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Terpenoids from Kenyan *Leonotis mollissima*

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Abstract
Leonotis mollissima belongs Leonotis genus that comprises of ten species. It is called *kipserere* by Marakwets of Kenya. No phytochemicals have been reported on the Laikipia Kenya species. The leaves were collected from Laikipia University Kenya, identified and voucher specimen kept for reference in Biological Department, Egerton University, Kenya. Dry powder of leaves was successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy two hours. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). With repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane three compounds, 4, 7-dimethoxy-5-methylchromen-2-one (1) [an aromatic compound], 12*β*-acetoxy-20-hydroxy-3, 7, 11, 15-tetraoxo-25, 26, 27-trisnorlanost-8-en-24-oic acid (2) [a triterpenoid] and (13R)-19*α*, 13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone (3)[a diterpenoid] were isolated. Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR (500 MHz) spectroscopy.

Keywords: *Leonotis mollissima*, Siderin, 20-hydroxylucidenicacid d2) labdane, leaves

Introduction
Plants are extremely important in the lives of people throughout the world and many people depend on them to satisfy basic human needs such as food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini *et al*., 2000) [4].
Leonotis mollissima belongs to the genus Leonotis that comprises of about 10 species and to the Lamiaceae family that has 7,200 species distributed in 236 genera (Nurdan and Aysel, 2007) [3]. They are known to treat cold, cough, fever, headache and asthma (Fowler 2006) [1]. The root decoction is used by the Marakwets to treat J. wound, festering sore and intestinal worms. Young leaves and buds are used to treat conjunctivitis and indigestion and are also chewed for cramp in the stomach (Kokwaro, 1976) [2]. No chemical composition and biological activity has not been reported on the Kenyan Laikipia *Leonotis mollissima*. In the course of this research, three compounds were isolated from the Dichloromethane extract of leaves.

Materials and Methods
Leonotis mollissima was collected from Laikipia University Kenya, in June 2014, and a voucher specimen deposited at the Department of Biological Sciences Herbarium Egerton University, Njoro Kenya. The leaves were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro Kenya and the masses taken using a Stanton electronic balance.
Dry powder of leaves (1,000 g) was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal tin. The solvents were evaporated under reduced pressure using a rotary evaporator. (Büchi type R-205) to give a greenish sticky residue. The dichloromethane leave extract (50 g) was subjected to a solvent step gradient of dichloromethane: methanol. Fractions containing more spots were purified by repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane respectively. The separated components were visualized under UV lamp (254 nm and 365 nm) and then sprayed with anisaldehyde reagent and heated in an oven for one minute at 70^{o}C.
The crude extracts were spotted on aluminium TLC plates (20x20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane, ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (Electrolux Struers) at 70^{o}C for one minute. The plates with the best R_f values were used to determine the best solvent system for the separation.
Crude extracts were then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker).

Further purification was achieved by repeated thin layer chromatography and column chromatography.

Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR spectroscopy. NMR spectra were recorded at room temperature on a 500 MHz Bruker AVANCE NMR spectrometer at the School of Biomedical and Molecular Sciences, University of Surrey at Guildford UK. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling (*J*) are given in Hz.

Results and Discussion

Compound 1 (7.7 mg) was isolated as a white powder with a brown spot on visualization with anisaldehyde reagent and UV active with an R_f of 0.3 in 5% methanol in dichloromethane. The ^{1}H NMR spectrum showed resonances between δ 2.58 ppm to δ 6.64 ppm (Table 1). The spectrum showed the presence of one high intensity peak indicating presence of one methyl group at δ 2.58 (sp^2) ppm corresponding to ^{13}C NMR signal at δ 23.73 ppm (Table 1). Two methoxy groups showing ^{1}H resonance at δ 3.90 ppm and δ 3.81 ppm corresponding to the ^{13}C NMR resonance at δ 55.76 ppm and δ 56.17 ppm respectively were also observed (Table 1).

The twelve carbon resonances observed in the ^{13}C NMR spectrum were characterized by DEPT experiment (Table 1). This indicated that the compound was aromatic with three methyl groups, three methine groups and six quaternary groups (two attached to methoxy groups).

^{1}H NMR proton signals at δ 6.63 ppm and δ 6.68 ppm indicated presence of aromaticity (Table 1). In the HMBC spectrum the correlations between ^{1}H and ^{13}C confirmed the position of the two methoxy groups and the carbonyl group (Table 1). COSY and NOESY spectra showed the correlations between the protons thus confirmation of compound 1 as 4, 7-dimethox-5-methylchromen-2-one (Table 1).

