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## New mono-ether of glycerol and triterpenes with DPPH radical scavenging activity from Cameroonian propolis

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### ABSTRACT

The extracts of some propolis samples were analysed by GC-MS and then purified by column chromatography. The latter led to the isolation of a new mono-ether of glycerol, 1'-O-eicosanyl glycerol and a new triterpene, methyl-3 $\beta$ ,27-dihydroxycycloartan-24-en-26-oate together with known triterpenoids namely betulin, 3 $\beta$ -hydroxylanostan-9,24-dien-21-oic acid, mangiferonic acid, a mixture of ambolic acid and  $\beta$ -sitosterol, 3 $\beta$ -hydroxycycloartan-12,24(25)-diene and 27-hydroxymangiferonic acid. The DPPH radical scavenging potential of some extracts and compounds were measured. The radical scavenging activity varied from Hexane extract of Fouban propolis (IC<sub>50</sub> = 5.6 mg/mL) to Methanol extract of Fouban propolis (IC<sub>50</sub> = 1.07 mg/mL) for the extracts and from 3 $\beta$ -hydroxylanostan-9,24-dien-21-oic acid (IC<sub>50</sub> = 1.22 mg/mL) to 1'-O-eicosanyl glycerol (IC<sub>50</sub> = 0.93 mg/mL) for the compounds. Activities of samples were moderate as they remained closer to those of the standard antioxidants Gallic acid (IC<sub>50</sub> = 0.30 mg/mL) and vitamin C (IC<sub>50</sub> = 0.80 mg/mL), especially 1'-O-eicosanyl glycerol, the most active compound.

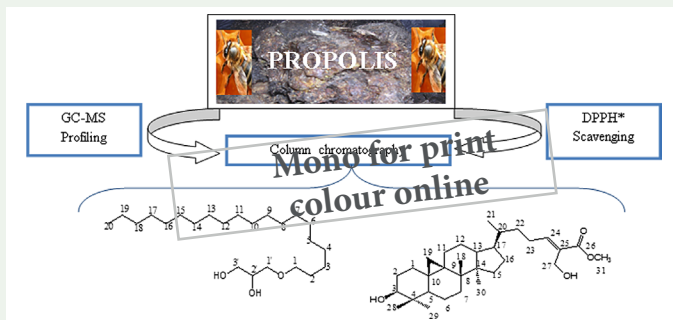
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Propolis; GC-MS profiles; NMR analysis; triterpenes; 1'-O-eicosanyl glycerol; DPPH radical scavenging activity



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## 1. Introduction

Propolis is a resinous, gummy and balsamic substance collected by bees from plants and used to close cracks in their hives, reduce the size of the hive entry, or to embalm dead animals that have entered into the hive. Propolis have been shown to possess many biological activities such as antimicrobial (Popova et al. 2013), anti-diabetic (Oršolić et al. 2012), anti-cancerous (Li et al. 2008) antioxidant and anti-ulcer (Socha et al. 2015; Tamfu et al. 2016). It is due to these important biological properties of propolis that it has been used by man for a wide range of purposes and finds applications in cosmetics, agriculture, food technology, human and veterinary medicine. Reports by several authors support the fact that the chemical composition and biological activities of propolis depends on many different factors such as the geographical region, collecting time, and plant source (Meneses et al. 2009). From different botanical and geographical origins of world, more than 300 compounds including volatile organic compounds, flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpenes, quinones, coumarins, steroids, amino acids, pterocarpanes, fatty acid esters and triterpenoids were reported to have been isolated from propolis (Li et al. 2008; Oršolić et al. 2012; Tamfu et al. 2016) and propolis types from unexplored regions of the world have the potential to provide valuable leads to secondary metabolites with important bioactivities (Popova et al. 2013). In this work, extracts were prepared from propolis samples and their GC-MS profiles established. The DPPH radical scavenging activity of the extracts was evaluated and the extracts purified to obtain pure compounds of which some were also tested for DPPH radical scavenging activity.

