Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

RADICAL SCAVENGING ACTIVITY OF TRITERPENE STEROIDS FROM STEM OF*POLYGONUM PULCHRUM* Bl

SAHIDIN[1*], NOHONG[2], ASRUL SANI[2], MARIANTI ANGGRENIMANGGAU[3], ASEP SUKOHAR[4], HARTO WIDODO[5], SYARULNATAQAIN BAHARUM[6]

[1]Faculty of Pharmacy, Halu Oleo University, Kendari 93232, Indonesia, [2]Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari 93232, Indonesia, [3]Pharmacology, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia, [4]Faculty of Medicine, Lampung University, Lampung 35145, Indonesia, [5]Medicinal Plants and Traditional Medicine Research and Development Centre (NIHRD), Jl. Raya LawuNo.10 Tawangmangu Central of Java 57792 Indonesia, [6]Institute of Systems Biology, University Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
Email: sahidin02@yahoo.com

Received: 20 Jun 2014 Revised and Accepted: 25 Jul 2014

ABSTRACT

Objective: *P. pulchrum* grows abundantly in Kendari (Sulawesi Tenggara province, Indonesia).However, there is no report neither chemical contents nor biological activitiesof the plant. This project studiesthe isolation, structure elucidations, and radical scavenging activity evaluation of triterpene steroids from stems of *P. pulchrum.*

Methods: The isolation of the compounds was carried out by using chromatography method, i.e., vacuum liquid chromatography (VLC) and radial chromatography (RC) with silica gel as an adsorbent and various solvents as eluent. The compound structures were evaluated by spectroscopic data (FTIR and NMR data) and then the results were compared with the existing data from references. The antioxidant activity of these compounds was evaluated towards DPPH (1,1-diphenyl 2-picryl-hydrazyl).

Results: Four triterpene steroids; namely, (**1**) 6β-hydroxystigmasta-4,22-dien-3-one, (**2**) stigmasterol,(**3**) stigmasta-4,22-dien-3-one, and (**4**) ergosterol peroxide, were isolated and identified from stems of *P. pulchrum* Bl. The antioxidant activities of all compounds were indicated by IC_{50} value of the compounds. The values of IC_{50}(μM) of6β-hydroxystigmasta-4,22-dien-3-one, stigmasterol, Stigmasta-4,22-dien-3-on,ergosterol peroxide, and vitamine C (standard) toward DPPH were obtained at233.4 ± 0.28; 372.3 ± 0.33; 144.80 ± 0.24; 1083.1 ± 0.38; and 68.9 ± 0.12, respectively.

Conclusions: We found that Stigmasta-4,22-dien-3-onewas the most active compound toward DPPH.

Keywords: *P. pulchrum* Bl., 6β-hydroxystigmasta-4, 22-dien-3-one, Stigmasterol, Stigmasta-4, 22-dien-3-one, Ergosterol peroxide and DPPH.

INTRODUCTION

In our previous studies on chemical and pharmacological aspects of traditional medicinal plants of South East Sulawesi Indonesia, we have reported several chemical contents and biological activities of Dipterocarpaceae[1-5] and *Jatropha* (Euphorbiaceae)[6-10].In this study we focus on chemical and pharmacological aspects of *Polygonum* (Polygonaceae) plants.

Polygonum(Polygonaceae) plant has a large species as well as traditional benefits. This genus comprises about150-300speciesandit generally grows in wet locations (swamp).~~,~~ The plant is often used as traditional remedies, flavors in cooking, and ingredient of perfume [11]. For example, the biological activities of *P.tinctorium* extracts have activities as anti-anticancer and antioxidant [12], *P. multiflorum*is used in antiaging process[13], *P.hydropiper*is an active plant against gonorrhea, arthritis, diarrhea, and intestinal parasitoses[14], *P.maritimum* has an active extract as antioxidant [15], and *P. jucundum*is used as anti rheumatism by Chinese people[16]. In addition, *P.minus*is widely used as a spice in Malays'cookingand it has a great potency for the ingredient of perfume [17].

