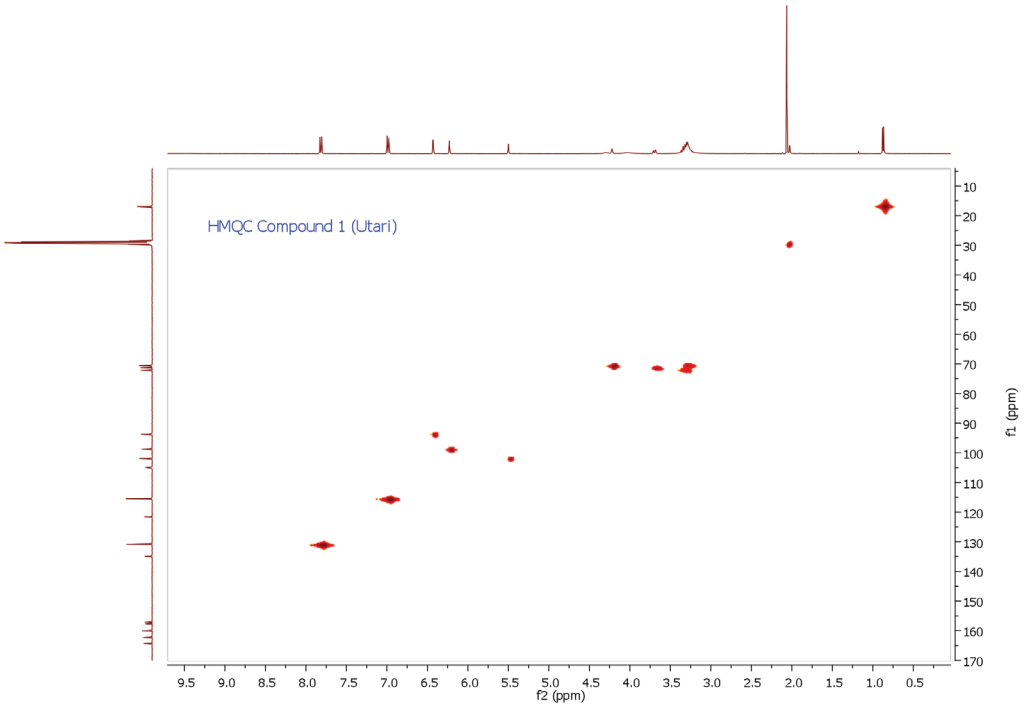
1H-NMR Spectrum of Compound 1



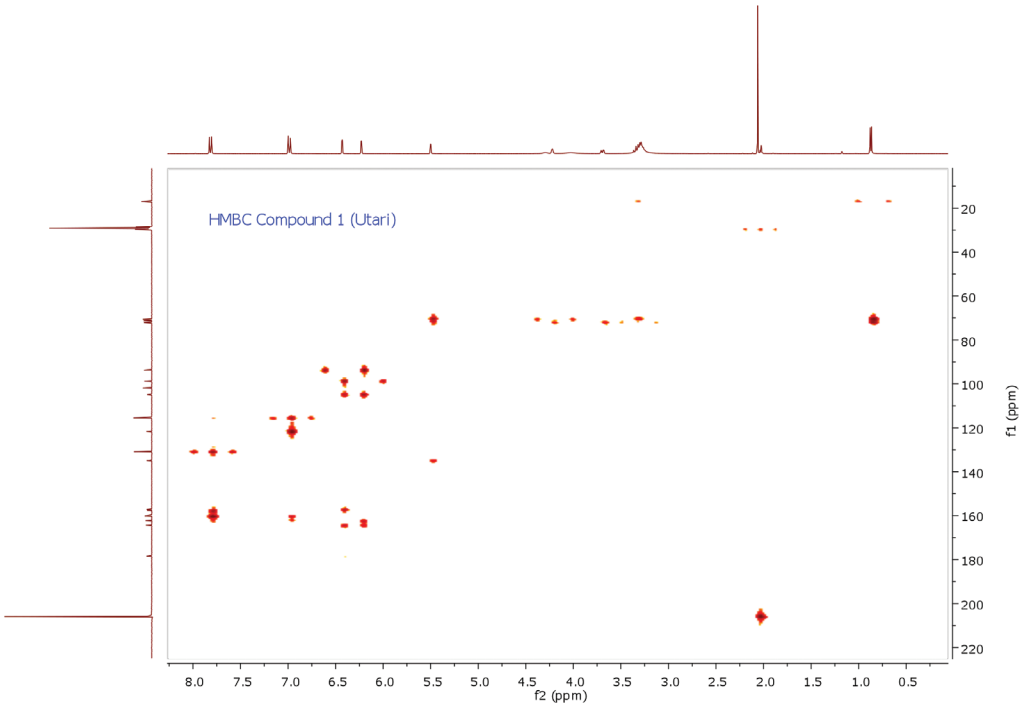
13C-NMR Spectrum of Compound 1