## 2. Results and discussion

The unprecedented resolving power of capillary GC (gas chromatography) and the valuable structural information provided by EIMS have proved to be still useful and GC-MS (gas chromatography/mass spectrometry) makes recently a remarkable comeback (Sforcin & Bankova 2011). The GC-MS investigation of different extracts of Fouban propolis revealed the presence of over 30 compounds belonging to a variety of classes of natural products and reported in Tables 1–3. Summarily, the extracts are rich principally in triterpenes, fatty

**Table 1.** GC-MS profile of ethyl acetate extract of Fouban propolis (silylated sample).

rT	Compound	% of TIC	rT	Compound	% of TIC
23.33	Glycerol	1.9	54.57	Heptadecatrienyl resorcinol	0.7
35.73	Vanillic acid	0.1	54.73	Heptadecyl resorcinol	5.7
32.63	Hydroxybenzoic acid	0.4	56.63	Anacardic acid (C17:2)	0.7
36.96	Dihydroxybenzoic acid	0.3	56.92	Anacardic acid (C17:1)	1.6
41.16	Hexadecanoic acid	0.9	57.34	Nonadecyl resorcinol	1.8
44.31	Oleic acid	0.6	61.92	Triterpenic ketone	1.8
44.72	Octadecanoic acid	0.2	62.35	Lanosterol	1.8
48.88	Pentadecyl phenol	0.3	62.65	Amyrenone	8.9
51.03	Docosanoic acid	0.2	63.01	β-amyrin	3.0
51.54	Heptadecenyl phenol	0.4	63.18	Triterpenic ketone	2.6
51.86	Heptadecyl phenol	1.3	63.60	Amyrenone + Triterpene [496 (100%), 481 (21), 253 (18), 223 (48), 170 (70)]	13.6
51.96	Pentadecenyl resorcinol	1.0	63.87	Cycloartenol	12.0
52.03	Pentadecyl resorcinol	0.6	66.14	Amyrin acetate	2.3
53.85	Fatty acid 440	0.8		Monosaccharides (sum)	0.6
54.43	Heptadecadienyl resorcinol	3.5			

**Table 2.** GC-MS profile of methanol extract of Fouban propolis (silylated sample).

rT	Compound	% of TIC
38.65	Triterpenic ketone	0.7
40.19	Triterpenic ketone	1.1
40.73	Triterpenic ketone	3.3
41.14	Triterpenic ketone	1.9
41.96	$\beta$ -Amyrenone	17.3
42.95	$\alpha$ -Amyrenone	9.1
43.67	Triterpenic ketone (lanostane type)	31.2
44.01	Cycloartenol	5.4
44.52	Amyrine	10.0
46.21	$\beta$ -Amyrine acetate	1.5
48.09	$\alpha$ -Amyrine acetate	3.5
51.60	Triterpenic aldehyde	1.1

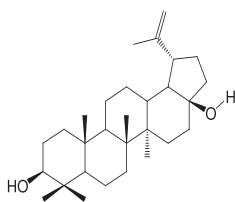
**Table 3.** GC-MS profile of hexane extract of Fouban propolis (silylated sample).

rT	Compound	% of TIC
23.34	Glycerol	13.3
30.67	Cinnamic acid	0.4
32.61	Hydroxybenzoic acid	0.6
37.27	Pinitol	3.7
41.16	Hexadecanoic acid (palmitic)	2.6
44.30	Octadecenoic acid	0.7
44.71	Octadecanoic acid (stearic)	0.6
47.98	Eicosanoic acid (arachidic)	2.3
51.03	Docosanoic acid (behenic)	2.2
54.38	Heptadecadienyl resorcinol	0.3
54.69	Heptadecenyl resorcinol	0.7
62.58	Triterpene [496 (100%), 481 (21), 253 (18), 223 (48), 170 (70)]	3.8
62.93	$\beta$ -amyrine	1.8
63.73	$\alpha$ -amyrine	2.6
63.79	Cycloartenol	3.7
	Monosaccharides (sum)	21.3
	Disaccharides (sum)	1.4

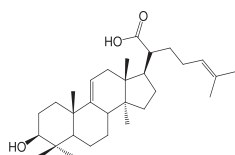
**Table 4.** IC<sub>50</sub> of DPPH<sup>•</sup> scavenging activity of some extracts and compounds.