The phytochemical study of *Polygonum* has reported that approximately24 species of *Polygonum* plants produced more than one hundred of compounds with a range of biological activities. Those compounds include anthraquinones, flavonoids, stilbenes, chromons and terpenoids [11]. For example, anthraglycoside B from *P. cuspidatum* is an antibacteri of *Streptococcus mutans* and *S. sobrinus*[18], flavonol-glucuronides from of *P. aviculare* is an antioxidant and anti-inflammatory[19], quercetin from *P. hydropiper* is active towards human gastric carcinoma cells (BGC-823)[20], and anti-proliferative effect [21], and also resveratrol from *P. cuspidatum* is useful for anti-oxidative, anti-cancer, and anti-inflammatory drugs [22]. Some compounds that belong to the group of triterpene steroids have been isolated from *Polygonum* plants; such ascycloartane-3,24-dione, 24 (E)-ethylidenecycloartanone, 24 (E)-ethylidenecycloartan-3α-ol, γ–sitosterol, β-sitosterone and 24-methylenecycloartanone from rhizomes of *P. bistorta*[23],β-sitosterol from rhizomes of *P. bistorta*[23] and *P. nepalense*[24], stigmasterol from *P. flaccidum*[25], and 3-*O*-glucosyl-β-sitosterol from *P. spectabile*[26].

The biological activities of triterpene steroids that have been reported are β-sitosterol as anthelminthic and anti mutagenic acivities [27], hyper cholesterolemia [28], anti-cancer fibro sarcoma[29], and anti-proliferation in human leukemia cells [30], and γ-sitosterol as cytotoxic against *Artemiasalina*[31]. However, triterpene steroids from *P. pulchrum* Bl. and their biological activities, in particular theirradical scavenger, have not been reported ~~yet~~. The main objective of this paper is to inform the isolation, structure elucidation, and radical scavenger evaluation of triterpene steroids from stems of *P. pulchrum* Bl.

MATERIALS AND METHODS

General

The process of isolation was carried out at Halu Oleo University by using vacuum liquids chromatography methods (VLC)and radial chromatography (RC). VLC and RC methods were equipped with Merck Si-gel 60 GF254 and TLC analysis on pre-coated Si-gel plates with Merck Kiesel gel 60 F254, 0.25 mm. UV spectra was measured using Cary Varian 100 conc. and IR spectra using Perkin-Elmer Spectrum One FT-IR Spectrophotometer.[1]H and [13]C NMR spectra

were recorded with a JEOL ECP 500 spectrometer and operated at 500 MHz (^{1}H) and 125 MHz ([13]C).This work was conducted at LIPI (Institute of Sciences of Indonesia).

Plant material

Samples of the stems of *P. pulchrum* Bl. were collected from "Pusat Kole ksidan Pengemb angan Tanaman Obat Tradisional Masyarakat Sulawesi Tenggara *Arboretum Prof. Mahmud Hamundu* Universit as Halu Oleo" in April 2012. The plant was identified in Herbarium Bogoriense, Bogor Indonesia, and a voucher specimen was deposited at the Herbarium. The radical scavenger activity of the compounds was determined at Pharmacology Laboratory, Faculty of Pharmacy Hasanuddin University Makassar Indonesia.

Isolation

Isolation of compounds from stems of *P. pulchrum* Bl.

The powder (230-270 mesh) of stems of *P. pulchrum* Bl.(5,0 kg) was macerated by methanol (MeOH) 3 x 3 L for 3 x 24 hs. The methanol extract was concentrated by vacuum rotary evaporator at low pressure until we got a dark green gum (450 g) was obtained. All methanol extract was fractionated by VLC using a column Φ 10 cm, adsorben: Si-gel (150 g) and mixture of ethylacetate:*n*-hexane (20-100%, MeOH 100%) as eluent, to give 5 fractions i.e. F1 (5.1 g), F2 (18.0 g), F3 (14.3 g), F4 (10.2 g) and F5 (275 g), respectively. F2 was refractionated using VLC with a column Φ 10 cm, adsorben: Si-gel (150 g) and mixture of ethylacetate: *n*-hexane (30-100%, MeOH 100%) as eluent, provide 5 fractions i.e. F21 (1.2 g), F22 (1.0 g), F23 (3.8 g), F24 (3.2 g) and F25 (6.6 g). F23 (1.0 g) was purified by RC, adsorbent: Si-gel and eluen mixture of chloroform:MeOH (95%-5%, MeOH 100%), to give compound **1** (0.2 g), a white needle crystal. Compound **2** (0.8 g), a white needle crystal, was isolated from F24by using the same method as for compound **1** with mixture of chloroform:MeOH (90%-10%, MeOH 100%) as eluent. F3 was refractionated by conducting VLC with a column Φ 10 cm, adsorben: Si-gel (150 g) and mixture of ethylacetate: *n*-hexane (30-100%, MeOH 100%) as eluent, to yield 4 fractions, i.e. F31 (1.3 g), F32 (2.2 g), F33 (2.8 g), and F34 (7.2 g). F32 (1.0 g) was purified by RC, adsorbent: Si-gel and eluen mixture ofn-hexane-etilacetate (85%-15%, MeOH 100%), to give compound **3** (0.1 g), a white amorf. Compound **4** (0.1 g), a white amorf, was isolated from F33by using the same method as for compound **3** with mixture of n-hexane:ethylacetate (75%-25%, MeOH 100%) as eluent.

