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Submission date: 08-Nov-2021 12:15PM (UTC+0800)

Submission ID: 1696208933

File name: C-27 - Nat Prod J_naskah.pdf (548.41K)

Word count: 4999

Character count: 23443



Research Article

Amine Derivative from the Aerial Part of *Spilanthes acmella* Murr. and their Alkaline Phosphatase Activity



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Abstract:

Objectives: *Spilanthes acmella* Murr. is included in Asteraceae used as a traditional remedy for tooth-aches, and originated from Africa, America, Borneo, India, Sri Lanka, Bangladesh, China, Japan, Thailand, and Indonesia. The present research aims to isolate the amine derivative from the ethyl acetate layer of this plant and evaluate the isolated compounds on alkaline phosphatase activity as marker of bone formation.

Methods: The air-dried plants of *Spilanthes acmella* Murr. were extracted with methanol, then partitioned with *n*-hexane and ethyl acetate successively by using liquid-liquid extraction, and repeated the chromatographic techniques, such as silica gel, octadecyl silylated silica gel, and HPLC. The isolated compounds were determined by spectrometric analysis using ultraviolet, infrared, high-resolution electrospray ionization mass spectrometry, 1D and 2D NMR.

Results: A benzenepropanoic acid, 4 hydroxy-2-oxo-3 piperidinyl ester (1), was isolated from the ethyl acetate layer of whole plants of *Spilanthes acmella* Murr. together with dendranthemoside A (2), uridine (3), icariside B2 (4), chicoriin (5), dendranthemoside B (6), and ampelopsionoside (7) from their butanol layer.

Conclusion: An amine derivative, a benzenepropanoic acid that determined as 4 hydroxy-2-oxo-3 piperidinyl ester (1) was isolated and reported for the first time from the ethyl acetate layer of *Spilanthes acmella* naturally. All the isolated compounds from this plant stimulated alkaline phosphatase activity as marked of bone formation up to 128%.

Keywords: *Spilanthes acmella* Murr., Asteraceae, benzenepropanoic, ethyl acetate, structure elucidation, alkaline phosphatase activity

1. Introduction

Spilanthes acmella Murr. is included in Asteraceae and used as a traditional remedy for tooth-aches, skin diseases, sexual deficiencies [1], dysentery, snake bite remedies [2], rheumatism, fever [3], influenza, cough, rabies diseases, tuberculosis, swelling, arthritis, purgative, sprain, tonsillitis, and other mouth related trouble [4]. Some bioactivity studies reported that this plant can be functioned as antipyretic [5–6], analgesic [7–8], local anesthetic [6], antimicrobial [9,10], antifungal [9–12], antimalarial [13–16], antioxidant [3,10,17–18], vasorelaxant [1,3,10,19], diuretic [20–22], immunostimulant [23,24], anti-inflammatory [17,25], hepatocurative, and hepatoprotective activities [26]. In Sumatra, it is used as panacea and in Java, it is used as stomatitis [27]. This plant is originated from Africa, America, Borneo, India, Sri Lanka, Bangladesh, China, Japan, Thailand, and Indonesia. It is an annual or short-term herb with a height of 40-60 cm. It grows in moist areas and has a low rate of germination or poor vegetative propagation. The flowers are yellow and have pungent taste accompanied by tingling and numbness on the tongue.

Generally, *Spilanthes acmella* is dominated by the presence of alkamide (spilanthol), phenolic, coumarin (scopoletin), sesquiterpenoids (polygodial), phytosterol (stigmasterol, amyriins), essential oil (limonene, β -caryophyllene, *Z*- β -ocimene, γ -cadinen, thymol, germacrene D and myrcene), vanillic acid, *trans*-ferulic acid, *trans*-isoferulic acid, scopolelin, 3-acetylaleuritolic acid, β -sitostenone, and amide derivatives [5,10]. Several studies showed that the major constituent in this plant is spilanthol (N-isobutylamide) and triterpenoids [28]. Suthikrai *et al.* (2010) reported that it contains 0.59-1.39 ng/g of phytotestosterone [29]. In our present study, we herein report an amine derivative, a benzenepropanoic acid that determined as 4 hydroxy-2-oxo-3 piperidinyl ester (1) was isolated from the ethyl acetate layer of this plant. It was reported for the first time from this species and naturally isolated. Meanwhile, dendranthemoside A (2), uridine (3), icariside B2 (4), chicoriin (5), dendranthemoside B (6), and ampelopsionoside (7) were isolated from the butanol layer of this plant (Fig. 1). All isolated compounds were evaluated their alkaline

phosphatase (ALP) activity as an initial marker of osteogenic differentiation.

