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Antimicrobial Terpenoids from *Erigeron sumatrensis*

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ABSTRACT

The leaves of *Erigeron sumatrensis* Retz is reported to exhibit antimicrobial properties. The study was conducted to isolate the dichloromethane soluble constituents of the plant which may contribute to this activity. The dichloromethane extract of the air-dried leaves of *E. sumatrensis* afforded (E)-β-farnesene 1, neophytadiene 2, spathulenol 3, and spinasterol 4. The structures of 1 and 2 were elucidated by extensive 1D and 2D NMR spectroscopy, while the structures of 3 and 4 were deduced by comparison of their ^{1}H and ^{13}C NMR spectra with those found in the literature. Compound **1** indicated moderate antifungal activity against *C. albicans* and low activity against *E. coli, P. aeruginosa,* and *T. mentagrophytes.* It was inactive against *S. aureus, B. subtilis,* and *A. niger.* Compound **2** exhibited moderate antifungal activity against *C. albicans,* low activity against *A. niger, T. mentagrophytes, E. coli,* and *P. aeruginosa,* and was found inactive against *S. aureus* and *B. subtilis.* Compounds **3** and **4** exhibited low activity against *E. coli, P. aeruginosa, S. aureus, C. albicans,* and *T. mentagrophytes.* They were inactive against *B. subtilis* and *A. niger.*

Keywords: *Erigeron sumatrensis, Compositae,* β-farnesene, neophytadiene, spathulenol, spinasterol, antimicrobial

INTRODUCTION

Erigeron sumatrensis Retz is a weed found throughout the Philippines. The plant is used in the treatment of skin disease, influenza, and headache (Flores *et al.*, 1981). An earlier study reported that the 70% ethanol extract of the air-dried leaves of *E. sumatrensis* exhibited strong antibacterial activity against *S. aureus* and *E. coli* and moderate activity against *C. albicans* (Hansel & Lagare, 2005). Previous studies on the aerial part of *E. sumatrensis* Retz. reported the isolation of (7R)-opposite-4(15)-ene-1β,7-diol, 15-methoxyisodauc-3-ene-1β,5α-diol, eudesm-4(15)-ene-1β,6α-diol, 6α-methoxy eudesm-4(15)-ene-1β-ol, 4α,15-epoxyeudesmane-1β-6α-diol, 1α-hydroxy isodauc- 4-en-15-al, aromadendrane-4β,10β-diol, and (5E)-germacra-5,10(14)-dien-1β,4β-diol (Iijima *et al.,* 2003a), and a new cyclopentenone derivative, erigerenone B (Iijima *et al.,* 2003b). Another study also reported the isolation of triterpenoids and sterols from plants of the genus *Erigeron* (Iijima *et al.,* 2002).

We report here the isolation and antimicrobial activities of (E)-β-farnesene **1,** neophytadiene **2,** spathulenol **3,** and spinasterol **4** from the dichloromethane extract of the air-dried leaves of *Erigeron sumatrensis* Retz (Figure 1). To the best of our knowledge this is the first report on the isolation of these compounds from *E. sumatrensis.*