Determination of Pure Compound Structures

The structure of pure compounds were set up by using spectroscopy methods including FTIR and NMR 1-D (^{1}H and [13]C).

Compound 1, a white needle crystal. Spectrum of ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 1.69 (1H, *m*, H-1a); 2.02 (1H, *m*, H-1b); 2.35 (1H,*brt*, H-2a); 2.50 (1H, *brt*, H-2b); 5.80 (1H, *s*, H-4); 4.33 (1H, *brt*, H-6); 1.22 (1H, *m*, H-7a); 1.96 (1H, *m*, H-7b); 1.21 (1H, *m*, H-8); 1.51 (1H, *m*, H-9); 0.81 (1H, *m*, H-11a); 1.49 (1H, *m*, H-11b); 1.13 (1H, *m*, H-12a); 2.03 (1H, *m*, H-12b); 0.98 (1H, *m*, H-14); 1.11 (1H, *m*, H-15a); 1.60 (1H, *m*, H-15b); 1.26 (1H, *m*, H-16a); 1.84 (1H, *m*, H-16b); 1.09 (1H, *m*, H-17); 0.75 (3H, *s*, H-18); 1.37 (3H, *s*, H-19); 2.04 (1H, *m*, H-20); 0.92 (3H, *d*, 6,5Hz, H-21); 5.14 (1H, *dd*, 15 Hz, H-22); 5.02 (1H, *dd*, 15Hz, H-23); 1.53 (1H, *m*, H-24); 1.67 (1H, *m*, H-25); 0.84 (3H, *m*, H-26); 0.82 (3H, *m*, H-27); 1.02 (1H, *m*, H-28a); 1.29 (1H, *m*, H-28b); dan 0.87 (3H, *m*, H-29). Spectrum of [13]C NMR ($CDCl_3$, 125 MHz) □ (ppm) 37.2 (C1); 34.3 (C2); 200.6 (C3); 126.5 (C4); 168.7 (C5); 73.4 (C6); 38.7 (C7); 29.9 (C8); 53.8 (C9); 38.1 (C10); 21.1 (C11); 39.8 (C12); 42.7 (C13); 56.1 (C14); 24.3 (C15); 28.3 (C16); 56.3 (C17); 12.1 (C18); 19.7 (C19); 36.3 (C20); 18.9 (C21); 138.0 (C22); 129.8 (C23); 46.0 (C24); 26.3 (C25); 21.0 (C26); 20.0 (C27); 34.1 (C28) and 23.2 (C29).

***Compound* 2,** a white needle crystal, m.p. 169-171^{o}C. Spectrum of ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 1.82 (1H, *m*,H-1^a); 1.15 (1H, *m*, H-1b); 1.95 (1H, *m*, H-2a); 1.85 (1H, *m*, H-2b); 3.35 (1H, *m*, H-3); 2.27 (1H, *m*, H-4a); 2.22 (1H, *m*, H-4b); 5.35 (1H, *br d*, H-6); 1.93 (2H, *m*, H-7; 1.49 (1H, *m*, H-8); 0.91 (1H, *br d*, H-9); 1.47 (2H, *m*, H-11); 2.02 (1H, *m*, H-12); 0.97 (1H, *m*, H-14); 1.54 (2H, *m*, H-15); 1.27 (1H, *m*, H-16); 1.08 (1H, *m*, H-17); 0.84 (1H, *br d*, H-18a); 0.79 (1H, *br d*, H-18b); 0.67 (1H, *br s*, H-18c); 1.00 (3H, *br s*, H-19); 1.97 (1H, *m*, H-20); 1.00 (3H, *br s*, H-21); 5.15 (1H, *dd*, 15, H-22); 5.02 (1H, *dd*, 15Hz, H-23); 0.91 (1H, *m*, H-24); 1.66 (1H, *m*, H-25), 1.00 (1H, *br s*, H-26a), 0.81 (2H, *br d*, H-26b); 0.91 (1H, *br d*, H-27a); 0.81 (1H, *br d*, H-27b); 0.69 (1H, *br s*, H-27c); 1.44 (2H, *m*, H-28); 0.84 (1H, *br d*, H-29a); 0.79 (1H, *br d*, H-29b); 0.67 (1H, *br s*, H-29c). Spectrum of[13]C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 37.4 (C1); 31.8 (C2); 71.9 (C3); 42.5 (C4); 141.9 (C5); 121.9 (C6); 32.1 (C7); 32.1 (C8); 50.3 (C9); 36.7 (C10); 21.2 (C11); 39.9 (C12); 42.5 (C13); 56.9 (C14); 24.4 (C15); 28.4 (C16); 56.2 (C17); 12.0 (C18); 21.3 (C19); 40.7 (C20); 21.3 (C21); 138.5 (C22); 129.4 (C23); 51.4 (C24); 31.1 (C25); 19.2 (C26); 19.0 (C27); 26.3 (C28); and 12.2 (C29).

