Novel Triterpenes and Bioactive Compounds Isolated from *Smilax canariensis* Brouss

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Article

Novel Triterpenes and Bioactive Compounds Isolated from *Smilax canariensis* Brouss

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Abstract: The aerial parts of *Smilax canariensis Brouss.*, an endemic plant species of the Canary Islands and Madeira, were chemically investigated, resulting in the isolation of multiple known and novel compounds. These include three known flavonol glycosides: quercetin-3-*O*-rutinoside, rutin (**7**), quercetin-3-*O*-rutinoside decaacetate (**7a**), kaempferol-3-*O*-rutinoside nonaacetate, nicotiflorin acetate (**8**), 1-*O*-*p*-coumaroylglycerol triacetate (**10**) and *trans*-resveratrol (**9**). Additionally, a new sterol, 24,24- dimethy-5α-cholesta-7, 25-dien-3-one (**1**), and two novel dammarane–type triterpenes, 24-hydroxy-24-methyl-dammara-20,25-dien-3-one (**2**) and 3-acetyl-25-methyl-dammara-20,24-diene (**3**), were identified. In addition, stigmasterol, sitosterol and stigmast-4-en-3-one (**4**) were obtained. The structural elucidation of these compounds was achieved via 1D and 2D NMR spectroscopy, mass spectrometry, and comparison with literature data. This study provides the first phytochemical profile of *S. canariensis* and highlights its potential as a source of bioactive compounds for pharmacological applications.

Keywords: *Smilax canariensis* Brouss.; flavonol glycosides; triterpenes; sterols

1. Introduction

The genus *Smilax* (Liliaceae) comprises over 350 species distributed widely across tropical and temperate regions, with notable applications in traditional medicine, particularly in East Asia and North America [1]. Many of them have long been used as medicinal herbs, especially as traditional Chinese medicines in China [2], for their diuretic, laxative, depurative, and hypoglycemic properties, whereas only four species are found in Europe: *Smilax aspera*, located in the Mediterranean basin; *Smilax azorica*, native to the Azores Islands; *Smilax excelsa*, in the Black Sea and Caspian Sea region; and *Smilax canariensis* Brouss. ex Willd, endemic to the Canary Islands and Madeira, which is the subject of our study.

Morphologically, S. *canariensis* is characterized by leaves with 3 to 5 nerves and a simple umbel inflorescence. It is an evergreen, lauriform plant that is narrower at the ends of the older branches, and there are few or no thorns on the older stems. Notably, when a fruit ripens, it acquires a dark color.

Traditional medicine in the Canary Islands has extensively utilized endemic plants, including *S. canariensis*, commonly known as "zarzaparrilla sin espinas." This plant is well regarded for its diuretic, laxative, depurative, and hypoglycemic properties. Despite these traditional uses, the phytochemical composition of *S. canariensis* has not been extensively studied, presenting a significant opportunity to explore its chemical constituents and potential bioactivities [2]. This study aims to address this gap by isolating and characterizing the secondary metabolites present in the aerial parts of this species, thereby uncovering potential pharmacologically active compounds and contributing

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to the understanding of their chemical diversity. To the best of our knowledge, this plant has not previously been the subject of phytochemical analysis.

2. Results and Discussion

Compound **1** was obtained as colorless needles, mp 138–140 °C. Its molecular formula was determined to be $C_{30}H_{48}O$ by its HRESIMS at m/z 447.3609 (calcd for $[C_{30}H_{48}O+Na]^+$, 447.3603), together with its ^{13}C NMR data. The ^1H NMR spectrum of **1** indicated two tertiary methyl protons at δ_H 0.59 and 0.80 ppm, one secondary methyl proton at δ_H 1.06 (d, J = 6.5 Hz) and an olefinic proton at δ_H 5.16 (br dd, J = 5.6 and 2.0 Hz), which are characteristic signals of H_3-18, H_3-19, H_3-30 and H-7 of **1** having a lophenol ring system [3]. The carbon signals (Table 1) due to the ring systems of **1** were almost identical to those of **4** obtained after ketonization of 24(R)-ethyllophenol [4]. The presence of the ketone at C-3 was evident in the ^{13}C NMR spectrum since it showed a carbonyl signal at δ_C 210.5, which presented cross-peaks between the protons at C-1, C-2, and C-4 and the methyl group at C-4 in the HMBC spectrum. The structure of **1** was further elucidated by analysis of its HMBC spectrum (Figure 2). The C-18 and C-19 methyl groups were positioned at the ring junctions C-13 and C-10 on the basis of the HMBC correlations of H_3-18/C-12, C-13, C-14, and C-17 and H_3-19/C-1, C-5, C-9, and C-10, respectively.

