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Secondary Metabolites from *Brachylaena Merana*, an Endemic Plant from Madagascar Rain Forest

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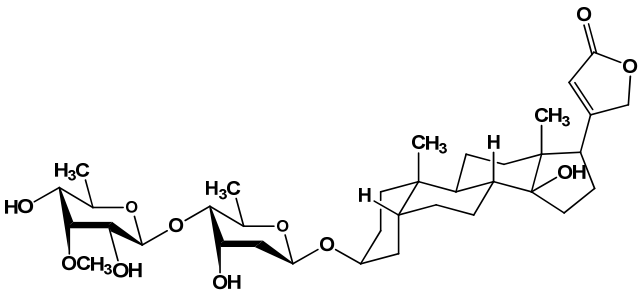
Abstract
New cardenolide 1 and two known compounds oleanan-3-one 2 and sucrose have been isolated from the aerial parts of *Brachyleana Merana* (Asteraceae) an endemic species from Madagascar. Structure elucidations of the compounds were elucidated through spectroscopic methods (1-D and 2-D NMR, ESI-MS) chemical methods and comparison with literature data.

Keywords: Cardenolide, Triterpene, *Brachyleana Merana*, Asteraceae, NMR.

1. Introduction
The genus *Brachyleana* belongs to the family Asteraceae, it is native mainly to East Africa and Madagascar. This family has 11 species but only 4 species are endemic of Madagascar [1]. These medicinal plants are reported to be rich in Sesquiterpenoids and Triterpenoids [2], tannins [3], Sesquiterpenoid lactone [4, 5] but cardenolide glycosides are on state of traces [3] [6]. Cardenolide glycosides are an important class of natural products that can be used as drugs as well as toxins, and have been used as arrow poisons, emetics or heart tonics. Cardenolide glycosides are used in the treatment of congestive heart failure. The cytotoxicity and structure characterization of various cardenolide glycosides have been extensively studied [7, 8].
Brachyleana merana is a traditional medicinal plant from East of Madagascar. The leaves of this plant are used as a folk medicine for treating gastric, pulmonary affection, also known as tonic and aperitive which usually administered as a decoction. This paper presents the isolation and structure elucidation of a new cardenolide 1 and known oleanan-3one 2.

Results and Discussion
Structural characterization of new compounds 1 (fig. 1) were carried out using combination of 1D ^{1}H, ^{13}C and various 2D NMR experiments. Spin systems were identified in COSY experiment. Subsequently these spin systems and quaternary carbons were found connected in the DEPT and HMBC spectrum. In our chemical investigation, a new cardenolid and two known compounds oleanan-3-one were isolated from the aerial parts of *Brachyleana merana* Baker by column chromatograph.
Compound 1 gave a positive reaction with Kedde reagent [9], suggesting that it contains a butenolide ring. Isolated as a white amourphous powder obtained from the Ethanolic extract, components of the EtOAc soluble fraction. Its negative ions ESIMS revelated a pseudomolecular ion peak at m/z: 648.4 $[M + HCOO]^-$, corresponding to a molecular formula of $C_{36}H_{56}O_{10}$ for **1.** Its ^{1}H NMR spectrum in CD_3OD indicated compound 1 to be cardenolide with two sugar units, with signal for two anomeric protons at δ_H 4.94 ppm (1H,s), and δ_H 4.32 ppm (1H, dd, J = 18.2, 1.5 Hz) (table 1). The ^{13}C NMR spectrum contained 36 signals, which included signals for 1 methoxyl, 3 méthyls, 11 methylenes, 16 methynes (including oxymethynes) and 5 quaternary carbons (including 1 oxyquaternary carbon, 1 olefinic carbon, as indicated by an HMQC spectrum (table 1). The above data suggested that 1 is a cardiac glycoside with two sugar moieties.
In the aglycone of 1, two spin systems CH_2-CH_2-CH-CH_2-CH-CH_2-CH_2-CH-CH-CH_2-CH_2 ((H_2-1 through H_2-2, H-3, H_2-4, H-5, H_2-6, H_2-7, H-8, H-9, and H_2-11 to H_2-12) in rings A, B, and C, and CH_2–CH_2–CH (H_2-15 through H_2-16 to H-17) in ring D (Fig. 2) were identified in

the COSY spectra. Long-range correlations from H_3-19 to C-1, C-5, C-9, and C-10, and from H_2-1 to C-9 indicated the connectivity of rings A and B. The relationship between rings C and D was established by the observation of correlations from H_3-18 to C-12, C-13, the oxygenated quaternary carbon at C-14 and C-17, as well as those observed from H_2-12 to C-17, and H_2-15 to C-8. The α,β-unsaurated γ-lactone was deduced to be connected at C-17 by the HMBC correlation of H-17 to C-18, C-20, C-21, and C-22



