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Antimicrobial Terpenoids from *Elephantopus mollis*

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ABSTRACT

The leaves of *Elephantopus mollis* HBK, commonly known as malatabako 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 air-dried leaves of *E. mollis* afforded molephantin (**1**), molephantinin (**2**), 2-deethoxy-2-hydroxyphantomolin (**3**), stigmasterol (**4**), ά-amyrin fatty acid ester (**5a**), and lupeol fatty acid ester (**5b**). The structures of the sesquiterpenes (**1-3**) were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR data with those found in the literature, while the structures of **4**, **5a**, and **5b** were deduced by comparison of their ^{1}H and ^{13}C NMR spectral data with those reported in the literature. Antimicrobial tests on **1-3** indicated that they are moderately active against the fungus, *C. albicans* and slightly against the bacteria: *E.coli* and *P. aeruginosa* and the fungus, *T. mentagrophytes*. Compounds **1** and a mixture of **1** and **2** exhibited slight activity against *S. aureus*, while **3** and a mixture of **1** and **2** were slightly active against *B. subtilis*. All compounds were found inactive against *A. niger*.

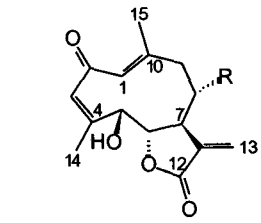
Keywords: *Elephantopus mollis,* Asteraceae, molephantin, molephantinin, 2-deethoxy-2-hydroxyphantomolin, ά-amyrin fatty acid ester, lupeol fatty acid ester, antimicrobial

INTRODUCTION

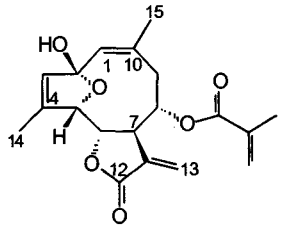
Elephantopus mollis is a weed found throughout the Philippines. The leaves are applied to wounds as a vulnerary, while a decoction of the plant is given as diuretic and febrifuge (Quisumbing, 1978). An earlier study reported that the 70% ethanol extract of the air-dried leaves of *E. mollis* exhibited antibacterial activity against *E. coli* (Hansel & Lagare, 2005). A previous study on the plant reported the isolation of three new sesquiterpenoid lactones: 2,5-epoxy-2β-hydroxy-8α-(2-methylpropenoyloxy)-4(15),10(14),11(13)-germacra trien-12,6α-olide,(4βH)-8α-(2-methylpropenoyloxy)-2-oxo-1(5),10(14),11(13) -guaiatrien-12,6α-olide and (4*H*)-5-hydroxy-8-(2-methylpropenoyloxy)-1(10),11(13)-guaiadiene-1,2,6-olide together with molephantin, elephantopin, isoelephantopin and 2-deethoxy-2β-methoxyphantomolin which exhibited potent leishmanicidal activities (Fuchino *et al.,* 2001). Other studies on *E. mollis* reported the isolation of elephanmollen and 2,5-epoxy-2β-hydroxy-8α-(2methylbut-2-enoyloxy)-4(15),10(14),11(13)-germacratrien-12, 6α-olide (Tabopda *et al.,* 2007); lupeol, lupeol acetate, epifriedelinol, molephantin, 2-deethoxy-2-methoxyphantomolin and deethoxy-2-hydroxy phantomolin

(But *et al.*, 1996); a derivative of desoxyelephantopin (Jakupovic *et al.*, 1987); methyl ethers of phantomolin (Banerjee *et al.*, 1986); and 1-hydroxy-15-senecioyloxy-α-curcumin (Bohlmann & Zdero, 1976). The following antitumor agents from *E. mollis* have also been reported: molephantinin and phantomolin were potent inhibitors of Ehrlich ascites carcinoma and Walker 256, while molephantinin also exhibited antileukemic activity (Lee *et al.*, 1980); molephantinin showed inhibitory activity against the Walker 256 carcinosarcoma in rats at 2.5 mg/kg (Lee *et al.*, 1975); and phantomolin (McPhail *et al.*, 1974) and molephantin (Lee *et al.*, 1973) exhibited cytotoxic activities.

We report here the isolation, identification and antimicrobial activities of molephantin (**1**), a mixture of **1** and molephantinin (**2**), and 2-deethoxy-2-hydroxyphantomolin (**3**) from the air-dried leaves of *Elephantopus mollis*. Stigmasterol (**4**) and a mixture of α-amyrin fatty acid ester (**5a**) and lupeol fatty acid ester (**5b**) were also isolated from the plant.



1 R = 2-methylpropenoyloxy
2 R = 2-methylbut-2-enoyloxy



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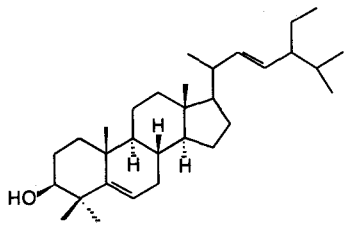

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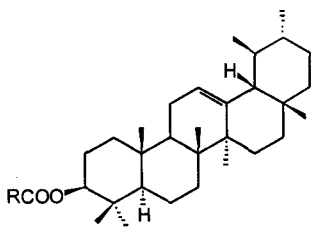
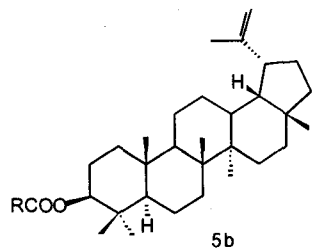


5a

R = long chain fatty acid



5b

R = long chain fatty acid

Figure 1. The five terpenoids and a sterol isolated from *Elephantopus mollis*: molephantin (**1**), molephantinin (**2**), 2-deethoxy-2-hydroxyphantomolin (**3**), stigmasterol (**4**), α-amyrin fatty acid ester (**5a**) and lupeol fatty acid ester (**5b**).