The HMBC correlations placed the side chain at C-17 of the lophenol skeleton (Figure 1). This was evidenced by the methine proton (δ_H 1.20, m, H-17) correlating with carbons at δ_C 28.6 (C-16), 31.3 (C-22), 43.9 (C-13), and 55.5 (C-14), along with δ_C 19.6 (C-21). Furthermore, the methyl protons H3-21 (δ_H 0.99, d, J = 6.5 Hz) were strongly correlated with δ_C 56.6 (C-17), 37.5 (C-20), and 31.3 (C-22), confirming the structure of the side chain. In addition, the ^1H-NMR spectrum of **1** showed a vinyl methyl signal at δ_H 1.71 (3H, brs) and a terminal methylene proton at δ_H 4.85 and 4.88 (1H, each, brs, H-26a, b) due to an isopropenyl group and two tertiary methyl protons at δ_H 1.08 (6H, s, 28-H_3, 29-H_3). The cross peaks observed between the vinylic protons H-26 a, b and the carbon signals at δ_C 152.4 (C-25), 20.0 (C-27) and with the methyl protons at δ_H 1.08 (6H, s, 28-H_3, 29-H_3) and C-25, C-26 and C-23 were consistent with the placement of the extra two methyl groups at C-24. The NMR spectroscopic features of compound **1** were analogous to those of 4α,24,24-trimethyl-5α-cholesta-7,25-dien-3β-ol (24,24-dimethyl-25-dehydrolophenol) (**1a**), which was isolated as the acetyl derivative from the unsaponifiable lipid of *Clerodendrum inerme* [5], except that **1** had a keto carbon at δ_C 210.5, where **1a** had 3β-acetate; hence, **1a** was considered to have the structure 24,24- dimethy-5α-cholesta-7,25-dien-3-one.

Table 1. ^1H and ^{13}C NMR data of 24, 24-dimethyl-5α-cholesta-7, 25-dien-3-one (1).

Position	$^1H^a$	$^1H^b$	$^{13}C^a$	$^{13}C^b$
1a	2.05 m	1.13 m		
1b	1.25 m	1,74 m	39.8	39.8
2a	2.48 td (14.3, 5.7)	2.21 ddd (13.6, 3.9, 2.6)	38.3	38.3
2b	2.30 m	2.09 td (13.6, 5.6)		
3			213.4	210.5
4	2.13 m	1.88 m	45.8	45.9
5	1.44 m	1.73 m	50.5	50.5
6a	2.15 m	1.92 m	28.1	28.5
6b	1.90 m	1.54 m		
7	5.21 brs	5.16 br dd (5.6, 2.0)	117.4	118.4
8			139.4	139.3
9	1.75 m	1.48 m	49.5	49.8
10			35.4	35.5
11a	1.61 m	1.38 (2H, m)	22.1	22.1
11b	1.55 m			
12a	2.16 m	1.99 dt (12.5, 3.7)	40.1	40.2