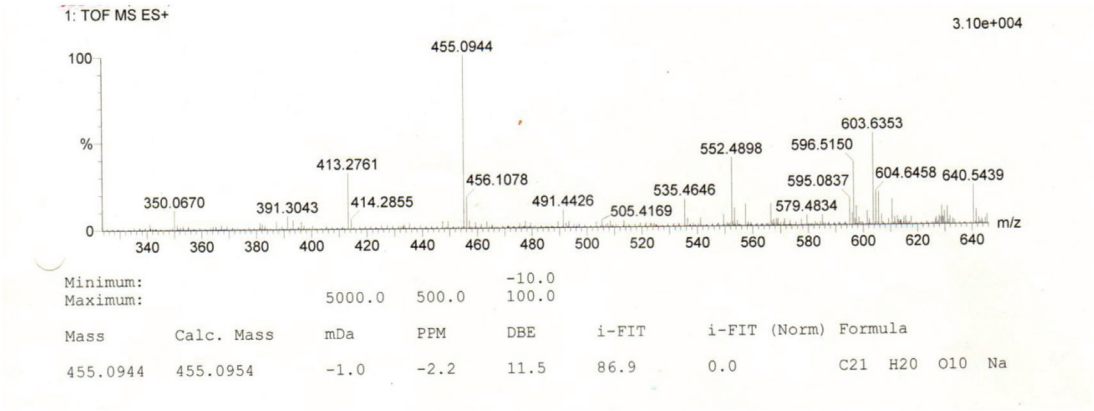
COSY Spectrum of Compound 1



HMQC Spectrum of Compound 1

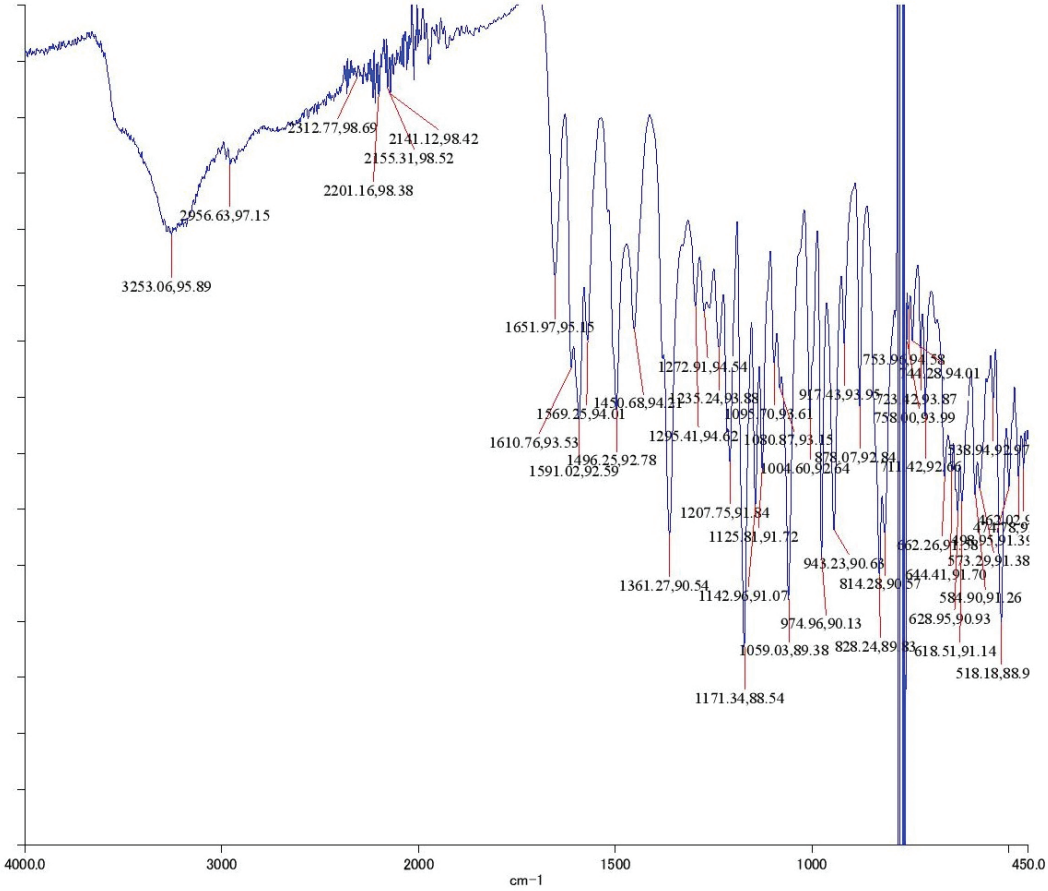


