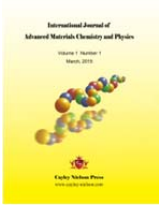
International Journal of Advanced Materials Chemistry and Physics, 2015, 1(1): 26-29

International Journal of
Advanced Materials Chemistry and Physics



Constituents of sphaeranthus africanus sphaeranthus africanus linn. of the family asteraceae

Consolacion Y. Ragasa

Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Binan City, Laguna, Philippines

ABSTRACT

*Chemical investigation of the dichloromethane extract of the leaves of Sphaeranthus africanus afforded carvotanacetone derivatives (**1, 3a** and **3b**), chrysosplenol D (**4**), squalene (**5**), spinasterol (**6**), and stigmasterol (**7**). The structures of **1** and **3a-7** were identified by NMR spectroscopy.*

Keywords: *Sphaeranthus africanus,* Asteraceae, chrysosplenol D, squalene, spinasterol, stigmasterol

INTRODUCTION

Sphaeranthus africanus Linn. of the family Asteraceae is a weed found in open damp places throughout the Philippines. The plant is used as a tonic, vermifuge, emollient and diuretic, while a decoction of the leaves is used as antiblennorrhagic and the juice is employed as a gargle in inflammation of the throat [1]. The whole plant is used in ayurvedic treatment of pitta epilepsy, migraine, jaundice, fever, cough, hemorrhoids, helminthiasis, skin diseases and as nervine tonic [2]. It is also used for cough, depurative, toothache and tumor (breast) [3].

We earlier reported the isolation of four new carvotanacetone derivatives (**1**, **2**, **3a**, and **3b**) from the leaves of *S. africanus*. Compounds **1**–**3** exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger* [4]. Previous studies reported the isolation of carvotanacetone derivatives from congeners of the plant, *S. suaveolens* D. C. [5], S. bullatus [6], and the aerial part of *S. confertifolius* Robyns [7].

We report herein the isolation of **1**, **3a-3b**, chrysosplenol D (**4**), squalene (**5**), spinasterol (**6**), and stigmasterol (**7**), (Fig. 1) from the leaves of *S. africanus*. To the best of our knowledge, this is the first report on the isolation of **4-7** from the plant.

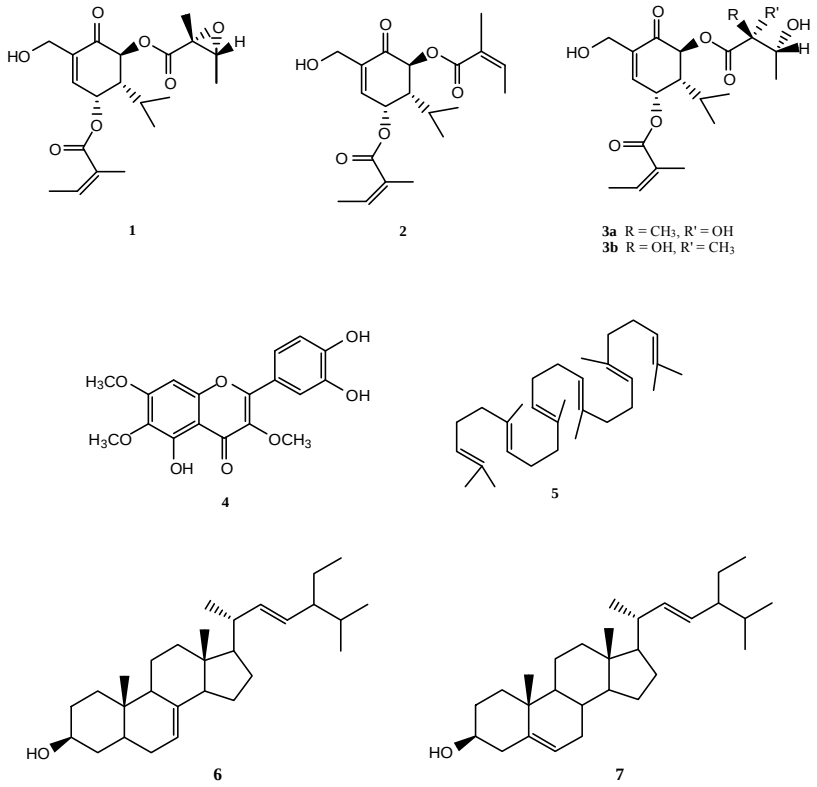


Fig. 1. Chemical constituents of *Sphaeranthus africanus*: carvotanacetone derivatives (1-3b), chrysosplenol D (4), squalene (5), spinasterol (6), and stigmasterol (7)

EXPERIMENTAL SECTION

General Experimental Procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 followed by warming.