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12b	1.48 m	1.13 m		
13			43.6	43.9
14	1.80 m	1.73 m	55.1	55.5
15a	1.53 m	1.55 m	23.7	23.7
15b	1.42 m	1.43 m		
16a	1.79 m	1.90 m	29.8	28.6
16b	1.27 m	1.28 m		
17	1.25 m	1.20 m	56.1	56.6
18	0.56 m	0.59 s	12.07	12.4
19	1.08 s	0.80 s	13.9	13.8
20	1.33 m	1.33 m	36.8	37.5
21	0.92 d (6.4)	0.99 d (6.5)	19.2	19.6
22a	1.24 m	1.33 m	30.6	31.3
22b	0.88 m	0.98 m		
23a	1.40 m	1.45 m	37.5	38.0
23b	1.20 m	1.23 m		
24			28.9	39.3
25			152.5	152.4
26a	4.73 brs	4.88 brs	109.5	110.6
26b	4.66 brs	4.85brs		
27	1.69 brs	1.71 brs	19.6	20.0
28	1.01[c]	1.06 d (6.5)	11.6	12.5
29	1.01 s	1.08 s	27.7	27.8
30	1.02 s	1.08 s	27.4	28.1

[a] $CDCl_3$, 600, 150 MHz. [b] C_6D_6, 600, 150 MHz. [c] Partially hidden.

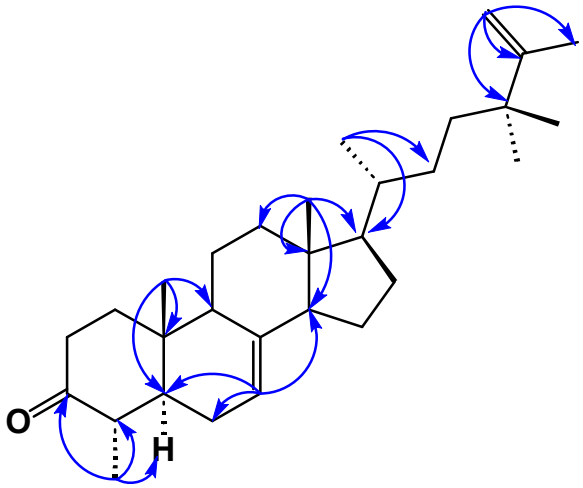

Figure 1. Key HMBC correlations of compound **1**.

Compound **2** was isolated as a viscous oil, and its molecular formula is $C_{31}H_{50}O_2$, as deduced from its positive HRESIMS (found $[M + Na]^+$ m/z 477.3706, calcd 477.3709) and ^{13}C NMR data, which are indicative of seven indices of hydrogen deficiency. The ^{13}C NMR (Table 2) data revealed 31 carbon resonances, which were classified by the HSQC spectrum as seven methyl, 12 methylene (two of them as terminal double bonds), four methine, and eight quaternary carbons, including two olefinic carbons at δ_C 153.8 (C-20) and 150.9 (C-25) and one carbonyl at δ_C 215.5 (C-3). Three of the implied six degrees of unsaturation were explained by multiple bonds, two by carbon–carbon double bonds and one by a carbon–oxygen double bond; consequently, **2** was a tetracyclic triterpene. Detailed analysis of the ^{13}C NMR data confirmed that compound **2** belongs to the dammarane-type triterpene class, characterized by the presence of an additional methyl group. The ^{1}H NMR spectrum exhibited seven

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distinct tertiary methyl signals at δ_H 0.70, 0.80, 1.64, 1.16, 0.99, 1.09, and 0.87 ppm (each integrating for three protons, singlets), which is consistent with this classification. These data corresponded to a dammarane triterpene skeleton similar to that of 24-methyldammara-20,25-dien-3-one isolated from *Copernicia prunifera* [6] and from *Cissus quadrangularis* [7]. The gross structure of **2** was deduced from its HMBC spectrum (Figure 2). The HMBC correlations between the tertiary methyl protons and their neighboring carbons were used to establish the dammarane backbone.