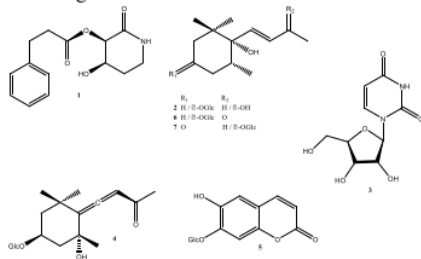


Figure 1: Isolated compound from *Spilanthes acmella*

2. Material and Methods

2.1. General experimental procedures

Infrared (IR) and ultraviolet (UV) spectra were recorded on a HORIBA FT-720 and JASCO V-520 UV/Vis spectrophotometer, respectively. The ^1H - and ^{13}C -NMR spectra were recorded using a Bruker Ultrashield 600 spectrometer at 600 MHz and 150 MHz, respectively, with TMS as an internal standard. Positive ion HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSprayTM. Silica gel open column chromatography (CC) and reversed phase (ODS) CC were performed on silica gel 60 (E. Merck, Darmstadt, Germany). HPLC was performed on an ODS column (Inertsil ODS-3, GL Science, Tokyo, Japan; $\Phi=6$ mm, $L=250$ mm), and monitored with a JASCO RI-930 intelligent detector and a JASCO PU-1580 intelligent pump.

2.2. Plant materials

The whole plant of *Spilanthes acmella* Murr. was collected in late June 2007 from Kebun Raya Purwodadi, Malang, Indonesia, and voucher specimens were deposited at the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University.

2.3. Extraction and isolation

The air-dried plants (2.0 kg) were extracted with methanol (MeOH, 10.0 L \times 3). The methanol solution was concentrated and adjusted to 95% aq. methanol by the addition of water and then partitioned with *n*-hexane (1.0 L \times 3, 23.5 g). The remaining aqueous methanol layer was evaporated and suspended in 0.5 L of water and then partitioned with ethyl acetate (1.0 L \times 3, 40.4 g) and 1-butanol (1.0 L \times 3, 47.5 g), successively.

The ethyl acetate layer (39.0 g) was subjected on silica gel (300 g) CC with hexane- CHCl_3 (1:1) and then increasing

amounts of MeOH in CHCl_3 [4 L, CHCl_3 -MeOH (50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 7:1, 5:1, 3:1, 2:1, 2 l), 500 mL fractions being collected], yielding 19 fractions (Fr. Sae1–Sae12). The fraction Sae5 (710 mg) was subjected to ODS CC in 10% aq. MeOH (400 mL)–100% MeOH (400 mL), linear gradient, lead 10 fractions (Fr. Sae5-1–Sae5-10). The residue of fraction Sae5-4 (231 mg) was purified by 60% aq. MeOH with HPLC to give **1** (benzenepropanoic acid, 4-hydroxy-2-oxo-3-piperidinyl ester, 11.1 mg).