*Compound**3***, a white amorf. Spectrum of^{1}H NMR ($CDCl_3$, 500 MHz) □ (ppm) 2.37 (2H, *m*, H-2); 6.62 (2H, d. *J*=8,5, H-6); 0.57 (3H, *s*, H-18); 1.01 (3H, *s*, H-19); 1.02 (3H, *d*,*J*=6.5, H-21); 5.15 (1H, *dd*. J=14.8, 8.4, H-22); 5.03 (1H, *dd*. *J*=14.8, 8.4, H-23); 0.79 (3H, *d*. *J*=6.5, H-26); 0.84 (3H, *d*. *J*=7.1, H-27); 0.93 (3H, *d*.*J*=7.1, H-28); 0.81 (3H, *t*.*J*=8,2, H-29). Spectrum of [13]C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 37.3 (C1); 31.6 (C2); 212.2 (C3); 121.1 (C4); 170,2(C5); 32,6 (C6); 33.9 (C7); 31.7 (C8); 50.2 (C9); 36.5 (C10); 20.9 (C11); 38.6(C12); 42.2 (C13); 56.6 (C14); 24.1 (C15); 28.2 (C16); 56.1 (C17); 11.6 (C18); 19.4 (C19); 40.8 (C20); 21.5 (C21); 138.8 (C22); 129.5 (C23); 51.4 (C24); 32.2 (C25); 19.2 (C26); 21.3 (C27); 25.7 (C28); 12.5(C29).

*Compound**4***, a white amorf. Spectrum of^{1}H NMR ($CDCl_3$, 500 MHz) □ (ppm) 1.74 (2H, *dd*.*J*=13.6, 3.9, H-1); 3.78 (1H, *m,* H-3); 6.62 (1H, d. *J*=8.5, H-6); 6.22 (1H, d. *J*=8.5, H-7); 1.24 (1H, *m*, H-11a); 1.54 (1H, *m*, H-11b); 1.27 (1H, *m*, H-12a); 1.96 (1H, *m*,.H-12b); 1.54 (1H, *m*, H-14); 1.41 (1H, *m*, H-15a); 1.65 (1H, *m*, H-15b); 1.35 (1H, *m*, H-16a); 1.80 (1H, *m*, H-16b); 1.25 (1H, *m*, H-17); 0.82 (3H, *s*, H-18); 0.88 (3H, *s*, H-19); 2.06 (1H, *m*, H-20); 1.02 (3H, *d*.*J*=6,5, H-21); 5.19 (1H, *dd*. J=15.6, 7.1,H-22); 5.28 (1H, *dd*. J=15.5, 7.8, H-23); 1.87 (1H, *m*, H-24); 1.50 (1H, *m*, H-25); 0.83 (3H, *d*.*J*=7.1, H-26); 0.84 (1H, *d*. *J*=7.1, H-27); 0.93 (1H, *d*. *J*=7,H-28). Spectrum of [13]C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 35.8(C1); 31.2 (C2); 66.3 (C3); 38.1 (C4); 82.4 (C5); 136.6 (C6); 131.2 (C7); 79.5 (C8); 52.2 (C9); 37.8 (C10); 24.1 (C11); 40.3 (C12); 45.2 (C13); 52.8 (C14); 21.4 (C15); 29.5 (C16); 57.1 (C17); 13.3 (C18); 18.6 (C19); 40.7 (C20); 20.1 (21); 136.5 (C22); 132.9 (C23); 43.8 (C24); 33.9 (C25); 20.4 (C26); 21.4 (C27); and 18.1 (C28).