All expected correlations (Me-18→C-8, 7, 9, 14; Me-19→C-10, 1, 5, 9; and Me-30→C-14, 8, 13, 15) displayed strong cross-peaks. Furthermore, the HMBC spectrum placed the keto carbon at C-3 (Figure 2), from which the methyl proton at δ_H 0.99 (Me-29) correlated with the carbon at δ_C 28.7 (C-28), 47.6 (C-4), 55.5 (C-5) and 215.3 (C-3). The side chain was placed at C-17 of the main skeleton from the HMBC correlation of the methine proton at δ_H 2.31 (m, H-17), with the carbons at δ_C 29.9 (C-16), 29.5 (C-22), 46.2 (C-13), 40.9 (C-14), 108.1 (C-21) and 153.8 (C-20), and the terminal olefinic methylene protons at δ_H 4.97 brs and 4.93 brs (2H, H-21), with the carbons at δ_C 29.5 (C-22), 48.5 (C-17) and 153.8 (C-20). The HMBC experiments also confirmed the presence of a methyl group at δ_H 1.16 S, HSQC 28.7/28.68, and a methyl group on an oxygen-bearing carbon atom at C-24 (δ_C 75.38/75.32), since long-range couplings were observed between C-24 and the protons of the vinyl methyl group (H-27 at δ_H 1.651/1.639 brs), the terminal methylene (H-26a, δ_H 5.12 brd (1.3 Hz) and H-26b at δ_H 4.86 dd (2.7 and 1.3 Hz). The ^{13}C NMR spectrum of compound **2** showed splitting of the signals of certain carbons (Table 2). The splitting of these carbon signals is consistent with the presence of a mixture of C-24 epimers [8]. On the basis of this combined evidence, the structure of **2** is defined as a 24-epimer mixture of 24-hydroxy-24-methyldammara-20,25-dien-3-one.

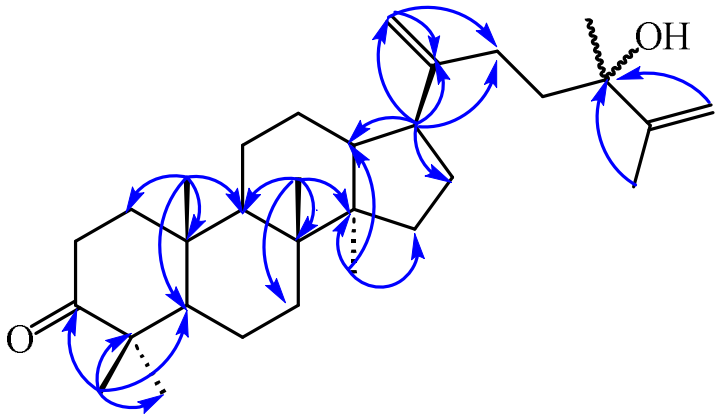

Figure 2. Key HMBC correlations of compound **2**.

Compound **3** was obtained as a white amorphous solid, mp (120–122 °C, hexanes). Its molecular formula was determined to be $C_{34}H_{56}O_2$ on the basis of its HRESIMS peak at m/z 519.4174 $[M+Na]^+$ (calcd for $C_{34}H_{56}O_2$, 519.4178). Its ^{1}H and ^{13}C NMR (Table 2) spectra were very similar to those of **2.** The structural difference between **3** and **2** was the functional group at C-3. The C-3 ketone in **2** was replaced by an acetoxy group in **3**, which was evidenced by the lack of a carbonyl signal, while acetoxy group signals at δ_H 2.05 (3H, s) and δ_C 21.39, 171.12 were present in the NMR spectrum of **3**. The ^{1}H-^{1}H COSY correlations between H-2 and H-3 (δ_H 4.49) and the HMBC correlations from H-3 to C-4 (δ_C 38.1) and the acetoxy carbonyl (δ_C 171.12) and from H3-28/H3-29 to C-3 (δ_C 80.9), C-4 (δ_C 38.1) and C-5 (δ_C 56.3) confirmed the above assignment (Figure 3). The large coupling constant of H-3 (dd, J =10.7, 3.0 Hz) suggested that the acetoxyl group at C-3 was β-oriented. The ^{1}H NMR spectrum of **3** also showed side chain signals at δ_H 1.08 (9H, s, H-26, H-27 and H-32) and at δ_H 4.88 and 4.71 (each 1H, brs, H2-31). The side chain structure was corroborated through HMBC correlations, including signals from H-21a (δ_H 4.76, br s) and H-21b (δ_H 4.74, br s) with carbons at δ_C 17 and δ_C 20. The deshielded t-butyl singlet at δ_H 1.08 further indicated the presence of an additional methyl group

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attached to C-25 and linked to the double bond. These signals were consistent with a 24-methylene-25-methyl-side chain [9]. The combined data suggested that compound **3** was 25-methyldammara-20, 24-diene-3-β-yl-acetate.