The 1-butanol layer (40.0 g) was subjected on silica gel (300 g) CC with hexane- CHCl_3 (1:1) and then increasing amounts of MeOH in CHCl_3 [4 L, CHCl_3 -MeOH (50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 7:1, 5:1, 3:1, 2:1, 2 l), 500 mL fractions being collected], yielding 19 fractions (Fr. Sab1–Sab12). The fraction Sab11 (2.75 g) was subjected to ODS CC in 10% aq. MeOH (400 mL)–100% MeOH (400 mL), linear gradient, lead 10 fractions (Fr. Sab11-1–Sab11-10). The residue of fraction Sab11-4 (123 mg) was purified by 35% aq. MeOH with HPLC to give **2** (dendranthemoside A, 3.71 mg). The fraction Sab10 (1.81 g) was subjected to ODS CC in 10% aq. MeOH (400 mL)–100% MeOH (400 mL) and lead 7 fractions (Fr. Sab10-1–Sab10-7). The residue of fraction Sab10-1 (770 mg) also was purified by HPLC (100% aqua, YMC column). Compound **3** (uridine, 27.5 mg) was collected. The remaining residue of fraction Sab10-2 (193 mg) was purified by HPLC (20% aq. MeOH) to give **4** (icaraside B2, 6.12 mg) and **5** (cichoriin, 2.99 mg). The residue of fraction Sab10-3 (142 mg) was purified by HPLC (35% aq. MeOH) to give **6** (dendranthemoside B, 4.31 mg). The fraction Sab5 (710 mg) was subjected to ODS CC in 10% aq. MeOH (400 mL)–100% MeOH (400 mL) and lead 10 fractions (Fr. Sab5-1–Sab5-10). The mixtures of fraction Sab6, Sab7, Sab8, and Sab9 (2.06 g) were subjected to ODS CC in 10% aq. MeOH (400 mL)–100% MeOH (400 mL) and lead 10 fractions (Fr. Sab6-9-1–Sab6-9-10). The residue of fraction Sab6-9-4 (114 mg) was purified by HPLC (40% aq. MeOH) to give **7** (ampelosisinoside, 5.43 mg).

Dendranthemoside A (2) [30]

Yellow powder; ^1H NMR (600 MHz, methanol- d_4) δ : 0.84 (3H, d, J 7.0 Hz, H₃-13), 0.88 (3H, s, H₃-12), 0.96 (3H, s, H₃-11), 1.24 (3H, d, J 7.0 Hz, H₃-10), 1.49 (1H, dd, J 12.0, 7.0 Hz, H-4 β), 1.56 (1H, ddd, J 12.0, 5.1, 2.0 Hz, H-2 β), 1.67 (1H, dd, J 12.0, 7.0 Hz, H-2 α), 1.82 (1H, m, H-4 α), 1.95 (1H, m, H-5), 3.13 (1H, dd, J 8.0, 7.0 Hz, H-2'), 3.26 (1H, t,

J 7.0 Hz, H-5'), 3.27 (1H, t, J 7.0 Hz, H-4'), 3.34 (1H, d, J 7.0 Hz, H-3'), 3.65 (1H, m, H-6' α), 3.86 (1H, m, H-6' β), 3.95 (1H, m, H-3), 4.29 (1H, d, J 7.0 Hz, H-9), 4.35 (1H, t, J 7.0 Hz, H-1'), 5.55 (1H, dd, J 16.0, 7.0 Hz, H-7), 5.73 (1H, dd, J 16.0, 7.0 Hz, H-8); ^{13}C NMR (150 MHz, methanol- d_4) δ : 16.6 (C-13, CH₃), 24.3 (C-10, CH₃), 25.3 (C-12, CH₃), 26.0 (C-11, CH₃), 35.7 (C-5, CH), 38.3 (C-4, CH₂), 40.6 (C-1), 42.7 (C-2, CH₂), 63.0 (C-6', CH₂), 69.4 (C-9, CH), 71.9 (C-4', CH), 75.3 (C-2', CH), 75.8 (C-3, CH), 78.0 (C-5', CH), 78.2 (C-3', CH), 78.4 (C-6), 102.8 (C-1', CH), 133.9 (C-7, CH), 135.7 (C-8, CH); positive HR-ESI-MS m/z 337.0684 [M+Na]⁺ (calcd. for C₁₇H₁₄O₆Na 337.0683).