Sample	IC <sub>50</sub> mg/mL
Hexane extract of Fouban propolis	5.60
Ethyl acetate extract of Fouban propolis	1.40
Methanol extract of Fouban propolis	1.07
Ethyl acetate extract of Ndian propolis	1.65
Hexane extract of Ndian propolis	4.00
1'-O-eicosanyl glycerol	0.93
3 $\beta$ -hydroxylanostan-9,24-dien-21-oic acid	1.22
Mangiferonic acid	1.09
Methyl-3 $\beta$ ,27-dihydroxycycloart-24-en-26-oate	0.98
Vitamin C	0.80
Gallic acid	0.30

acids, alkenyl phenols and resorcinols. Cameroonian propolis just like propolis from tropical areas, have shown rich triterpene profile unlike propolis from temperate regions such as France containing mainly aromatic acids and their esters, flavonoids and Sugars with only traces of triterpenes and acids (Popova et al. 2014). Quantitative analyses on propolis samples from Brazil and Poland indicate that it contains mainly phenolic compounds (Inui et al. 2014;



**Figure 1.** Betulin.

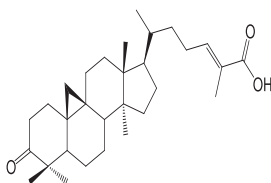


**Figure 2.** 3β-hydroxyylanostan-9,24-dien-21-oic acid.

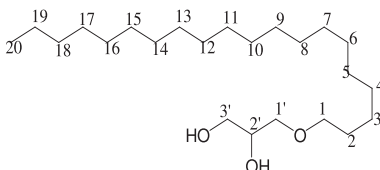
Socha et al. 2015). The chemical composition of propolis varies from one region to another depending on the local flora. In addition to the triterpenes, Cameroonian propolis is equally rich in fatty acids, alkenyl phenols and alkenyl resorcinols (Kardar et al. 2014; Zhang et al. 2014; Tamfu et al. 2016) which can this far be considered as characteristic in the GC-MS chemical profile of Cameroonian propolis.

The column chromatographic separation of two samples of propolis from two geographical region of Cameroon led to the isolation and characterisation of eight compounds. The structures of the compounds were established based on their spectroscopic data and by comparison with some data existing in literature. The structures of the known compounds were elucidated as; betulin, Figure 1 (Zheng-Fei et al. 2014), 3β-hydroxyylanostan-9,24-dien-21-oic acid, Figure 2 (Mosa et al. 2011), mangiferonic acid, **3** (Kardar et al. 2014), mixture of ambolic acid (Kardar et al. 2014) and stigmasterol (Chaturvedula & Indra 2012) Figures 5(a) and 5(b) respectively, 3β-hydroxycycloart-12,25(26)-diene, Figure 6 (Sakava et al. 2014) and 27-hydroxymangiferonic acid, Figure 7 (Tamfu et al. 2016).