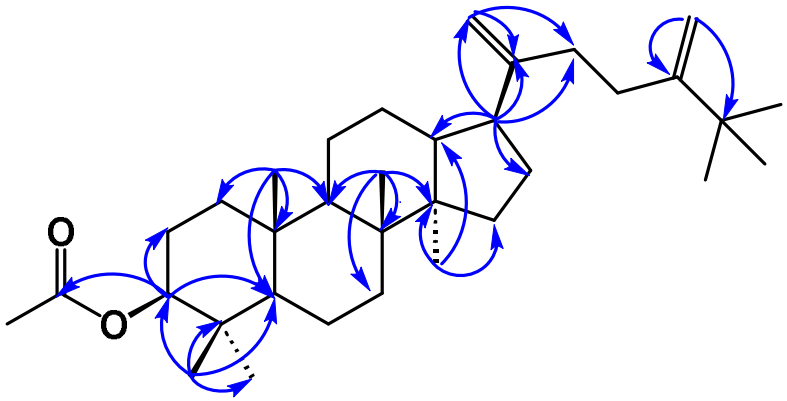

Figure 3. Key HMBC correlations of compound **3**.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of 24-hydroxy-24-methyl-dammara-20, 25-dien-3-one **2** and 3-acetyl-25-methyl-dammara-20, 24-diene **3,** in $CDCl_3$.

	δ_H (*J* values are given in Hz)		δ_C	
atom/position	(**2**)	(**3**)	(**2**)	(**3**)
H-1a	1.87 m	1.71 m	40.1	
H-1b	0.95 m	1.06 m		38.7
H-2a	2.27 m	1.64 m	34.4	
H-2b	2.23 m	1.64 m		23.9
H-3	C-3	4.49 d d (10.7, 5.5)	215.5	80.9
			47.6	38.1
H-5	1.10 m	0.85 m	55.6	56.3
H-6a	1.31 m	1.51 m	20.1	
H-6b	1.22 m	1.46 t d (12.8, 3.0)		18.1
H-7a	1.40 m	1.59 m	35.3	
H-7b	1.14 m	1.28 m		35.4
			40.7	40.5
H-9	1.15 m	1.33 dd (12.3, 3.0)	50.7	50.9
			37.2	37.1
H-11a	1.17 m	1.52 m	22.35/22.33[a]	
H-11a	1.05 m	1.20 m		21.3
H-12a	1.56 (2H, m)	1.58 m	25.6	
H-12b	1.56 (2H, m)	1.08 m		25.0
H-13	1.79 m	1.67 m	46.26/46.07[a]	45.6
			49.9	49.5
H15a	1.60 m	1.60 m	31.96/31.94[a]	
H-15b	1.08 m	1.11 m		31.3
H-16a	1.99 m	1.93 m		29.2

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H16b	1.57 m	1.41 m	29.9/29.8[a]	
H-17	2.31 m	2.23 t d (10.6, 6.8)	48.58/48.47[a]	48.06
Me 18	0.80 brs	0.98 s	16.3	15.82
Me-19	0.70 s	0.87 s	16.4	16.3
			153.86/153.81[a]	153.7
H-21a	4.97 brs	4.76 brs		
H-21b	4.93 m	4.74 brs	108.12/108.05[a]	107.61
H-22a	2.23 m	2.12 m		
H-22b	2.07 m	2.12 m	29.56/29.55[a]	34.1
H-23a	1.77 (2H, m)	2.18 m		
H-23b	1.77 (2H, m)	2.18 m	39.7/40.0[a]	30.3
			75.38/75.32[a]	158.3
			150.9	36.3
H-26a	5.12 brd (1.3)			
H-26b	4.86 dd (2.7, 1.3)	1.08 s	110.4	29.5
Me-27	1.651/1.639 brs	1.08 s	19.95	29.5
Me-28	1.16 s	0.86 s	28.71/28.68[a]	28.2
Me-29	0.99 s	0.85 s	21.5	16.5
Me-30	1.09 s	0.87 s	27.1	15.9
Me-31	1.16 s	4.88 br s H31a	28.7/28.68[a]	106.14
		4.71 brs H31b		
Me-32		1.08 s		29.5
Ac		2.05 (3H, s)		21.39, 171.12

[a] Value on the right corresponds to the minor isomer.