Radical scavenging activity

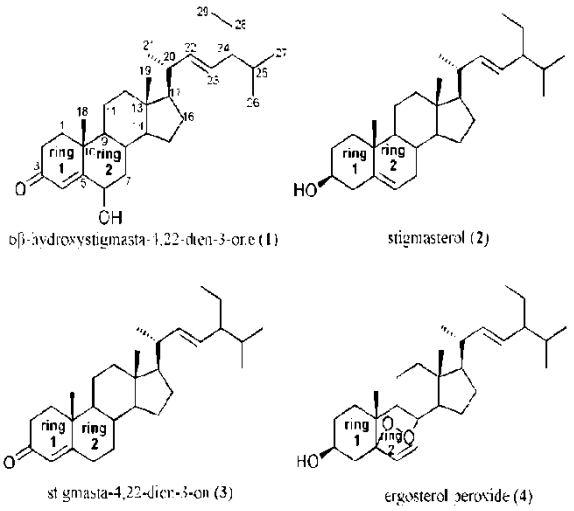
The potency of isolated compounds as radical scavengers was evaluated against inhibition of DPPH reduction. The reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl or 2,2-diphenyl-1-(2,4,6-trinitro phenyl)-hydrazyl radical was analyzed by using both qualitative and quantitative methods. The qualitative analysis was determined by TLC (Thin Layer Chromatography) autographic spray. The procedures of TLC autographic assay were as follow. After developing and drying, TLC plates (amount of samples ranging 0.1 – 100 μg) were sprayed with 0.2 % (2 mg/mL) of DPPH solution in methanol. Then, the plates were examined for 30 minutes after sprayed. Active compounds appeared as yellow spots with a purple background [32]. The quantitative procedure was adopted from Bios method [33]with minor modification. One ml of 500 μM (0.2 mg/mL) DPPH in methanol was mixed with the same volumes of the tested compounds at various concentrations. They were mixed well and kept in the dark for 30 minutes.

The absorbance at 517 nm was monitored in the presence of different concentrations of the samples. The blank experiment, i.e., with only solvent and DPPH (i.e. 2 mL of 500 μM in methanol), was also carried out to determine the absorbance of DPPH before interacting with the compounds. The amount of sample in mg/mL at which the absorbance at 517 nm decreased to half of its initial value was used as the IC_{50} value of compounds. The analysis was done in triplicate for standard and compounds.

RESULTS AND DISCUSSION

Four known triterpene steroids have been isolated from stems of *P. pulchrum*. Structure elucidations of all compounds were determined by comparing the spectroscopic data (^{1}H and [13]C NMR data) of the

isolated compounds with the published relevant data and references there in.



Compound **1**was isolated as a white crystal compound. Spectra of [13]C NMR of compound **1**displayed 29 signals for 29 carbon atoms.

The four important [13]C NMR signals were chemical shifts at δ_C126.5, 168.7, 138.0 and 129.8 ppm which indicated two pairs of carbon double bonds or carbon atoms with hybride orbitals *sp²*. Moreover, a carbon atom has δ_C200.6 ppm, showed a carbon of carbonyl group (C=O). According to the [13]C NMR spectra, it can be concluded that the compound is a triterpene which has two pairs of double bonds and one carbonyl unit. Spectra of ^{1}H-NMR showed that compound **1** comprised of 46 protons and three of them had chemical shifts at δ_H 5.02; 5.14; and 5.80 ppm, which were bigger than the others. It indicated that the protons were more deshielding due to the induction effects of neighbor atoms. Protons at δ_H 5.02 and 5.14 ppm had the same coupling constant at *J* = 15 Hz, referring to two protons attached to double bonds or carbon atoms, i.e., protons atC-22 andC-23.The identities were the characters of steroids group such as stigmasterols. However, stigmasterol did not have carbon atom atδ_C 200 ppm, which is the character of carbonyl group. In conclusion, compound **1** is similar to stigmasterol which has a carbonyl group.

According to NMR 1D (^{1}H and [13]C) spectra, compound**1**is 6β-hydroxystigmasta-4,22-dien-3-one. It is supported by high similarity parameters of^{1}H and [13]C NMR data betweencompound**1**and 6β-hydroxystigmasta-4,22-dien-3-one (**1***), as presented in Table**1**.