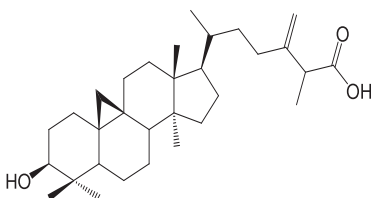
Compound **4**, whose structure is given as Figure 4 was isolated as a white powder and melted between 68 and 69.2 °C. It showed a pseudo-molecular ion peak  $[M + Na]^+$  at  $m/z$  395.4 on its ESI-TOF MS spectra (positive mode) and another diagnostic peak  $[2 M + Na]^+$  at  $m/z$  767.5 from which the molecular mass of compound 4 was deduced as 372.4 g/mol for  $C_{23}H_{48}O_3$  with a zero double bond equivalence. The  $^1H$  NMR (600.13 MHz) spectrum showed chemical shifts of oxymethine protons at  $\delta_H$  3.88, 3.55, 3.53 and 3.70 ppm. Thus, at  $\delta_H$  = 3.55 ppm (2H, t) and  $\delta_H$  = 3.88 ppm (2H, d) corresponding to the methylene protons of the aliphatic (H-1) and glycerol (H-1') moieties of the ether function, respectively. The peaks at  $\delta_H$  = 3.53 ppm (1H, m) and  $\delta_H$  = 3.67 ppm (2H, dd) are attributable to the oxymethine protons H-2' and H-3' of the glycerol moiety. The  $^{13}C$  NMR spectra, the three C-atoms of glycerol appear at  $\delta_C$  72.57 ppm, 71.88 ppm and 70.37 ppm attributable to C-1', C-2' and C-3', respectively. An aliphatic carbon atom C-1 linked to the oxygen atom of the ether function appears at  $\delta_C$  64.35 ppm while a terminal methyl of a long chain appears at  $\delta_C$  14.14 ppm. Summarily, the  $^{13}C$  NMR (150.92 MHz) spectra on reveals carbon atoms of the long aliphatic chain linked through an ether function to a molecule of glycerol as follows:



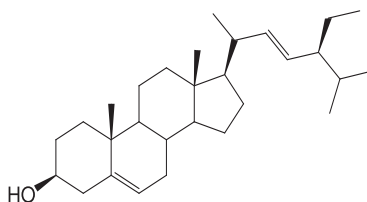
**Figure 3.** Mangiferonic acid.



**Figure 4.** 1'-O-eicosanyl glycerol.

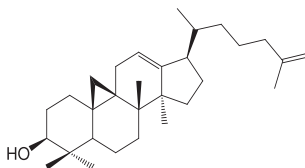


(a)



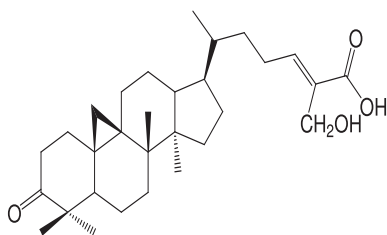
(b)

**Figure 5.** Ambolic acid (a) and stigmasterol (b).

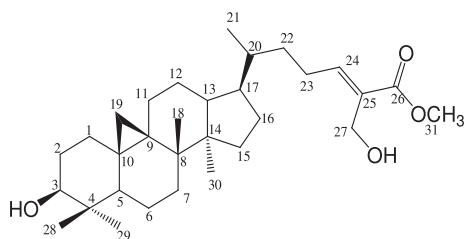


**Figure 6.** 3β-hydroxycycloart-12,25(26)-diene.

$\delta_c$  71.88 ppm (C-1'), 72.57 ppm (C-2'), 70.37 ppm (C-3'), 64.35 ppm (C-1), 31.95 ppm (C-2), between 29.72 and 29.77 ppm (C-3 to C-19), 14.14 ppm (C-20). The connectivities of aliphatic and glycerol moieties were elucidated as follows: HSQC ( $^1J_{H-C}$ ) showed correlation between



**Figure 7.** 27-hydroxymangiferonic acid.



**Figure 8.** Methyl-3β,27-dihydroxycycloart-24-en-26-oate.

$\delta_{\text{C}}$  14.14 ppm and  $\delta_{\text{H}}$  0.80 ppm;  $\delta_{\text{C}}$  29.72 ppm  $\delta_{\text{H}}$  1.20;  $\delta_{\text{C}}$  71.88 ppm and  $\delta_{\text{H}}$  3.88;  $\delta_{\text{C}}$  64.35 ppm and  $\delta_{\text{H}}$  3.55. HMBC showed correlations between C-19 and H-20, C-2 and H-3. Important COSY ( $^1\text{H}$ – $^1\text{H}$ ) correlations were also observed between the following pairs of protons: H-19 and H-20; H-1 and H-2; H-2 and H-3; H-1' and H-2'; H-2' and H-3' just to mention the major correlations.