HMBC Spectrum of Compound 1

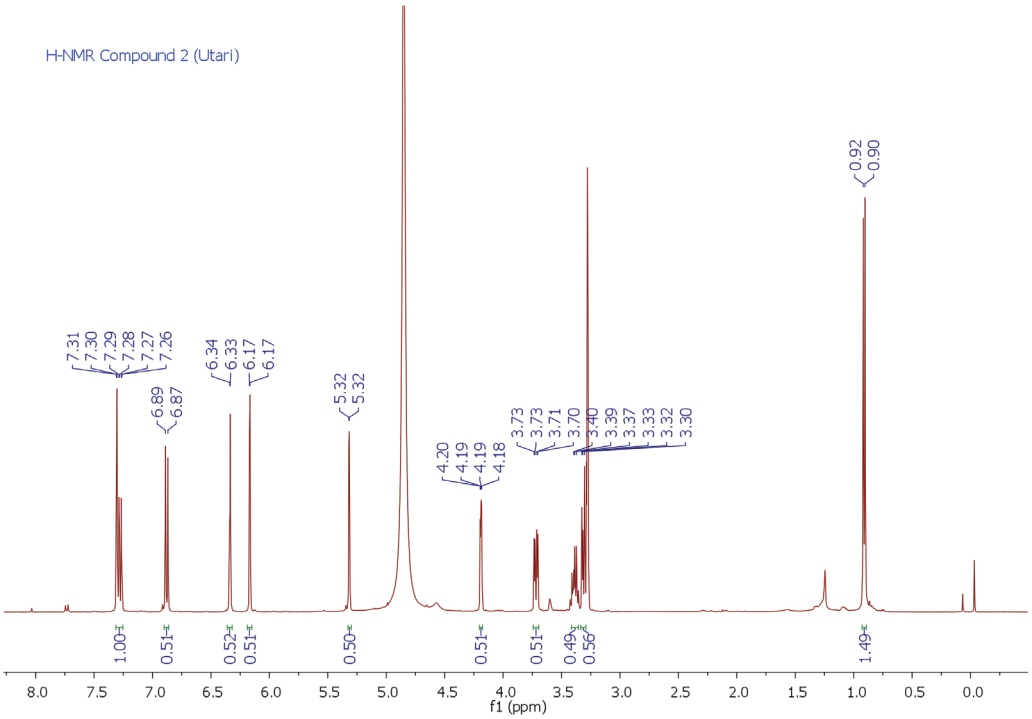


MS Spectrum of Compound 1