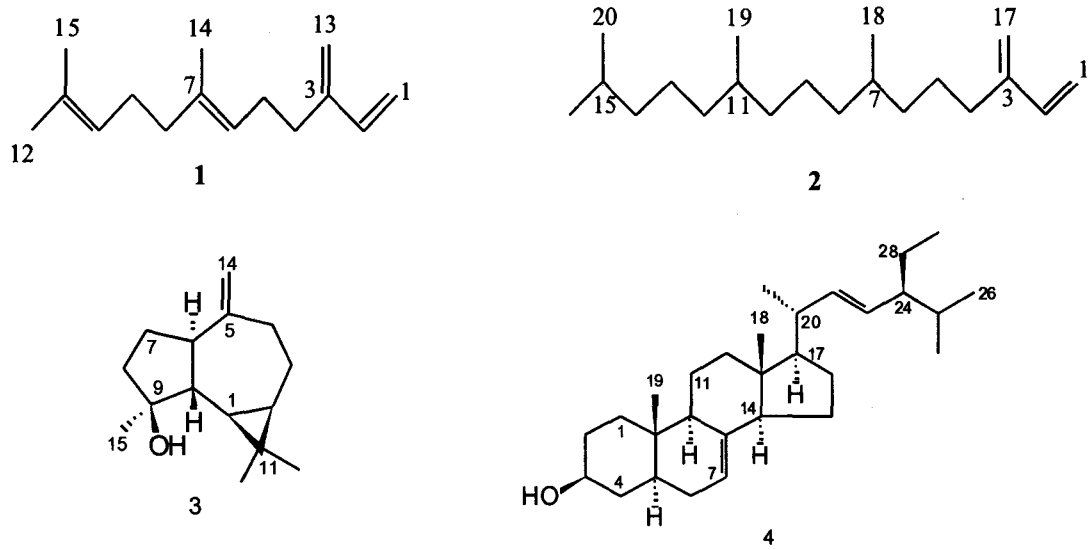

Figure 1. The three terpenoids and a sterol isolated from *Erigeron sumatrensis*: (E)-β-farnesene **1**, neophytadiene **2**, spathulenol **3**, and spinasterol **4**.

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Varian Unity Inova spectrometer in $CDCl_3$ at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh); TLC was performed with plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin sulfuric acid and warming.

Sample collection

The plant material was collected from Tanay, Rizal in May 2008. It was identified as *Erigeron sumatrensis* Retz at the Institute of Biology, University of the Philippines-Diliman. A voucher specimen no. 140 is deposited at the Chemistry Department, De La Salle Univesity-Manila.

Isolation

The air-dried leaves of *Erigeron sumatrensis Retz.* (1 kg) were ground in an osterizer, soaked in dichloromethane for three days, then filtered. The filtrate was concentrated under vacuum to afford a crude extract (155 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment. The dichloromethane fraction was rechromatographed (7×) in petroleum ether to afford **1** (8 mg) and **2** (10 mg). The 10-20% acetone in dichloromethane fractions were rechromatographed (5×) in 5% ethyl acetate in petroleum ether, followed by 10% ethyl acetate in petroleum ether. The more polar fractions afforded **3** (15 mg), while the less polar fractions afforded **4** (20 mg).

Antimicrobial Tests

The microorganisms used were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* (UPCC 1244), *Bacillus subtilis* (UPCC 1149), *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Candida albicans* (UPCC 2168), *Trichophyton mentagrophytes* (UPCC 4193) and *Aspergillus niger* (UPCC 3701). The test compound was dissolved in 95% ethanol. The antimicrobial assay reported in the literature was employed (Guevara and Recio, 1985). The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

RESULTS AND DISCUSSION

The aerial part of *Erigeron sumatrensis* afforded (E)-β-farnesene (**1**), neophytadiene (**2**), spathulenol **3**, and spinasterol **4** by silica gel chromatography. The structures of **1-2** were elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectral data of **1** (Table 1) showed resonances for olefinic protons at δ 5.05, 5.23, and 6.38 which were coupled to each other, and weakly coupled to a broad singlet at 5.00 (2H). Two other vinylic resonances were found at δ 5.14 and 5.10; three allylic methyl groups at δ 1.69 (3H), and 1.62 (6H); and four allylic methylene groups at δ 2.00, 2.08, 2.20, and 2.22. The ^{13}C NMR spectral data (Table 1) indicated fifteen carbon atoms with the above-mentioned functionalities. The ^{1}H and ^{13}C assignments of **1** (Table 1) were verified by HSQC and their connectivities were verified by HMBC. Literature search revealed that **1** is (E)-β-farnesene as evidenced by similar ^{1}H and ^{13}C NMR data (Baeckstrom and Li, 1991).

The ^{1}H NMR spectral data of **2** (Table 1) gave resonances for olefinic protons at δ 5.05, 5.23, and 6.38 which were coupled to each other. These vinylic protons were also coupled to the broad singlets at 5.00 and 4.98, indicating the same conjugated system as in **1**. This was supported by the ^{13}C NMR resonances for vinylic carbons at δ 113.0, 139.1, 115.4, and 146.7, in addition to 16 aliphatic ^{13}C resonances. There was only one allylic methylene group at δ 2.18 and there was no allylic methyl singlet. Four methyl doublets were found at δ 0.82 (J = 7.0 Hz), 0.84 (J = 7.0 Hz), 0.85 (J = 7.0 Hz) and 0.86 (J = 7.0 Hz). The rest of the ^{1}H NMR resonances at δ 1.22-1.63 were attributed to methine and methylene protons in **2.** The ^{1}H and ^{13}C assignments of **2** (Table 1) were verified by HSQC. The ^{13}C NMR spectral data of **2** were compared with those found in the literature for phytol (Goodman *et al.,* 1973). Compound **2** and phytol have similar resonances, except at the region where their structures differ, i.e. C-1 to C-4 and C-17. Thus, **2** is neophytadiene.