Sample Collection

The leaves of *Sphaeranthus africanus* Linn. were collected from Damarinas, Cavite, Philippines in June 2009. The sample was collected and identified by one of the authors (DDR).

Isolation of the Chemical Constituents of S. africanus

General Isolation Procedure

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 18 inches in height and 1.0 inch internal diameter was used for the crude extracts from the leaves. Ten milliliter fractions were collected. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns.

Isolation

The air-dried leaves (568.8 g) of *S. africanus* were ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (32.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatograped

(3×) in petroleum ether to afford **5** (4 mg). The 30% to 40% acetone in CH_2Cl_2 fractions were combined and rechromatograped (3×) in petroleum ether to afford **6** (10 mg) and **7** (6 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatograped (4×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) to afford **1** (4 mg) after washing with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatograped (5×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford **4** (3 mg) after washing with petroleum ether. The 90% acetone in CH_2Cl_2 fraction was rechromatograped (6×) in CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7 by volume ratio) to afford a mixture of **3a** and **3b** (5 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *Sphaeranthus africanus* afforded **1** and **3a-7.** The structures of the carvotanacetone derivatives (**1, 3a-3b**) [2]**,** chrysosplenol D (**4**) [8,9], squalene (**5**) [10], spinasterol (**6**) [11,12], and stigmasterol (**7**) [13] were identified by comparison of their ^{1}H and ^{13}C NMR data with those reported in the literature [4, 8-13].

Although no biological activity tests were conducted on the isolated compounds, literature search revealed that **4-7** exhibited the following bioactivities.

Bioactivity-guided isolation on *Vitex negundo* led to the identification of chrysoplenetin and chrysosplenol D (**4**) as the active constituents with PC_{50} values of 3.4 µg/mL and 4.6 µg/mL, respectively against PANC-1 human pancreatic cancer cells. These compounds induced apoptosis-like morphological changes in PANC-1 cells [14]. Flavonoid **4** inhibited the growth of KB cells with an ED_{50} = 13.95 µg/mL [15]; markedly inhibited the incorporation of 32P into phospholipids when HeLa cells are stimulated by 12-O-tetradecanoylphobol-13-acetate (TPA) [16]; and inhibited the growth of tsFT210 cells with an IC_{50} = 3.5 µg/mL by inducing apoptosis [17]. Another study reported that **4** exhibited strong inhibitory activity against *P. fluorescens* which was comparable with that of ampicillin sodium (MIC, 500 $\mu g \cdot mL^{-1}$). It showed weak activities against *E. coli*, *B. subtilis* and *M. tetragenus*, with (MIC) values of 500, 500 and 250 $\mu g \cdot mL^{-1}$, respectively [18]. Flavonoid **4** from *Artemisia annua* was linked to the *in vitro* anti-plasmodial activity of either whole plant or cell cultures [19].

Squalene (**5**) significantly suppresses colonic ACF formation and crypt multiplicity. This strengthens the hypothesis that squalene possesses chemopreventive activity against colon carcinogenesis [20]. It has cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [21].

Spinasterol (**6**) exhibited cytotoxicity against HCT 116 with an IC_{50} value of 40.52 µg/mL [13]. Furthermore, **6** isolated from Pueraria root showed antitumor activity [22]. It demonstrated a positive vascular damage activity and showed possible anti-angiogenic properties characterized by capillary hemorrhaging and ghost vessels, which eventually led to a non-functional chorioallantoic membrane [11]. Another study reported that **6** exhibited antibacterial action against *Streptococcus mutans* and *S. sorbrinus* [23].

Stigmasterol (**7**) lowered plasma cholesterol levels, inhibited intestinal cholesterol and plant sterol absorption, and suppressed hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [24]. It showed therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [25].