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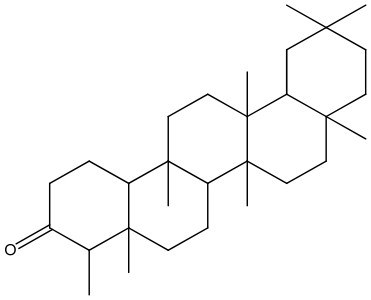

2

Fig 1: Structures assigned to cardenolide **1** and oleanan-3-one **2** the aerial part of *Brachyleana merana*

More over the assigned ^{13}C NMR chemical shifts and 2D NMR studies of the aglycone of **1** in CD_3OD are very similar to those of digitoxigenin [10, 11].

The presence of two sugar units in **1** was indicated by the presence of two anomeric protons signals at δ_H 4.95 and 4.35 ppm. Their spin systems were determined by COSY correlations. H-1′- H_2-2′- H-3′- H-4′- H-5′-H_3-6′ and H-1"- H-2"- H-3"- H-4"- H-5"-H_3-6". Again the interglycosidic linkage and the point of attachment to the genin were established by HMBC correlations from H-1′ to C-3 and H-1" to C-4′.

The HMBC correlation between the proton 3.47 ppm and C-3″ (δ_C : 76.9 ppm) placed the methoxygroup at C-3″.

Therefore, the structure of **1** was determined as digitoxigenin-3-O-β-digitalopyranosyl-(1-4)-O-β-digitoxopyanoside.

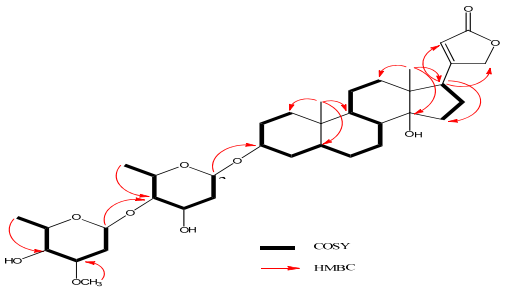

Fig 2: COSY and HMBC correlations of compound **1**

Compound **2** gave a positive Leibermann-Burchard test indicating triterpenoids. Isolated as a white powder obtained from the hexanic extract**.** Its positive ions ESIMS revelated a pseudomolecular ion peak at m/z: 427.368 $[M + H]^+$, corresponding to a molecular formula of $C_{30}H_{50}O$ for compound **2**. And Infrared gave absorption at 1715 cm^{-1} indicated carbonyl vibration C=O. The ^{13}C shift values compared with literature [14] indicated that compound 2 is 3-oxo- friedelin or oleanan-3-one [13].

Table 1: NMR spectroscopie data ^1H and ^{13}C chemical shift values and assignments δ for 1 and 2

Position	1		2	
	δ_C, type	**δ_H, (J in Hz)**	**δ_C, type**	**δ_C, literature [14]**
1	31.09	1.46, m, 2H	22.29	22.3
2	28 .7	2.12m, 1.62 m, 2H	41.54	41.5
3	76.5	4.02, s br, 1H	213.6	213.2
4	31.82	1.44m, 2H	58.2	58.2
5	41.2	1.66, m, 1H	42.1	42.1
6	26.9	1.88 m, 1.25 m, 2H	41.2	41.3
7	22.6	1.71 m, 1.22 m, 2H	18.24	18.2
8	47.32	1.65 m, 1H	53.5	53.1
9	43.6	1.74 m, 1H	37.4	37.4
10	37.4	-	59.4	59.4
11	36.1	1.46 m, 1.19 m, 2H	35.3	35.6
12	39.2	1.49 m, 1.35 m, 2H	30.5	30.5
13	49.6	-	39.25	39.7
14	84.7	-	38.29	38.3
15	33.91	2.11 m, 1.62 m, 2H	32.09	32.4
16	26.5	2.11 m, 1.87 m, 2H	36.0	36.0
17	50.5	2.78 m, 1H (9.0)	30.00	30.0
18	14.9	0.87 s, 3H	42.78	42.8
19	15.62	0.92 s, 3H	35.6	35.3
20	175.8	-	28.12	28.1
21	73.9	5.01, dd, (17.8, 1.7), 1H 4.80, dd, (17.8, 1.7), 1H	32.7	32.7
22	116.4	5.87, s br, 1H	39.7	39.2
23	176.9	-	6.84	6.8
24	-	-	14.66	14.6
25	-	-	17.96	17.9
26	-	-	20.27	20.2
27	-	-	18.68	18.6
28	-	-	32.4	32.1
29	-	-	35.04	35.0
30	-	-	31.79	31.8
Digitoxose				
1′	95.46, CH	4.94, dd, (9.7, 1.8), 1H		
2′	37.4, CH_2	1.97 m, 1.73 m, 2H		
3′	69.9, CH	4.24 m, 1H		
4′	82.5, CH	3.24 m, (2.8), 1H		
5′	68.3, CH	3.76 dq, (9.6, 6.1), 1H		
6′	15.5, CH_3	1.22 d, 3H		
Digitalose				
1″	104.7, CH	4.32, dd, (9.6, 1.9), 1H		
2″	67.12, CH	2.1 m, 1.65 m, 2H		
3″	82.9, CH	3.84 d, 1H		
4″	68.3, CH	3.22 m, (2.9),81H		
5″	70.2, CH	3.93 dq,(9.5, 6.2), 1H		
6″	17.2, CH_3	1.20 d, (6.2), 3H		
O – CH_3	55.9	3.47 s, 3H		