This study represents the first comprehensive phytochemical investigation of *Smilax canariensis*, leading to the identification of novel triterpenes and sterols, as well as several known compounds. These findings underscore the chemical and pharmacological potential of this endemic species, paving the way for further studies on its bioactive properties.

3. Experimental

3.1. General

Melting points were measured on a Reichert Thermovar apparatus without correction. Optical rotations were recorded on a Perkin Elmer 2H polarimeter equipped with a 1 dm cell. NMR spectra (^{1}H and ^{13}C) were obtained via Bruker Advance II 500 and Bruker Advance III 600 spectrometers in $CDCl_3$, C_6D_6, CD_3COCD_3, and C_5D_5N, with residual solvent signals serving as internal references, (δ_H 7.26; δ_C 76.7), (δ_H 7.16; δ_C 128.39), (δ_H 2.05, δ_C 29.92), and (δ_H 8.74, 7.58, 7.22; δ_C 150.35, 135.91, 123.87), respectively. The pulse conditions for 1D and 2D NMR were as follows [10]. Mass spectrometry (ESI-TOF and EI-MS) was performed using a Micromass LCT Premier XE and a Micromass Autospec instrument at 70 eV, respectively. Column chromatography was performed on Amberlite XAD 2 (Supelco XAD -2-3019), Sephadex LH-20 Pharmacia (ref. 17-0090-01), silica gel (Merck 2300-400 mesh), octadecyl-functionalized silica gel (Aldrich 377635-1006) and analytical TLC Merck Kieselget 60 F254. HPLC separations were carried out on a JASCO Pu-980 series pumping system equipped with a JASCO UV-975 detector and a Waters Kromasil Si 5 mm (10 × 250 mm) column. A Mackerey-Nagel VP 250/10 nucleodur Sphinx RP 5 μm column was used for HPLC–RP chromatography; chromatograms were visualized under UV light at 255 and 366 nm and/or sprayed with oleum

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followed by heating. All the solvents were distilled before use. For acetylations, dry phenolic material was dissolved in the minimum volume of pyridine. Two times the amount of acetic anhydride was added, and the mixture was left to stand overnight at ambient temperature. The mixture was then diluted with H_2O and extracted three times with ethyl acetate. The organic phase was evaporated at reduced pressure, and the residue was further purified by HPLC (SiO_2 column) using EtOAc-hexane as the eluent. NMR spectra are given in the Supplementary Material (Figures S1-S19).

3.2. Plant Material

The aerial parts of *Smilax canariensis Brouss. Ex Willd* were collected in April 2011 from Las Nieves (Velhoco) and Santa Cruz de La Palma (UTM coordinates: 228115/3177048). The plant was identified by Prof. Pedro Luis Pérez de Paz from the Department of Botany, Faculty of Pharmacy, University of La Laguna, where a voucher specimen (TFC: 49.941) has been deposited.

3.3. Extraction and Isolation of the Constituents

The fresh aerial parts of *Smilax canariensis* (7.5 kg) were finely divided and subjected to exhaustive extraction with ethanol at ambient temperature for two weeks. The resulting extract was filtered and concentrated under reduced pressure, yielding 240 g of brown residue. This residue was dissolved in two liters of distilled water and extracted with dichloromethane to yield 30.4 g of residue, followed by n-butanol extraction, which yielded 11 g of residue. The remaining aqueous layer was concentrated to yield 125 g of residue.