Uridine (3) [31]

Yellow powder; ^1H NMR (600 MHz, pyridine- d_6) δ : 4.20 (1H, dd, J 12.0, 2.0 Hz, H-5' β), 4.31 (1H, dd, J 12.0, 2.0 Hz, H-5' α), 4.66 (1H, m, H-4'), 4.92 (2H, d, m, H-2', 3'), 5.80 (1H, d, J 8.1 Hz, H-5), 6.83 (1H, d, J 4.0 Hz, H-1'), 8.54 (1H, d, J 8.1 Hz, H-6); ^{13}C NMR (150 MHz, pyridine- d_6) δ : 62.1 (C-5', CH₂), 71.6 (C-3', CH), 76.5 (C-2', CH), 86.7 (C-4', CH), 90.8 (C-1', CH), 102.8 (C-5, CH), 141.5 (C-6, CH), 152.7 (C-2), 164.8 (C-4); positive HR-ESI-MS m/z 267.0587 [M+Na]⁺ (calcd. for C₉H₁₂O₆N₂Na 267.0587).

Icariside B2 (4) [32]

Colorless solid; ^1H NMR (600 MHz, pyridine- d_6) δ : 1.10 (3H, s, H₃-12), 1.52 (3H, s, H₃-11), 1.53 (3H, s, H₃-13), 1.67 (1H, dd, J 12.1, 2.0 Hz, H-4 β), 2.21 (3H, s, H-10), 2.39 (1H, ddd, J 12.1, 4.0, 2.0 Hz, H-2 α), 2.88 (1H, ddd, J 12.1, 4.0, 2.0 Hz, H-4 α), 3.92 (1H, m, H-5'), 4.10 (1H, t, J 8.3 Hz, H-2'), 4.28 (1H, t, J 9.2 Hz, H-3'), 4.31 (1H, t, J 9.2 Hz, H-4'), 4.42 (1H, dd, J 12.1, 2.0 Hz, H-6' β), 4.54 (1H, dd, J 12.1, 5.0 Hz, H-6' α), 4.98 (1H, m, H-3), 5.12 (1H, d, J 7.0 Hz, H-1'), 5.91 (1H, s, H-8); ^{13}C NMR (150 MHz, pyridine- d_6) δ : 26.9 (C-10, CH₃), 29.6 (C-11, CH₃), 31.5 (C-13, CH₃), 32.4 (C-12, CH₃), 36.7 (C-1), 47.5 (C-4, CH₂), 48.5 (C-2, CH₂), 63.2 (C-6', CH₂), 71.7 (C-5), 72.2 (C-4', CH), 72.3 (C-3, CH), 75.8 (C-2', CH), 78.8 (C-5', CH), 79.1 (C-3', CH), 100.9 (C-8, CH), 103.5 (C-1', CH), 120.3 (C-6, CH), 198.2 (C-7), 210.1 (C-9); positive HR-ESI-MS m/z 409.1836 [M+Na]⁺ (calcd. for C₁₉H₃₀O₈Na 409.1833).

Cichoriin (5) [33]

Colorless solid; ^1H NMR (600 MHz, pyridine- d_6) δ : 4.13 (1H, ddd, J 7.0, 6.2, 2.0 Hz, H-3'), 4.28 (1H, d, J 7.0 Hz, H-2'), 4.30 (1H, d, J 9.3 Hz, H-4'), 4.36 (1H, d, J 9.3 Hz, H-5'), 4.39 (1H, dd, J 11.1, 5.0 Hz, H-6' α),

4.58 (1H, dd, J 11.1, 2.0 Hz, H-6' β), 5.61 (1H, d, J 8.0 Hz, H-1'), 6.24 (1H, d, J 9.3 Hz, H-3), 7.13 (1H, s, H-8), 7.60 (1H, br s, H-4), 7.69 (1H, s, H-5); ^{13}C NMR (150 MHz, pyridine- d_6) δ : 62.9 (C-6', CH₂), 71.7 (C-4', CH), 75.4 (C-2', CH), 78.9 (C-5', CH), 79.7 (C-3', CH), 104.7 (C-1', CH), 104.9 (C-8, CH), 111.9 (C-10), 113.0 (C-3, CH), 116.9 (C-5, CH), 144.6 (C-7), 144.7 (C-7), 152.5 (C-6), 154.3 (C-9), 161.8 (C-2); positive HR-ESI-MS m/z 363.0686 [M+Na]⁺ (calcd. for C₁₅H₁₆O₉Na 363.0687).