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound 1 ($CDCl_2$)

S/N	^{1}H ppm	^{13}C ppm	DEPT	HMBC (H → C)	COSY	NOESY
1		162.1	C			
2	5.50	87.9	CH	3		10
3		170.0	C			
4		115.9	C			
5		137.7	C			
6	6.58	115.9	CH (d) *J*=2.50 Hz		11	
7		162.1	C			
8	6.64	98.9	CH (d) *J*=2.50 Hz			12
9		156.9	C			
10	3.90	55.8	CH_3 (s)	3		2
11	2.58	23.7	CH_3 (s)		6	
12	3.81	56.8	CH_3 (s)	7		8

Compound 2 (7.1 mg) was isolated as a dirty white powder with a brown spot, UV active, with an R_f of 0.3 in 35% ethyl acetate in hexane. The ^{1}H NMR spectrum showed the presence of seven high intensity peaks indicating presence of seven methyl groups at δ 1.27 ppm (two), δ 1.57 ppm, δ 2.13 ppm, δ 1.23 ppm (two), δ 1.78 ppm corresponding to ^{13}C NMR signal at δ 15.8 ppm, δ 23.7 ppm, δ 21.6 ppm, δ 17.2 ppm and δ 24.9 ppm respectively. In ^{13}C NMR, four keto groups at δ 214.5 ppm and δ 198.8 ppm, an acetoxy group at δ 170.6 ppm, a carboxyl group at δ 198.8 ppm and a hydroxyl at δ 69.3 ppm were observed (Table 2).

The twenty nine carbon resonances observed in the ^{13}C NMR spectrum were characterized by DEPT experiment. This indicated that compound 2 was a triterpenoid with seven methyl groups, six methylene groups, four methine groups and twelve quaternary carbon (Table 2).

In the HMBC spectrum, the correlations between ^{1}H and ^{13}C confirmed the position of the acetoxy, keto and the carboxyl groups. COSY and NOESY spectral showed the correlations between the protons thus confirming compound 2 as 12*β*-acetoxy-20-hydroxy-3, 7, 11, 15-tetraoxo-25, 26, 27-trisnorlanost-8-en-24-oic acid.

Table 2: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound 1 ($CDCl_2$)

S/N	^{13}C	^{1}H	DEPT	HMBC H → C	COSY	NOESY
1	33.4	2.76, 2.62	CH_2	2, 10, 19	5	
2	32.1	2.13, 2.35	CH_2	1, 3, 4, 10	17, 5	
3	214.5		C			
4	39.4		C			
5	36.2	1.96	CH	1, 4, 6, 10, 27, 28	1, 2, 6, 26	
6	33.4	2.76, 2.62	CH_2	5, 7, 10	5	
7	198.8		C			
8	150.4		C			
9	150.6		C			
10	33.4		C			
11	198.7		C			
12	77.0	5.74	CH	11, 13, 14, 25		
13	42.3		C			
14	55.4		C			
15	214.5		C			
16	36.2	2.41, 1.96	CH_2	13, 15		
17	44.9	3.15	CH	13, 16, 18, 20	2	26, 29

18	15.8	1.27	CH_3	13, 14		
19	23.7	1.57	CH_3	1, 5, 9, 10		
20	69.3		C			
21	30.5	1.36	CH_2	21,22, 24		
22	33.4	2.62, 2.76	CH_2	21, 23		
23	198.8		C			
24	24.9	1.78	CH_3	17		
25	170.6		C			
26	21.6	2.13	CH_3	25		17
27	17.2	1.23	CH_3	3, 4, 5, 28		
28	17.2	1.23	CH_3	3, 4, 5, 27		
29	15.8	1.27	CH_3	8, 13, 14, 15		17

Compound 3 (21.2 mg) was isolated as white crystals with no R_f because it was UV inactive with no spot on visualization with anisaldehyde reagent. In the ^{1}H NMR spectrum a methyl doublet at δ 0.87 ppm was observed corresponding to ^{13}C NMR resonance at δ 17.6 ppm. Two other methyl groups at δ 1.29 ppm and δ 1.04 ppm corresponding to ^{13}C NMR resonance at δ 23.6 ppm and δ 23.2 ppm were also observed attached to the decalin. ^{1}H NMR signals between δ 1.46 ppm and δ 2.91 ppm indicated seven methylene groups. A doublet signals at δ 4.13 ppm and δ 4.26 ppm showed that one methylene was attached to oxygen in a cyclic ester (Table 3).