The dichloromethane extract (30.4 g) of *S. canariensis* was adsorbed onto silica gel and subjected to column chromatography on silica gel (Merck, 230–400 mesh, 300 g). The column was eluted with stepwise gradients of n-hexane and ethyl acetate, yielding 109 fractions (600 mL each). The evolution of this chromatography system was followed by the addition of CCF. Fractions $SH_{16–17}$ provided a white solid (24,24-dimethyl-5α-cholesta-7,25-dien-3-one) upon recrystallization in methanol, whereas subfractions $SH_{51–55}$ yielded a mixture of β-sitosterol and stigmasterol. Fractions SH_{1-15} (1.5 g) were eluted with pure n-hexane, which consists of a complex mixture of essential oils, and were not investigated further. Frs SH_{16-17} were purified by precipitation in MeOH, resulting in a white solid, which was identified as 24,24- dimethy-5α-cholesta-7,25-dien-3-one **1**. Fractions SH_{39-41} (152 mg) were purified via column chromatography over Sephadex LH-20 (hex:CH_2Cl_2:MeOH; 2:1:1) and an SHT column. Subfraction SHT_{5-6} was rechromatographed over a SiO_2 column and used as an eluent mixture of benzene–EtOAc, totaling 18 fractions, 10 mL each. Subfractions 8-9 yielded 17 mg of Stigmast-4-en-3-one **4,** which showed significant hypoglycemic activity [12].

Subfraction SHT_{8-9} (28 mg) was further purified on Sephadex LH-20 (hex:CH_2Cl_2:MeOH; 2:1:1) to yield 12 mg of 25-methyldammara-20, 24-diene-3-β-yl-acetate **3**. Fractions SH_{51-55} were eluted with a 7:3 hexane-EtOAc mixture, yielding 218 mg of colorless crystals with a melting point of 125 °C in acetone (lit. mp 130 °C) [11], identified as a 3:1 mixture (for the integral) of β-sitosterol **5** and stigmasterol **6,** respectively.

Fras SH_{58-67} (235 mg) were chromatographed on Sephadex LH-20 (hex:CH_2Cl_2:MeOH; 2:1:1) columns, and fractions of 15 mL each were collected. A total of 35 fractions were collected, which, according to their behavior in the CCF, were regrouped into SHS_{11-12} (25 mg) and SHS_{20-22} (14 mg). Subsequent purification of SHS_{20-22} by HPLC (hexane:ethyl acetate 9:1) allowed us to obtain the majority of the products (Tr = 40 min, flow rate of 2 mL min^{-1}), identified as ethyl *p*-hydroxybenzoate **11**. Subfractions SHS_{11-12} were obtained after chromatography via high-performance liquid chromatography (HPLC) (SiO_2, EtOAc-Hex 8:2 flow ratio of 2 mL min^{-1}, Tr 22 min), 12 mg of 24-hydroxy-24-methyl-dammara-20, 25-dien-3-one (**2**).

Fras SH_{89-97} (337 mg) were chromatographed on Sephadex LH-20 (hex:CH_2Cl_2:MeOH; 2:1:1, SHY column), and Subfracs SHY_{20-23} yielded 34 mg of a substance with strong UV absorption (R_f = 0.32, hexane: ethyl acetate 20%) at the melting point (114–116 °C, MeOH). This compound was identified from its physical and spectroscopic data as *p*-hydroxybenzaldehyde (**12**).

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A 5.5 g portion of the n-butanol extract was subjected to low-pressure chromatography on an RP C-18 column (7 × 40 cm) with a linear gradient of 40,500 ml H_2O–MeOH mixtures beginning with H_2O and ending with MeOH and an SM column. (Frs. SM_{20-24}, 740 mg) were rechromographed on Sephadex LH-20 (CH_2Cl_2:MeOH; 1:1, Sph column). Frs SpH_{8-9}, after precipitation in methanol/dichloromethane, yielded 16.5 mg of *trans*-resveratrol (**9**). Frs. SpH (22.5 mg) was purified by precipitation in a methanol–ethyl acetate mixture to yield rutin (**7**) (7.1 mg), and the mother liquor, which was composed of a complex mixture of flavonoids, was acetylated with acetic anhydride in pyridine for 12 h. After elimination of the solvent in vacuo, 12.5 mg of the crude product, which was rechromatographyed via HPLC (SiO_2, EtOAc–Hex 7:3, flow 2 mL min^{-1}), was obtained. Three pure products were obtained: quercetin-3-*O*-rutinose decaacetate (rutin acetate, **7a**, 3.7 mg, Tr: 20.2 min), kaempferol-3-*O*-rutinose nonaacetate (nicotiflorin acetate, **8**, 3.4 mg, Tr: 17.6 min) and 1-*O*-*p*-coumaroylglycerol triacetate (**10**), 2.8 mg (Tr: 9.76 min).