Experimantal Section

General Experimental Procedures.

Melting points were obtained using a Buchi 510 melting points, Opical rotation were recorded on a JASCO P-2000 polarimeter. NMR spectra were recorded with a Brüker AV-400 with a cryoprobe for ^{1}H, APT, COSY, HSQC, and HMBC. Chemical shift values are given in δ(ppm) using the peak signals of the solvent CD_3OD (δ_H 3.35; and δ_C 49.3) for **1** and $CDCl_3$ (δ_H 7.28 and δ_C 77.3) for **2** as references, and coupling constants are reported in Hz. ESIMS data were measured with a Finningan LCQ "Classic". Column chromatography was performed on silica gel 60 (6.3-20µm) (Merck, Darmstadt, Germany). Normal-phase silica gel 60 TLC plates (w/UV 254) were used for fraction detection. The spot were visualized using UV light at 254 nm and spraying with Kedde reagent for **1** and MeOH-sulfuric acid reagent for **2**.

Plant Material

Fresh aerial part of the plant *Brachyleana Merana* Backer was collected Ampitabe Moramanga wildlife area of Andaingomadinika at 1002m altitude, 18° 51' 52.4" latitude South and 48° 16' 29.7" longitude East, Madagascar, in August 2010. Collection was made by Jacquis Razakarivelo, with two botanists Randrianasolo Sennen and Rakotondrafara Andriamalala at CNARP. The herbarium specimen was from a tree of 5 m, diameter at breast height 6 cm. Duplicates of the voucher specimens have been identified and deposited at the Centre National d'Application de Recherche Pharmaceutique, under number SSR570.

Extraction and Isolation.

The collected biomass was air-dried at room temperature and then crushed to a coarse powder (1200g) and extracted with ethanol (EtOH: H_2O, 9:1, 2000mL) at room temperature for 5 days. The extract was filtered and solvent removed using rotary evaporator to give a dark green semi-solid residue (15.7g) was dispersed in water (250 mL) and fractionated successively with, with hexanes (3 × 200 mL) with $CHCl_3$ (3× 200 mL), ethyl acetate (3x200mL). Removal of the solvent gave the respective extracts in the following yields: hexane 3.4g, Chloroform 2 g and ethyl acetate 5 g.

*Isolation of compounds **1** and **2***

The ethyl acetate fraction (3g) was column chromatographed on silica gel (63 – 200 mesh size) using chloroform and chloroform: methanol mixtures of different proportions as eluents. The fractions were bulked according on their TLC profile on silica gel and eight fractions were obtained: F-1 to F-8, F-5 gave white powder as pure compound (25 mg) was concluded to be pure compound **1.**

Hexane extract was column chromatographed as the same as ethyl acetate extract, using hexane-ethyl acetate mixture. Compound **2** was obtained associated with some impurities on Fraction 2 (eluted with Hexane-Ethyl acetate 8:2, v/v); after purification by rechromatography over silica gel using Hexane-Ethyl acetate (9:1) as eluent, yield: (13mg).

Digitoxigenin-3-O-β-digitalopyranosyl-(1-4)-O-β-digitoxopyanoside

Compound **(1)** was a white amourpous solid powder**.** λ_{max} nm (ε) 215 (3.5); mp: 250 -253 °C, **ESIMS** *m/z*: 685 [M + HCOO], ^{1}H **NMR** (400 Mhz in CD_3OD, and ^{13}C NMR (125 Mhz in CD_3OD) see table 1.

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