Table 1: Comparison^{1}H and [13]C-NMR data between compound 1 (1) and6β-hydroxystigmasta-4,22-dien-3-one from reference (1*)

No. C/H	δ_C**1(ppm)**	δ_C**1*** **(ppm)**	δ_H**1** **(ppm, *mult*)**	δ_H**1*** **(ppm, *mult*)**
1	37.2	37.1	1.69, 2.02	1.69, 2.02
2	34.4	34.3	2.35, 2.50, *t*	2.35, 2.50, *t*
3	200.6	200.4	-	-
4	126.5	126.3	5.80	5.80
5	168.7	168.5	-	-
6	73.4	73.3	4.33, *t*	4.33, *t*
7	38.7	38.6	1.22, 1.96	1.22, 1.96
8	29.9	29.7	1.21	1.21
9	53.8	53.6	1.51	0.88
10	38.1	38.0	-	-
11	21.1	21.0	0.81, 1.49	0.81, 1.47
12	39.8	39.6	1.13, 2.03	1.13, 2.03
13	42.5	42.5	-	-
14	56.1	55.9	0.98	0.98
15	24.3	24.2	1.11, 1.60	1.11, 1.58
16	28.3	28.2	1.26, 1.84	1.27, 1.84
17	56.3	56.1	1.09	1.10
18	12.1	12.0	0.75	0.76
19	19.7	19.5	1.37	1.39
20	36.3	36.1	2.04	1.33
21	18.9	18.7	0.92, *d*	0.94, *d*
22	138.0	138.1	5.17, *dd*	5.13, *dd*
23	129.8	129.5	5.02, *dd*	5.01, *dd*
24	46.0	45.8	1.53	0.92
25	26.3	26.1	1.67	1.15
26	21.0	19.8	0.84, *d*	0.85, *d*
27	20.0	19.0	0.82, *d*	0.82, *d*
28	34.1	33.9	1.02, 1.29	1.03, 1.30
29	23.3	21.2	0.87, *t*	0.87, *t*

1. compound**1,** 1*.[34]

Since the structure determination of compounds**2, 3,** and **4** was carried out by using the similar procedures as the structure elucidation of compound **1**(6β-hydroxystigmasta-4, 22-dien-3-one), the compounds**2, 3,** and **4** were believed as stigmasterol [35], Stigmasta-4, 22-dien-3-on [36], and ergosterol peroxide [37], respectively.

The potentials of radical scavengers of6β-hydroxystigmasta-4,22-dien-3-one, stigmasterol, stigmasta-4,22-dien-3-on, and ergosterol peroxide towards DPPH assays are presented in Table 2.

Based on the data shown in Table 2 indicated that the ability of tested compounds in netralizing DPPH radicals.

Table 2: Activity of all compounds against DPPH

	IC_{50} (μM)				
	6β-hydroxystigmasta-4,22-dien-3-one	stigmasterol	stigmasta-4,22-dien-3-on	ergosterol peroxide	Ascorbic Acid
DPPH	233.4 ± 0.28	372.3 ± 0.33	144.80 ± 0.24	1083.1 ± 0.38	68.9 ± 0.12

Itcan be concluded that stigmasta-4,22-dien-3-on is the most active compound eventhough the activity of stigmasta-4,22-dien-3-on is less than that of the ascorbic acid. Aspredicted,this is due to delocalized electrons at ring **1** and **2** on compounds **1-3.** Meanwhile,ergosterol peroxide is the most inactive compound as an antioxidant agent since the compound has peroxide unit at ring 2. On the otherhand, compound 4isas an oxidator agent.

CONCLUSIONS

Four triterpene steroid shave been isolated and identified from stems of *P. pulchrum* Bl.; namely, 6β-hydroxystigmasta-4,22-dien-3-one (**1**), stigmasterol (**2**), Stigmasta-4,22-dien-3-on (**3**), andergosterol peroxide (**4**). The antioxidant activity of all compounds showed that stigmasta-4, 22-dien-3-onwas the most active compound.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

We want to express our thanks to Directorate General of Higher Education of Ministry of National Education of Republic of Indonesia for providing research grants under skim “Hibah Fundamental 2013 and Hibah Kompetensi 2014”.