Dendranthemaside B (6) [30]

Yellow powder; ^1H NMR (600 MHz, methanol- d_4) δ : 0.77 (3H, d, J 7.0 Hz, H₃-13), 0.83 (3H, d, J 7.0 Hz, H₃-11), 1.00 (3H, d, J 7.0 Hz, H₃-12), 1.47 (1H, dd, J 12.0, 7.0 Hz, H-4 β), 1.55 (1H, ddd, J 12.0, 5.1, 2.0 Hz, H-2 β), 1.67 (1H, dd, J 12.0, 7.0 Hz, H-2 α), 1.83 (1H, m, H-4 α), 2.08 (1H, m, H-5), 2.23 (3H, d, J 7.0 Hz, H₃-10), 3.10 (1H, dd, J 8.0, 7.0 Hz, H-2'), 3.23 (2H, t, J 7.0 Hz, H-4', 5'), 3.30 (1H, d, J 7.0 Hz, H-3'), 3.62 (1H, m, H-6' α), 3.83 (1H, m, H-6' β), 3.95 (1H, m, H-3), 4.33 (1H, t, J 7.0 Hz, H-1'), 6.31 (1H, dd, J 16.0, 7.0 Hz, H-8), 6.85 (1H, dd, J 16.0, 7.0 Hz, H-7); ^{13}C NMR (150 MHz, methanol- d_4) δ : 16.6 (C-13, CH₃), 25.2 (C-12, CH₃), 26.1 (C-11, CH₃), 27.5 (C-10, CH₃), 35.5 (C-5, CH), 38.0 (C-4, CH₂), 41.1 (C-1), 42.6 (C-2, CH₂), 63.0 (C-6', CH₂), 71.9 (C-4', CH), 75.3 (C-2', CH), 75.6 (C-3, CH), 78.0 (C-5', CH), 78.2 (C-3', CH), 79.2 (C-6), 102.9 (C-1', CH), 131.7 (C-8, CH), 154.4 (C-7, CH), 201.0 (C-9); positive HR-ESI-MS m/z 411.1991 [M+Na]⁺ (calcd. for C₁₉H₃₂O₆Na 411.1989).

Ampelosinioside (7) [34]

Yellow powder; ^1H NMR (600 MHz, methanol- d_4) δ : 0.90 (3H, d, J 6.0 Hz, H₃-13), 0.93 (3H, s, H₃-12), 0.99 (3H, s, H₃-11), 1.32 (3H, d, J 6.0 Hz, H₃-10), 1.82 (1H, d, J 14.0 Hz, H-2 β), 2.11 (1H, dd, J 13.0, 2.0 Hz, H-4 β), 2.28 (1H, m, H-5), 2.44 (1H, t, J 13.0 Hz, H-4 α), 2.87 (1H, dd, J 14.0, 3.0 Hz, H-2 α), 3.12 (1H, t, J 8.0 Hz, H-5'), 3.18 (2H, dd, J 9.0, 7.0 Hz, H-2'), 3.29 (1H, m, H-4'), 3.35 (1H, t, J 4.0 Hz, H-3'), 3.65 (1H, dd, J 11.0, 5.0 Hz, H-6' α), 3.84 (1H, m, H-6' β), 4.35 (1H, d, J 7.0 Hz, H-1'), 4.44 (1H, q, H-9), 5.73 (1H, d, J 16.0 Hz, H-7), 5.91 (1H, dd, J 16.0, 7.0 Hz, H-8); ^{13}C NMR (150 MHz, methanol- d_4) δ : 16.5 (C-13, CH₃), 21.5 (C-10, CH₃), 25.0 (C-11, CH₃), 25.4 (C-12, CH₃), 37.8 (C-5, CH), 43.9 (C-1), 46.4 (C-4, CH₂), 52.5 (C-2, CH₂), 62.7 (C-6', CH₂), 71.6 (C-4', CH), 75.4 (C-2', CH), 77.8 (C-6, C-9), 78.1 (C-5', CH), 78.2 (C-3', CH), 102.6 (C-1', CH), 134.0 (C-7,

CH), 134.9 (C-8, CH), 214.9 (C-3); positive HR-ESI-MS m/z 411.1990 $[M+Na]^+$ (calcd. for $C_{19}H_{32}O_6Na$ 411.1989).