In ^{13}C NMR spectrum the resonance at δ 174.7 ppm and δ 183.6 ppm showed presence of two carbonyl groups in a cyclic ester. The twenty carbon resonances observed in the ^{13}C NMR spectrum were characterized by DEPT experiment. This indicated that compound 3 was a diterpenoid with three methyl groups, eight methylene groups (one oxygenated), three methine groups (one oxygenated) and six quaternary carbons (two carbonyl carbons) Table 3.

In the HMBC spectrum, the correlations between ^{1}H and ^{13}C confirmed the position of the carbonyl and hydroxyl groups thus confirming that compound 3 as labdane diterpenoid. Further confirmation was done using COSY and NOESY spectral thus confirming the Compound 3 as (13R)-19*α*, 13*α*-epoxylabda-6*β* (19).16(15)-dioldilactone Table 3.

Table 3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound 1 ($CDCl_2$)

Position	^{13}C	^{1}H	DEPT	COSY	HMBC H⟶C	NOESY
1	29.5	1.25, 1.30 (m)	CH_2			17, 18, 20
2	18.2	1.77, 1.52 (m)	CH_2	11, 18		
3	29.1	1.46, 2.12 (m)	CH_2			
4	44.2		C			
5	46.2	2.07 (m)	CH	6	1, 3, 4, 10, 11, 19, 18, 20	
6	76.2	4.70 (m)	CH	5, 7,8	7, 8, 10	7,8,18
7	32.1	1.63 (m)	CH_2	6, 12		6
8	31.9	1.63 (m)	CH	6, 12		6
9	92.3		C			
10	39.1		C			
11	28.3	1.85, 2.11 (m)	CH_2	2, 12		6, 16
12	37.2	2.12 (m)	CH_2	7, 8, 11, 19	1, 9, 14, 16	14, 16, 17, 20
13	86.3		C			16
14	42.14	2.57, 2.91 (d, *J*=17.31 Hz)	CH_2		12, 13, 15, 16	12
15	174.68		C			
16	78.82	4.13, 4.26 (d, *J*=8.89 Hz)	CH_2		12, 13, 14, 15	11,12
17	183.62		C			
18	23.24	1.04 (s)	CH_3		1, 3, 5, 9, 10, 11	1, 6, 12
19	17.60	0.87 (d, J=6.25Hz)	CH_3	12	7, 8, 9	12
20	23.63	1.29 (s)	CH_3	2	1, 2, 3, 4, 10, 11, 17, 17, 20	1, 6

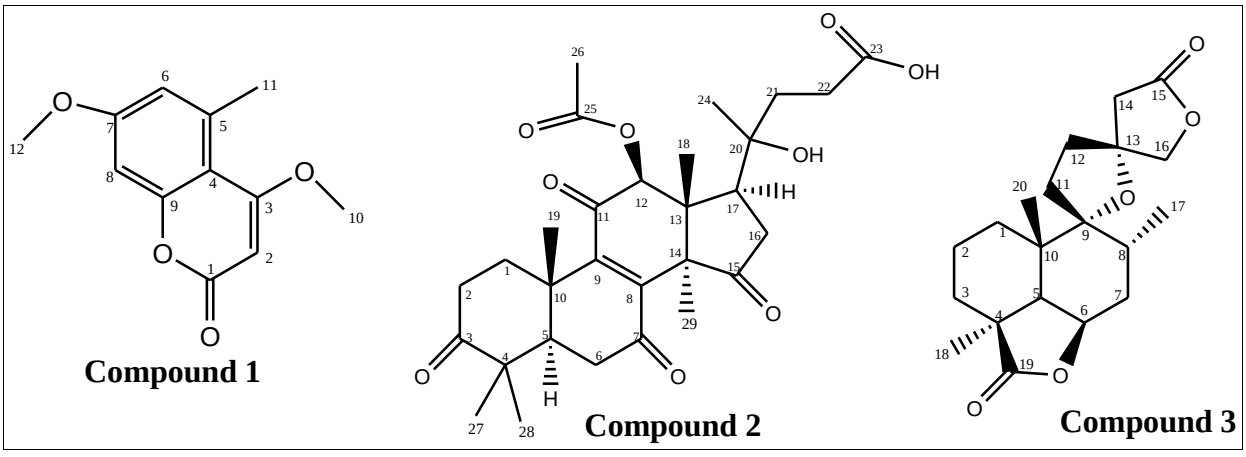

Fig 1: Compounds isolated from the leaves of *Leonotis mollissima*

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