REFERENCES

1. Sahidin, Hakim EH, Juliawaty LD, Syah YM, bin Din L, Ghisalberti EL, *et al*. Cytotoxic properties of oligostilbenoids from the tree barks of Hopea dryobalanoides. Zeitschrift fur Naturforschung C J of Bio Sci 2005;60(9-10):723-7.
2. Hakim EH, Syah YM, Juliawaty LD, Oligomer A, Lajis NH, Sains F, *et al*. Sahidin, bersifat Sitotoksikdari Ekstrak Aseton Kulit Batang Shoreaassamica Dyer. J ITB 2007;12(3):113-8.
3. Resverator S, Hakim EH, Syah YM, Juliawaty LD, Achmad SA, Din LB, *et al*. From Stem Bark of Hopeagregaria and Their Cytotoxic Properties of. J Bioorg Med Chem 2008;8(2):245-51.
4. Juliawati LD, Sahidin A, Hakim EH, Ahmad SA, Syah YM, Latip J, *et al*. 2-arylbenzofuran derivative from Hopea mengarawan. J Nat Prod Communication 2009;4(7):947-50.
5. Muhammad N, Din LB, Sahidin I, Hashim SF, Ibrahim N, Zakaria Z, *et al*. Acuminatol and other antioxidative resveratrol oligomers from the stem bark of Shorea acuminata. J Molecules (Basel, Switzerland) 2012;17(8):9043-55.
6. Nakazibwe S, Taher M, Saxena AK, Ichwan SJA, Australian J. Sahidin, Ardiansyah. Antiproliferation of curcusone B from Jatrophacurcas on human cancer cell lines. J Basic and Applied Sci 2011;5(8):47-51.
7. Sahidin I, Taher M, Manggau M, Universitas S. Ardiansyah, Terpenoids from the stem barks of Jatropha plants and their biological activities, Makara Seri. J Bioorg Med Chem 2011;15(2):106-10.
8. Dali N, Manggau MA, J. Sahidin, Ardiansyah,. Katecin dan jatrophone dari kulit batang jarak merah (Jatropha gossypifolia) dan aktivitas biologinya, Bulletinof. Society of Natural Products Chemistry;In Indonesian. J Bioorg Med Chem 2011;11(1):8-11.
9. Sabandar CW, Ahmat N, Jaafar FM, Sahidin I. Medicinal property, phytochemistry and pharmacology of several Jatropha species (Euphorbiaceae):a review. J Phytochemistry 2013;85:7-29.
10. Ginting S, Manggau MA, Int J. Sahidin, Yamin, Lukman. Cytotoxic potency of diterpenes from Jatropha plants. J Pharmacy and Pharm Sci 2013;5(3):417-20.
11. Manggau AM, Tawangmangu M. Sahidin, Nohong, Profil radical scavenger and antibacterial activities of stigmasterol and stigmasta-22-dien-3-on from stems of Polygonum pulchrum. J Proceeding of Int Symposium on Medicinal Plants and Traditional of Java Indonesia. 2014;4.
12. Heo BG, Park YJ, Park YS, Bae JH, Cho JY, Park K. Anticancer and antioxidant effects of extracts from different parts of indigo plant, Industrial Crops and Products. J Bioorg Med Chem 2014;56:9-16.
13. Kim HN, Kim YR, Jang JY, Choi YW, Baek JU, Hong JW, *et al*. Neuroprotective effects of Polygonum multiflorum extract against glutamate-induced oxidative toxicity in HT22 hippocampal cells. J Ethnopharmacol 2013;150(1):108-15.
14. Braga FG, Bouzada MLM, Fabri RL, de O Matos M, Moreira FO, Scio E, *et al*. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol 2007;111(2):396-402.
15. El-Haci IA, Bekkara FA, Mazari W, Hassani F, Didi MA. Screening of biological activities of Polygonum maritimum L. from Algerian coast. Asian Pacific J of Tropical Biomedicine 2013;3(8):611-6;discussion 5.
16. Zhang F, Gong X, Xiao B, Zhang C, Wang Z. Pharmacokinetics and tissue distribution of a bioactive sesquiterpenoid from Polygonum jucundum following oral and intravenous administrations to rats. J Pharm Biomed Anal 2013;83:135-40.
17. Bunawan and Noor. J Sanggar Kerja Penyelidikan Biologi Sistem in Malaysian 2010.
18. Ban S-H, Kwon Y-R, Pandit S, Lee Y-S, Yi H-K, Jeon J-G. Effects of a bio-assay guided fraction from Polygonum cuspidatum root on the viability, acid production and glucosyltranferase of mutans streptococci. J Fitoterapia 2010;81(1):30-4.
19. Granica S, Czerwinska ME, Granica BZ, Kiss AK, Polygonumaviculare L. Antioxidant and anti-inflammatory flavonolglucuronides from Fitoterapia. J Bioorg Med Chem 2010;91:180-8.
20. Chen Z, Liu Y-M, Yang S, Song B-A, Xu G-F, Bhadury PS, *et al*. Studies on the chemical constituents and anticancer activity of Saxifraga stolonifera (L) Meeb. J Bioorg Med Chem 2008;16(3):1337-44.
21. Chaabi M, Freund-Michel V, Frossard N, Randriantsoa A, Andriantsitohaina R, Lobstein A. Anti-proliferative effect of Euphorbia stenoclada in human airway smooth muscle cells in culture. J Ethnopharmacol 2007;109(1):134-9.
22. Zhang D, Li X, Hao D, Li G, Xu B, Ma G, *et al*. Systematic purification of polydatin, resveratrol and anthraglycoside B from PolygonumcuspidatumSieb. J Et Zucc Separation and Purification Technology 2009;66:329-39.
23. Manoharan KP, Benny TKH, Yang D. Cycloartane type triterpenoids from the rhizomes of Polygonum bistorta. J Phytochemistry 2005;66(19):2304-8.
24. Rathore A, Sharma SC, Tandon JS. Flavanones from Polygonumnepalense, Phytochemistry. J Bioorg Med Chem 1986;25(9):2223-5.
25. Ahmed M, Khaleduzzaman M, Islam MS. Isoflavan-4-ol, dihydrochalcone and chalcone derivatives from Polygonumlapathifolium, Phytochemistry. J Bioorg Med Chem 1990;29(6):2009-11.
26. Brandao GC, Kroon EG, Duarte MGR, Braga FC, Filho JDS, Oliveira AB. Antimicrobial, antiviral, and cytotoxic activity of extracts and constituents from Polygonumspectabile Mart. J Phytomedicine 2010;17(12):926-9.
27. Ferreira AA, Amaral FA, Duarte IDG, Oliveira PM, Alves RB, Silveira D, *et al*. Antinociceptive effect from Ipomoea cairica extract. J Ethnopharmacol 2006;105(1-2):148-53.
28. Richter WO, Geiss HC, Sonnichsen AC, Schwandt P. Treatment of severe hypercholesterolemia with a combination of beta-sitosterol and lovastatin, Current Therapeutic Research. J Bioorg Med Chem 1996;57:497-505.
29. Moon D-O, Lee K-J, Choi YH, Kim G-Y. Beta-sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. Int Immunopharmacol 2007;7(8):1044-53.
30. Moon D-O, Kim M-O, Choi YH, Kim G-Y. beta-Sitosterol induces G2/M arrest, endoreduplication, and apoptosis through the Bcl-2 and PI3K/Akt signaling pathways. J Cancer Lett 2008;264(2):181-91.
31. Khan MR, Mlungwana SM. sitosterol, a cytotoxic sterol from Markhamiazanzibarica and Kigeliaafricana, Fitoterapia. J Bioorg Med Chem 1998;70:96-7.