Compound **8**, whose structure is given as Figure 8 was isolated as an amorphous white powder and melted between 167 and 169.8 °C. The ESI-TOF MS spectra of compound **8** showed a pseudo-molecular ion peak  $[\text{M} + 2\text{Na}]^+$  at  $m/z$  532.3 from where the molecular formula of the compound was deduced as  $\text{C}_{31}\text{H}_{50}\text{O}_4$ . The  $^1\text{H}$  NMR (500 MHz) spectra of compound **8** showed characteristic peaks some of which are similar to those of mangiferolic acid. That is, at  $\delta_{\text{H}}$  6.80 ppm (1H, t) characteristic of a conjugated olefin corresponding to the methylene proton H-24,  $\delta_{\text{H}}$  4.20 ppm (2H, brs) attributable to the protons of the allylic carbon atom bonded to an oxygen atom (H-27), at  $\delta_{\text{H}}$  3.05 ppm a triplet (1H, t) attributable to the oxymethine proton in a  $\alpha$ -position with the hydroxyl in  $\beta$ -position H-3. A set of AB doublets at  $\delta_{\text{H}}$  0.15 ppm (1H, d) and 0.35 ppm (1H, d) characteristic of a cyclopropane methylene protons  $\text{H}_{\alpha}$ -19 and  $\text{H}_{\beta}$ -19, respectively, allylic methylene protons at  $\delta_{\text{H}}$  2.10 ppm (1H, m) and 2.25 ppm (1H, m) corresponding to  $\text{H}_{\alpha}$ -23 and  $\text{H}_{\beta}$ -23 and finally signals of five tertiary methyls  $\delta_{\text{H}}$  0.67 ppm (3H, s) H-29, 0.70 ppm (3H, s) H-30, 0.75 ppm (3H, d) H-21, 0.80 ppm (3H, s) H-28 and 0.82 ppm (3H, s) H-18 corresponding to the five angular methyl groups. In addition to these, was a broad singlet of 3H at 3.24 ppm attributable to methoxyl protons H-31.  $^{13}\text{C}$  NMR (125 MHz) spectrum compound **8** exhibited the following characteristic signal; five methyls  $\delta_{\text{C}}$  14.73 ppm (C-30), 18.65 ppm (C-18), 19.84 ppm (C-21), 21.14 ppm (C-29), 26.08 ppm (C-28), a cyclopropane methylene  $\delta_{\text{C}}$  29.19 ppm (C-19), two olefinic carbons  $\delta_{\text{C}}$  132.70 ppm (C-25) and 148.21 ppm (C-24), an  $\alpha\beta$ -unsaturated carbonyl ester carbon  $\delta_{\text{C}}$  170.17 ppm (C-26), a hydroxyl methylene at  $\delta_{\text{C}}$  79.56 ppm (C-3), an alkoxyl carbon at 48.84 ppm (C-31) confirming the presence of a methyl ester seen on the  $^1\text{H}$  NMR and finally

the oxymethylene at  $\delta_c$  56.67 ppm (C-27). These  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data closely resembled those of 28-hydroxymangiferonic acid except for the appearance of the signals due to the allylic oxymethylene ( $\delta_H$  4.20 ppm, 2H, brs, H-27;  $\delta_c$  56.67 ppm, C-27) and the methoxyl group of methylester ( $\delta_H$  3.24 ppm, 3H, brs, H-31;  $\delta_c$  48.84 ppm, C-31). The hydroxyl group bonded to the allylic methylene C-27 was confirmed on the basis of HMBC correlations between H and 27 ( $\delta_H$  4.20 ppm, 2H) with the two olefinic carbons at  $\delta_c$  148.21 ppm (C-24) and 132.70 ppm (C-25) and the methyl esterified carbonyl carbon at  $\delta_c$  170.17 ppm (C-26). Also, HSQC revealed a correlation between the broad singlet at  $\delta_H$  3.24 ppm H-31 and the carbon atom at 48.88 ppm C-31. Other HMBC correlations appeared between  $\delta_c$  79.56 ppm C-3 and the methyl protons at  $\delta_H$  0.67 ppm (3H, s) H-29 and  $\delta_H$  0.80 ppm (3H, s) H-28. Important COSY ( $^1\text{H}$ – $^1\text{H}$ ) correlations were also observed between the following pairs of protons: H-3 with H-2; H-21 with H-20; H-22 with  $\text{H}_\alpha$ -23 and  $\text{H}_\beta$ -23;  $\text{H}_\alpha$ -19 and  $\text{H}_\beta$ -19 amongst others.