2.4. Alkaline phosphatase (ALP) activity

Cell Culture. Osteoblast-like cell of MC3T3-E1 was purchased from Riken Cell Bank, Tsukuba, Japan. The cells were cultured in petri dish in α -MEM medium containing 10% fetal bovine serum (FBS), 100 μ g/mL streptomycin, and 100 units/mL penicillin in a 5% CO_2 incubator at 37°C and sub cultured every 3 days at a dilution of trypsin (0.25%).

ALP activity. The 5×10^4 MC3T3-E1 cells were seeded in 24-well plates and incubated in α -MEM medium containing 10 mM β -glycerophosphate, 10% FBS and 50 μ g/mL ascorbic acid with 6 days incubation period at 37°C under 5% CO_2 . Then, the cells were cultured for 3 days with α -MEM medium containing 0.3% bovine serum albumin (BSA) and isolated compounds (1–7). The medium was discharged and the cell monolayer was gently washed twice with phosphate buffer

saline (PBS). The cells were lysed with 0.2% triton X-100 for 10 minutes at 37°C and centrifuged at 14000 \times g for 5 min. The clear supernatant was used to measure ALP activity, which was determined using an ALP assay kit and the absorbance was evaluated using ELISA reader at 405 nm [35]. The 17 β -estradiol used as positive control at 0.02, 0.01 and 0.005 μ M.

3. Results and Discussion

Compound **1** was obtained in the form of yellow amorphous powder, with a molecular formula of $C_{14}H_{17}O_4NNa$ as determined by HR-ESI-MS at a m/z of 286.1052 $[M+Na]^+$ (calcd. for $C_{14}H_{17}O_4NNa$ 286.1050). The IR spectrum of **1** displayed adsorption bands at 3390 cm^{-1} and 1777 cm^{-1} indicating the presence of hydroxyl and carbonyl functionality. The UV spectrum revealed maximal absorption band at 210 nm and 267 nm.

Table 1: 1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) data of **1** in methanol- d_4

Carbon no.	δ_C	δ_H	DEPT 135	COSY	HMBC
2	180.3	-	C		4,6
3	74.4	4.03 (d, 2.1)	CH		5
4	82.4	4.95 (ddd, 8.0, 5.1, 2.1)	CH		5,6
5	24.7	2.35 (m) 2.23 (m)	CH2		3,6
6	29.4	2.59 (dd, 10.4, 7.2) 2.51 (dd, 10.4, 6.3)	CH2		
1'	173.8	-	C		3,2'
2'	41.9	3.46 (m)	CH2	3'	3'
3'	36.8	2.81 (m)	CH2	2'	2, 5',9'
4'	140.2	-	C		2',3'
5',9'	130.0	7.28 (dd, 6.7, 1.5)	CH		3',6',8'
6',8'	129.7	7.25 (dd, 6.7, 1.5)	CH	7'	5', 7',9'
7'	127.5	7.19 (t, 7.0)	CH	6',8'	5',9'

J-values were given in Hz

The 1H -NMR spectrum (Table 1) displayed protons of monosubstituted benzene ring in the region δ_H 7.19–7.28 and two oxygenated methine protons H-3 and H-4 (δ_H 4.03 and 4.95). At the same time, four methylene protons of H-5 and H-6 were observed as two sets of multiplet centered at δ_H 2.23 and 2.35 and double of doublet at δ_H 2.51 and 2.59, respectively. In addition, two methylene protons of H-2' and H-3' at δ_H 3.46 and 2.81 were analyzed. The ^{13}C -NMR and DEPT 135 spectra (Table 1) revealed presence of two carbonyls (δ_C 180.3 and 173.8; C-2 and C-1'), aromatic ring (δ_C 127.5, 129.7, 130.0 and 140.2; C-7', C-6', C-8', C-5', C-9' and C-4'), oxygenated methine (δ_C 74.4 and 82.4; C-3

and C-4) and methylene carbons (δ_C 24.7, 29.4, 36.8 and 41.9; C-5, C-6, C-3', and C-2').

