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Original Article

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

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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) □C (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°C. Spectrum of ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 1.82 (1H, *m*,H-1ª); 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) □H (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) □H (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

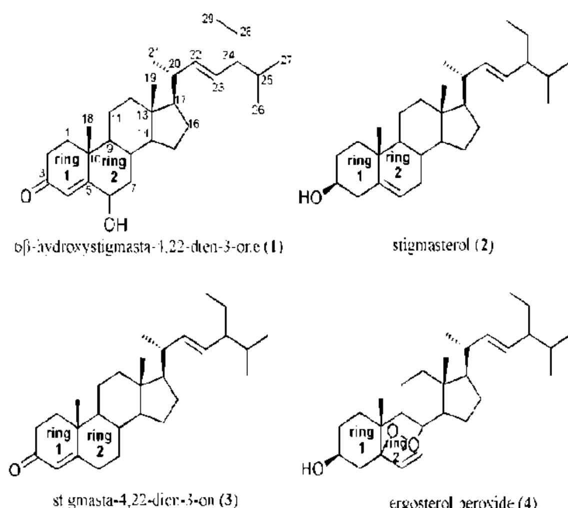
The potency of isolated compounds as radical scavengers was evaluated against inhibition of DPPH reduction. The reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl or 2,2-diphenyl-1-(2,4,6-trinitro phenyl)-hydrazyl radical was analyzed by using both qualitative and quantitative methods. The qualitative 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 *sp*^{2}. 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

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