It is accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability. Hence, DPPH radical was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H (Kumazawa et al. 2004). A comprehensive means of interpreting radical scavenging activity of each extract was to determine the  $\text{IC}_{50}$  of each extract which is the concentration at which the extract will have a percentage radical scavenging activity of 50. The  $\text{IC}_{50}$  is inversely proportional to % radical scavenging activity. Therefore, the order of decreasing radical scavenging activity is: hexane extract of Fouban propolis ( $\text{IC}_{50}$  = 5.6 mg/mL), hexane extract of Ndian propolis ( $\text{IC}_{50}$  = 4.00 mg/mL), ethyl acetate extract of Ndian propolis ( $\text{IC}_{50}$  = 1.65 mg/mL), ethyl acetate extract of Fouban propolis ( $\text{IC}_{50}$  = 1.40 mg/mL), 3 $\beta$ -hydroxyloganostan-9,24-dien-21-oic acid ( $\text{IC}_{50}$  = 1.22 mg/mL) methanol extract of Fouban propolis ( $\text{IC}_{50}$  = 1.07 mg/mL), mangiferonic acid ( $\text{IC}_{50}$  = 1.09 mg/mL) methyl-3 $\beta$ ,27-dihydroxycycloart-24-en-26-oate (0.98 mg/mL) 1'-O-eicosanyl glycerol ( $\text{IC}_{50}$  = 0.93 mg/mL), vitamin C ( $\text{IC}_{50}$  = 0.80 mg/mL) and gallic acid ( $\text{IC}_{50}$  = 0.30 mg/mL) as shown in Table 4. Although none of the samples showed antiradical activity greater than that of the standards, their activities remained nevertheless closer to those of the standard antioxidants gallic acid and vitamin C. It is observed that the pure compounds showed higher DPPH radical scavenging activity than the extracts except for methanol extract of Fouban propolis that was more active than mangiferonic acid and 3 $\beta$ -hydroxyloganostan-9,24-dien-21-oic acid. Socha and co-workers investigated the antioxidant activities of propolis samples from Poland and found the samples to possess antiradical activity which was highest in samples with higher phenolic and flavonoid contents. The antiradical activity measured towards DPPH radical varied from 1.92 to 2.69 mM TE/g and showed significant correlation with total phenolic and flavonoid contents (Socha et al. 2015). This difference with our results can be attributed to the difference in chemical compositions of the propolis samples. Although our extracts are void of phenolic compounds which are known antiradical agents, their activity might be attributed to triterpenes and alkenyl phenols and resorcinols they contain.

### 3. Experimental

#### 3.1. Extraction

About 550 g of propolis from Ndian, south-west region of Cameroon which was collected in February 2015 was chilled and ground. The powder was then extracted with a 10-folds



volume of 70% ethanol at room temperature for 48 h. This process was repeated three times to yield a hydro-alcoholic solution of the propolis which was concentrated to near dryness to give the ethanol extract. The ethanol extract was extracted successively by liquid–liquid extraction with hexane (3 times) and ethyl acetate (3 times) to obtain a hexane extract of Ndian propolis (70.1 g) and ethyl acetate extract of Ndian propolis (65 g), respectively. This process was repeated for 1 kg of propolis of Fouban, west region of Cameroon and gave hexane extract of Fouban propolis (362 g), ethyl acetate extract of Fouban propolis (150 g) and methanol extract of Fouban propolis (130 g).