The position of the benzenepropanoic moiety was deduced to be at C-3 by analysis of the HMBC data, showing correlations of H-3 to carbons at δ_C 173.8 (Fig. 2 and Table 1). Based on spectrum analysis and HMBC correlations, the structure of **1** was determined to be benzenepropanoic acid or 4 hydroxy-2-oxo-3 piperidinyll ester. The relative configuration of **1** was established by NOESY analysis. The correlation of H-4/H-3 and H-3' were suggested as *Z* configuration and it had 5 kcal/mole using MM2 analysis (Fig. 2). Nishiyama *et al.* 1992 reported that the *cis* conformation of piperidinone was naturally

high yield. The H-3 and H-4 protons that appeared at sharp doublet and broad ddd, respectively, were indicated as *cis* conformation [36].

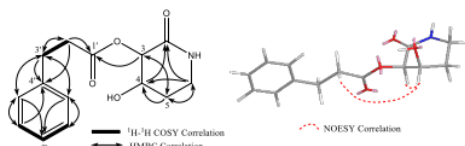


Figure 2: ^1H - ^1H COSY, HMBC and NOESY correlations of compound **1**.

The MC3T3-E1 is one of cell line that can be used for osteoblastic cell markers studies. The cell line describes high ALP activity with extracellular matrix mineralization *in vitro*. Therefore, MC3T3-E1 is a great candidate for assessing the osteoblast proliferation and differentiation. ALP activity is an initial marker of osteogenic differentiation. This enzyme is a mature matrix

and specific protein that is active in all cell membranes at higher level in osteoblasts to allow matrix mineralization and bone cell phenotype [37].

To evaluate the effects of 4 hydroxy-2-oxo-3 piperidinyl ester (**1**), dendranthemoside A (**2**), uridine (**3**), icarisiide B2 (**4**), chicoriin (**5**), dendranthemoside B (**6**), and ampelopsisionoside (**7**) on osteoblast function, ALP activity, which was related to the osteoblast differentiation, was determined. It was found that dendranthemoside A (**2**), uridine (**3**), and ampelopsisionoside (**7**) stimulated ALP activity, which markedly increased osteoblast growth and differentiation in osteoblastic MC3T3-E1 cells (Table 2). At concentrations of 25, 12.5 and 6.26 μM , they stimulated ALP activity up to 128% compared to control and had higher ALP activity than the positive control, 17β -estradiol at 0.02, 0.01 and 0.005 μM .

Table 2. The ALP activity of isolated compounds from *Spilanthes acmella* Murr.

Sample	ALP activity (%)		
	25 (μM)	12.5 (μM)	6.26 (μM)
Control	100 \pm 2.1	100 \pm 4.5	100 \pm 3.6
17β -estradiol	110 \pm 3.1	109 \pm 5.4	99 \pm 5.1
4 hydroxy-2-oxo-3 piperidinyl ester (1)	111 \pm 1.3	110 \pm 9.0	100 \pm 0.5
dendranthemoside A (2)	128 \pm 4.0	117 \pm 3.8	115 \pm 5.2
uridine (3)	123 \pm 3.1	121 \pm 7.7	111 \pm 1.3
icarisiide B2 (4)	108 \pm 1.8	106 \pm 8.8	105 \pm 7.3
chicoriin (5)	113 \pm 5.2	104 \pm 5.6	101 \pm 4.4
dendranthemoside B (6)	111 \pm 2.4	110 \pm 0.9	108 \pm 6.6
ampelopsisionoside (7)	116 \pm 6.4	113 \pm 8.0	112 \pm 1.8

Conclusion

An amine derivative, a benzenepropanoic acid that determined as 4 hydroxy-2-oxo-3 piperidinyl ester (**1**) was isolated and reported for the first time from the ethyl acetate layer of *Spilanthes acmella* naturally. All the isolated compounds from this plant stimulated alkaline phosphatase activity as marked of bone formation up to 128%.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Acknowledgments

This research was supported by Directorate General Higher Education of Indonesia as National Innovations Research Incentive Program Batch I (INSINAS) with grant No. 07/INS-1/PPK/E4/2018.

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