CONCLUSION

The compounds isolated from the leaves of *S. africanus*, chrysosplenol D (**4**), squalene (**5**), spinasterol (**6**), and stigmasterol (**7**), have been reported to exhibit anticancer activities. The antitumor property of the plant may be attributed to active principles which include **4-7.**

REFERENCES

[1] Quisumbing E, Medicinal plants of the Philippines. Manila: Bureau of Printing; **1978**, 998–999.
[2] S Rehel, **2013**, *Sphaeranthus africanus*. The IUCN Red List of Threatened Species. Version **2014**.1. <www.iucnredlist.org>. Downloaded on 05 July **2014**.
[3] T Johnson. CRC Ethnobotany Desk Reference. CRC Press LLC. **2000** Corporate Blvd. NW Boca Raton, Florida 33431, USA.
[4] CY Ragasa; P-W Tsai; C Galvez; C-C Shen, *Planta Med*., **2010**, *76*(2):146–151.
[5] AA Ahmed; AA Mahmoud, *Phytochem.,* **1997**, 45, 533–535.
[6] J Jacupovic; M Grenz; F Bohlman; GM Mungai, *Phytochem.,***1990**, 29, 1213–1217.
[7] C Zdero; F Bohlmann; GM Mungai, *Phytochem.,* **1991**, 30, 3297–3303.
[8] GD Browna; G-Y Liangc; L-K Sy, *Phytochem.,* **2003**, 64, 303–323.
[9] G Kraus; S Roy, *J. Nat. Prod.,* **2008,** 71, 1961–1962.
[10] P-W Tsai; K de Castro-Cruz; C-C Shen; CY Ragasa, *Phcog. J.,* **2012**, 4(31), 1–4.
[11] DD Raga, AA Herrera, AB Alimboyoguen, C-C Shen, CY Ragasa, *Philipp. Agric. Scient.,* **2011**, 94(2),103–110.
[12] CY Ragasa CY; OB Torres; C-C Shen; MGR Mejia; RJ Ferrer; SD Jacinto, *Phcog J.,* **2012**, 4(32), 29–31.
[13] J-MC Cayme; CY Ragasa, *Kimika.* **2004**, 20(1/2), 5–12.
[14] S Awale; TZ Linn; Feng Li, Y Tezuka; A Myint, A Tomida, T Yamori; H Esumi; S Kadota, *Phytother. Res.,* **2011**, 25(12), 1770–1775.
[15] M Arisawa; T Hayashi; M Shimizu; N Morita; H Bai; S Kuze; Y Ito, *J. Nat. Prod.,* **1991**, 54, 898–901. [16] M Arisawa; M Shimizu; Y Satomi; A Nishino; H Nishino; A Iwashima, *Phytother. Res.,* **1995**, 9, 222–224. [17] A Mori; C Nishino; N Enoki; S Tawata, *Phytochem.,* **1988**, *27*, 1017–1020.
[18] T-J Ling; W-W Ling; Y-J Chen; X-C Wan; T Xia; X-F Du; Z-Z Zhang, *Molecules,* **2010**, *15*, 8469–8477.
[19] KCS Liu; S-L Yang; ME Roberts; BC Elford; JD Phillipson, *Plant Cell Rep.,* **1992**, 11, 637–640.
[20] CV Rao; HLN Mark; BS Reddy, *Carcinogenesis*, **1998**, 19, 287–290.
[21] KHS Farvin; R Anandan; S Hari; S Kumar; KS Shing; S Mathews; TV Sankar; PGV Nair, *J Med. Food,* **2006**, 9(4), 531–536.
[22] GC Jeon; MS Park; DY Yoon; CH Shin; HS Sin; SJ Um, *Exp. Mol. Med*., **2005**, 37(2), 111–20.
[23] MJ Salvador; OLAD Zucchi; RC Candido; IY Ito; DA Dias, *Pharm. Biol*., **2004**, 42(2), 138–148.
[24] AK Batta; G Xu; A Honda; T Miyazaki; G Salen, *Metabolism*, **2006**, 55(3), 292–299.
[25] T Ghosh; TK Maity; J Singh, *Orient Pharm. Exp. Med*., **2011**, 11, 41–49.