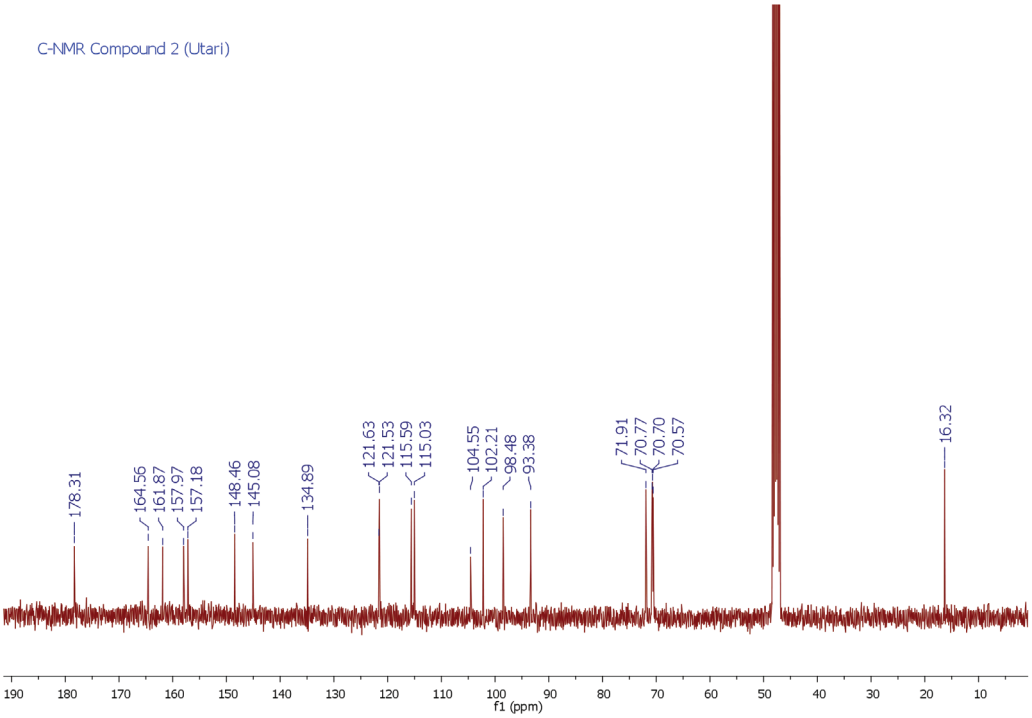
Compound 2



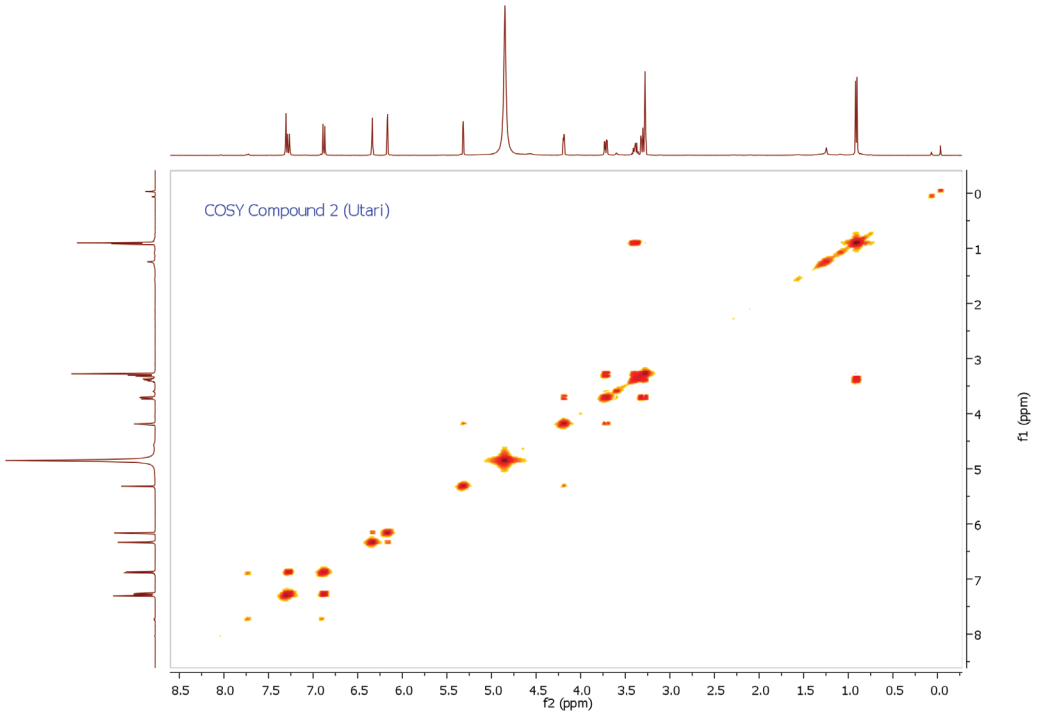
IR Spectrum of Compound 1



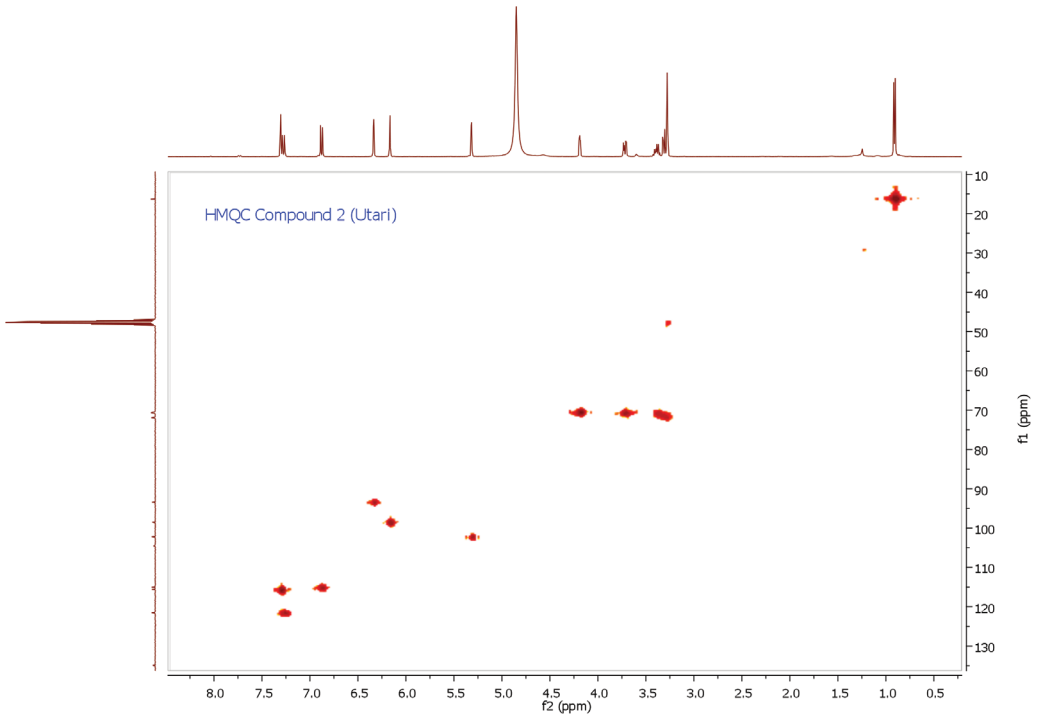