32. Chacha M, Bojase-Moleta G, Majinda RRT. Antimicrobial and radical scavenging flavonoids from the stem wood of Erythrina latissima. J Phytochemistry 2005;66(1):99-104.
33. Fidrianni I, Utari P, Ruslan KW. Evaluation of antioxidant capacities, flavonoid, phenolic, carotenoid content from various extracts of four kinds brassica herbs, International Journal of Pharmacy and Pharmaceutical Sciences. J Bioorg Med Chem 2014;6(2):268-72.
34. Kamrun N. Asha, and Steroids and Polyketides from Uvariahamiltonii stem bark, ActaPharmaceutica,;54. J Bioorg Med Chem 2004.
35. Yahya MAA, Yacob WA, Nazlina I, The G. Isolation of Chemical Constituents From Rhizomes of EtlingerasphaerocephalaVar. J of Analytical Sci 2011;15(1):22-6.
36. Changa C, Chang FR, Chang Y. The Constituents of Lindera glauca. J of the Chinese Chemical Society 2000;47:373-80.
37. Wu Q-P, Xie Y-Z, Deng Z, Li X-M, Yang W, Jiao C-W, *et al*. Ergosterol peroxide isolated from Ganoderma lucidum abolishes microRNA miR-378-mediated tumor cells on chemoresistance. J PLoS One 2012;7(8):e44579.