### 3.2. GC-MS analysis

The GC–MS analysis was performed with a Hewlett–Packard gas chromatograph 5890 series II Plus linked to a Hewlett–Packard 5972 mass spectrometer system equipped with a 30 m long, 0.25 mm i.d. and 0.5- $\mu$ m film thickness HP5-MS capillary column. The temperature was programmed from 60 to 300 °C at a rate of 5 °C/min, and a 10 min hold at 300 °C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:10, the injector temperature 280 °C, the interface temperature 300 °C and the ionisation voltage 70 eV, as described elsewhere (Popova et al. 2014; Tamfu et al. 2016).

### 3.3. Evaluation of antiradical activity on DPPH<sup>•</sup>

Anti-radical is based on the decrease in the absorbance when the 2,2'-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical is reduced at 517 nm. This was done according the method described by Talla and co-workers in 2014 (Talla et al. 2014).

### 3.4. Isolation of secondary metabolites

About 25 g of the ethanol extract of propolis from Fouban was subjected to column chromatography using silica gel using hexane-ethyl acetate (0–100%) then ethyl acetate-MeOH (0–60%) gradient system to afford 110 fractions which were later grouped into 7 pooled fractions (A–G) based on their TLC profiles. Fraction A (2 g) was purified on a Chromatographic column using hexane-ethyl acetate (5–10%) gradient system to betulin (Figure 1, 8 mg,  $R_f$  = 0.7). Fraction B (400 mg) crystallised into a whitish powder and was filtered out as 3 $\beta$ -hydroxylanostan-9,24-dien-21-oic acid (Figure 2, 65 mg). Fraction C (5 g) was purified by column chromatography using hexane-ethyl acetate (80/20, v/v) gradient system and afforded a mixture of a fatty acid ester and fatty alcohol. Further column chromatography of fraction D (0.5 g) using hexane-ethyl acetate (40/60, v/v) gave 3-oxo-cycloart-24-en-26-oic acid (Figure 3, 63 mg,  $R_f$  = 0.5) and finally, Fraction E (1.5 g) was purified by column chromatography using hexane-ethyl acetate (20/80, v/v) gradient system and afforded 1-O-eicosanyl glycerol (Figure 4, 30 mg,  $R_f$  = 0.2).

About 35 g of hexane extract of propolis from Ndian were subjected to column chromatographic separation with silica gel as absorbent using hexane-ethyl acetate (0–100%) and ethyl acetate-MeOH (0–20%) to give 302 fractions which were regrouped into 5 major fractions (A–E) based on their TLC profiles. Successive column Chromatographies using silica gel gave the following compounds from some of the fractions with their respective eluent systems as follows: fraction A (8.2 g) on a gradient system of hexane-ethyl acetate (95/5, v/v)

yielded a mixture of 3 $\beta$ -hydroxy-24-methylenecycloartan-26-oic acid and  $\beta$ -sitosterol (Figures 5(a) and 5(b), 17 mg). Fraction C (5.3 g) upon purification using a gradient system of hexane-ethyl acetate (40/60, v/v) gave 3-oxo-cycloart-24-en-26-oic acid (Figure 3, 13.8 mg).