The structures of **3** and **4** were deduced by comparison of their ^{1}H NMR and ^{13}C NMR data with those found in the literature for spathulenol (Ragasa *et al.,* 2003) and spinasterol (Ragasa *et al.,* 2005), respectively.

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, **1** and **2** were tested for antimicrobial activity against seven microorganisms (Table 2). Compound **1** exhibited moderate antifungal activity against *C. albicans* with an activity index (AI) of 0.3 and low activity against *E. coli, P. aeruginosa,* and *T. mentagrophytes* with AI of 0.1, 0.2, and 0.2, respectively. It was inactive against *S. aureus, B. subtilis*, and *A. niger.* Antimicrobial tests on **2** indicated that it has moderate antifungal activity against *C. albicans* with an AI of 0.4, and low activity against *A. niger* with an AI of 0.3 and *T. mentagrophytes* with an AI of 0.5. Compound **2** gave low antibacterial activity against *E. coli* and *P. aeruginosa* with an AI of 0.1, and was found inactive against *S. aureus* and *B. subtilis.* Compounds **3** and **4** exhibited low activity against *E. coli, P. aeruginosa, S. aureus, C. albicans, and T. mentagrophytes* with AI of 0.2, 0.2, 0.1, 0.2 and 0.2, respectively. They were inactive against *B. subtilis* and *A. Niger.*

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Table 1. 500 ^{1}H NMR and 125 MHz ^{13}C NMR Spectral Data of **1** and **2** in $CDCl_3$

Position	δ_C (1)	δ_H mult. (J Hz),* 1	δ_C, (2)	δ_H mult. (J Hz),* (2)
1	113.0	5.05 dd (10.8, 4.0), 5.23 dd (4.0, 17.6)	113.0	5.05 dd (10.8, 4.0), 5.23 dd (4.0, 17.6)
2	139.0	6.38 dd (17.6, 10.8)	139.1	6.38 dd (17.6, 10.8)
3	146.2	-	146.7	-
4	31.5	2.22	31.7	2.18
5	39.7	2.00	25.6	1.50
6	124.1	5.14	37.0	1.26, 1.35
7	138.4	-	32.7	1.40
8	26.66	2.20	37.4	1.26
9	26.74	2.08	24.5	1.28, 1.24
10	124.4	5.10	37.4	1.26
11	131.3	-	32.8	1.40
12	25.7	1.69 s (Me)	37.4	1.26
13	115.7	5.00 br s	24.8	1.32, 1.22
14	16.0	1.62 s (Me)	29.7	1.26
15	17.7	1.62 s (Me)	28.0	1.63
16			19.7	0.82 d (7.0, Me)
17			115.4	5.00 br s, 4.98 br s
18			22.7	0.86 d (7.0, Me)
19			22.6	0.85 d (7.0, Me)
20			19.6	0.84 d (7.0, Me)

*multiplet unless otherwise indicated

Table 2. Antimicrobial Test Results of 1-4.

Microorganism	Sample	Clearing Zone,[a] mm	AI
Escherichia coli	1	11	0.1
	2	11	0.1
	3	12	0.2
	4	12	0.2
	Chloramphenicol[b]	23	2.8
Pseudomonas aeruginosa	1	12	0.2
	2	11	0.1
	3	12	0.2
	4	12	0.2
	Chloramphenicol[b]	14	1.3
Staphylococcus aureus	1	-	0
	2	-	0
	3	11	0.1
	4	11	0.1
	Chloramphenicol[b]	25	3.2
Bacillus subtilis	1	-	0
	2	-	0
	3	-	0
	4	-	0
	Chloramphenicol[b]	20	2.3
Candida albicans	1	13	0.3
	2	14	0.4
	3	12	0.2
	4	12	0.2
	Clotrimazole[c]	18	0.8
Trichophyton mentagrophytes	1	12	0.2
	2	15	0.5
	3	12	0.2
	4	12	0.2
	Clotrimazole[c]	55	4.5
Aspergilus niger	1	-	0
	2	13	0.3
	3	-	0
	4	-	0
	Clotrimazole[c]	23	1.3

[a]Average of 3 trials, [b]Chloramphenicol disc - 6 mm diameter, [c]Contains 1% chlotrimazole