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 Limay, Bataan in May 2008. It was identified as *Elephantopus mollis* HBK at the Institute of Biology, University of the Philippines-Diliman. A voucher specimen no. 141 is deposited at the Chemistry Department, De La Salle University-Manila.

Isolation

The air-dried leaves of *Elephantopus mollis* HBK (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 (125 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment. The dichloromethane and 10% acetone in dichloromethane fractions were combined and rechromatographed in increasing proportions of ethyl acetate in petroleum ether at 5% increment. The less polar fractions were rechromatographed (5×) in petroleum ether to afford a mixture of 5a and 5b (21 mg). The more polar fractions were rechromatographed in 10% ethyl acetate in petroleum ether (3×) to afford 4 (32 mg) after washing with petroleum ether. The 40% to 70% acetone in dichloromethane fractions were rechromatographed (7×) in diethyl ether:acetonitrile:dichloromethane (1:1:8) to afford 1 (8 mg) and a mixture of 1 and 2 (14 mg). The more polar fractons were rechromatographed (4×) in diethyl ether:acetonitrile:dichloromethane 1.5:1.5:7 to afford 3 (12 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.

molephantin (**1**): 133.3 (C-1), 194.9 (C-2), 129.3 (C-3), 158.5 (C-4), 73.8 (C-5), 81.1 (C-6), 49.5 (C-7), 72.4 (C-8), 45.4 (C-9), 136.9 (C-10), 132.9 (C-11), 169.2 (C-12), 128.4 (C-13), 18.9 (C-14), 20.0 (C-15), 165.6 (C-1′), 135.7 (C-2′), 126.6 (C-3′), 18.3 (C-4′).

molephantinin (**2**): 133.2 (C-1), 195.0 (C-2), 129.3 (C-3), 158.7 (C-4), 73.7 (C-5), 81.2 (C-6), 49.5 (C-7), 72.3 (C-8), 45.5 (C-9), 137.1 (C-10), 133.0 (C-11), 169.3 (C-12), 128.3 (C-13), 18.9 (C-14), 20.0 (C-15), 165.2 (C-1′), 128.0 (C-2′), 138.8 (C-3′), 14.6 (C-4′), 12.1 (C-5′).

2-deethoxy-2-hydroxyphantomolin (**3**): 127.2 (C-1), 110.5 (C-2), 130.7 (C-3), 138.6 (C-4), 85.2 (C-5), 79.2 (C-6), 38.8 (C-7), 76.5 (C-8), 32.5 (C-9), 136.1 (C-10), 134.9 (C-11), 168.8 (C-12), 127.0 (C-13), 13.1 (C-14), 28.7 (C-15), 165.7 (C-1′), 133.4 (C-2′), 126.5 (3′), 18.4 (C-4′).

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *Elephantopus mollis* HBK afforded molephantin (**1**), molephantinin (**2**), 2-deethoxy-2-hydroxyphantomolin (**3**), stigmasterol (**4**), α-amyrin fatty acid ester (**5a**), and lupeol fatty acid ester (**5b**). The structures of **1-3** were elucidated by extensive 1D and 2D NMR and confirmed by comparison of their NMR spectral data with those found in the literature for molephantin (But *et al.,* 1996 and Tabopda *et al.,* 2007), molephantinin (Tabopda *et al.,* 2007), and 2-deethoxy-2-hydroxyphantomolin (But *et al.,* 1996), respectively. These sesquiterpene lactones have been previously reported as constituents of *Elephantopus mollis.* Molephantinin is a potent inhibitor of Ehrlich ascites carcinoma and Walker 256 and it exhibited antileukemic activity (Lee *et al.,* 1980). It also showed inhibitory activity against the Walker 256 carcinosarcoma in rats at 2.5 mg/kg (Lee *et al.,* 1975). On the other hand, molephantin exhibited cytotoxic activities (Lee *et al.,* 1973).

Compound **4** was identified as stigmasterol based on similar ^{1}H and ^{13}C NMR data with those found in the literature for stigmasterol (Cayme and Ragasa, 2003). The ^{1}H and ^{13}C NMR spectral data of **5a** and **5b** were compared with the literature data of α-amyrin fatty acid ester (Miranda *et al.,* 2006) and lupeol fatty acid ester (Barreiros *et al.,* 2002), respectively. The spectra matched in all essential respects, confirming the structures of **5a** and **5b**.

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, compounds **1** to **3** were tested for possible antimicrobial activities by the agar well method. Results of the study (Table 1) indicated that **1-3** are moderately active against the fungus, *C. albicans* and slightly active against the bacteria: *E. coli* and *P. aeruginosa* and the fungus, *T. mentagrophytes.* Compound **1** and a mixture of **1** and **2** exhibited slight activity against *S. aureus*, while **3** and a mixture of **1** and **2** were slightly active against *B. subtilis.* All compounds were found inactive against *A. niger.*

Acknowledgement

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Table 1. Antimicrobial Test Results of **1-3**.

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

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

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