1H-NMR Spectrum of Compound 2



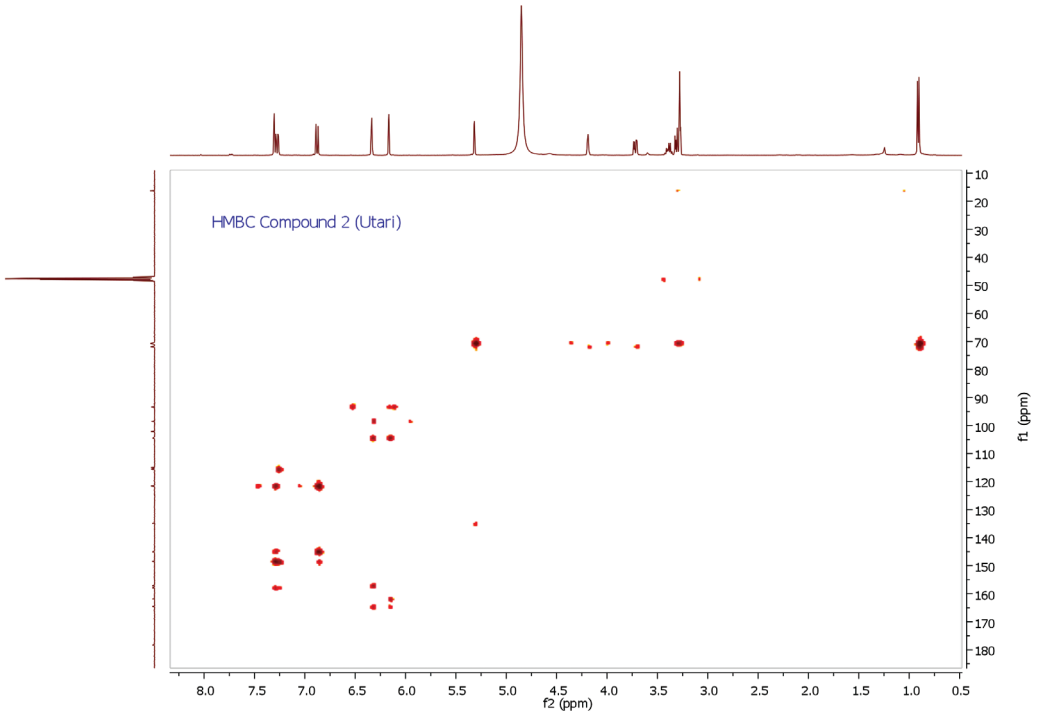
13C-NMR Spectrum of Compound 2



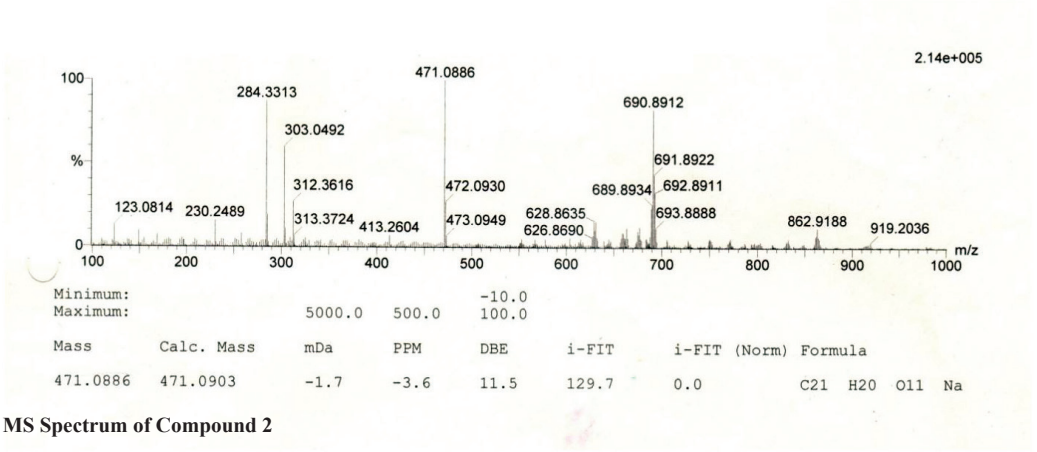
COSY Spectrum of Compound 2



HMQC Spectrum of Compound 2



HMBC Spectrum of Compound 2



MS Spectrum of Compound 2