About 35 g of ethyl acetate extract of propolis from Ndian were subjected to column chromatographic separation with silica gel as absorbent using hexane-ethyl acetate (20–100%) and ethyl acetate-MeOH (0–100%) to give 531 fractions. Fractions 1–318 were regrouped into 7 major fractions (I–VII) based on their TLC profiles. The fractions were each submitted to successive column chromatographies using silica gel and gave the following compounds from some of the fractions with their respective eluent systems as follows: Fraction I afforded 3 $\beta$ -hydroxycycloartan-12,24(25)-diene (Figure 6, 104 mg) on a gradient system of hexane-ethyl acetate (50/50, v/v). Fraction IV (12.3 g) yielded 3-oxo-27-hydroxycycloart-24-en-26-oic acid (Figure 7, 169 mg) on a gradient system of hexane-ethyl acetate (30/70, v/v). Finally, fraction V (2.5 g) on a gradient system of hexane-ethyl acetate (20/80, v/v) led to the isolation of 3 $\beta$ ,27-dihydroxycycloart-24-en-26-oic acid methyl ester (Figure 8, 28.6 mg). The structures of the compounds were established based on their 1D and 2D NMR spectra that is  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ - $^1\text{H}$  COSYqf45, HSQC and HMBC which were recorded on a Bruker AV500 and AV600 spectrometer (500 or 600 MHz for  $^1\text{H}$  and 125 or 150 MHz for  $^{13}\text{C}$ ) with TMS as internal standard and chemical shifts expressed in parts per million. ESI-MS spectra were measured on a Q-TOF Ultima spectrometer with an ionisation voltage of 3Kv. Their melting points were recorded on an Electrothermal 9100 device and are uncorrected.

### 3.5. NMR data of compounds 4 and 8 (see spectra in supplementary material)

#### 3.5.1. Methyl-3 $\beta$ ,27-dihydroxycycloart-24-en-26-oate (8)

$^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{C}}$  31.05 (C-1), 30.79 (C-2), 79.56 (C-3), 40.64 (C-4), 48.50 (C-5), 21.14 (C-6), 27.24 (C-7), 50.03 (C-8), 19.84 (C-9), 26.41 (C-10), 27.41 (C-11), 33.26 (C-12), 46.55 (C-13), 50.11 (C-14), 38.47 (C-15), 29.59 (C-16), 53.54 (C-17), 18.65 (C-18), 29.19 (C-19), 36.68 (C-20), 19.84 (C-21), 34.17 (C-22), 26.08 (C-23), 148.21 (C-24), 132.70 (C-25), 170.17 (C-26), 56.67 (C-27), 26.08 (C-28), 21.14 (C-29), 14.73 (C-30), 48.84 (C-31).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  1.57 (m, H-1), 1.75 (m, H-2), 3.05 (t, H-3), 1.30 (m, H-5), 1.75 (m, H-6), 1.33 (m, H-7), 1.45 (m, H-8), 1.85 (m, H-11), 1.62 (m, H-12), 1.25 (m, H-15), 1.88 (m, H-16), 1.65 (m, H-17), 0.82 (s, H-18), 0.15 & 0.35 (d, H-19), 1.42 (m, H-20), 0.75 (d, H-21), 1.67 (C-22), 2.10 & 2.25 (m, H-23), 6.80 (t, H-24), 4.20 (brs, H-27), 0.80 (s, H-28), 0.67 (s, H-29), 0.70 (s, H-30), 3.24 (brs, H-31).

#### 3.5.2. 1'-O-eicosanyl glycerol (4)

$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  64.35 (C-1), 31.95 (C-2), 29.77 (C-3), 29.76 – 29.72 (C-4 to C-19), 14.14 (C-20), 72.57 (C-1'), 71.88 (C-2'), 70.37 (C-3').  $^1\text{H}$  NMR (600.13 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  3.55 (t, 2H, H-1), 1.55 (m, 1H, H-2), 1.25–1.30 (brm, H-3 to H-18), 1.20 (m, 2H, H-19), 0.80 (t, 3H, H-20), 3.88 (m, H-1'), 3.55 (m, H-2'), 3.67 (dd, 2H, H-3').

## 4. Conclusion

The GC-MS chemical profiles of some propolis samples showed that they are rich in triterpenes, fatty acids, alkenyl phenols and resorcinols. Some triterpenes were isolated by column chromatography and their antiradical activity on DPPH tested alongside that of the extracts.

Some tested samples showed moderate anti-radical activity against DPPH radical acting as free radical terminators, hence can cause reduction in risk of several chronic diseases thereby increasing their importance. Further purification of the propolis extracts will be done with the aim of obtaining more compounds and testing their antioxidant activities using various methods. Hopefully, this work will attract the attention of scientists to further research on propolis.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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