Chemical structures of all identified products are provided in Figure 4.

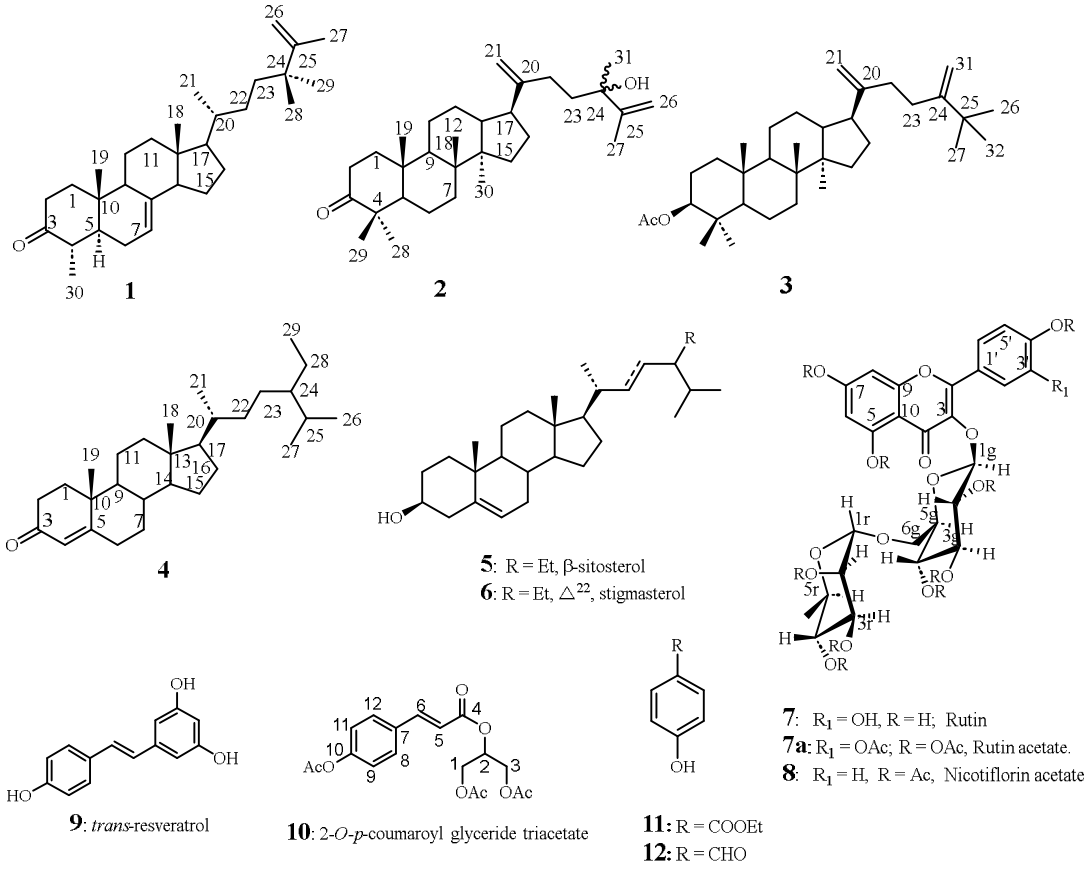

Figure 4. Structures of compounds **1-12**.

3.3.1. 24,24-Dimethyl-5α-cholesta-7,25-dien-3-one (1)

Colorless needles, mp 138–140 °C, MeOH. ^1H ^{13}C NMR (Table 1), HREI-MS, m/z 447.3610, (calcd for $C_{30}H_{48}O$, 447.3603) $[M+Na]^+$ (100%).

3.3.2. 24-Hydroxy-24-methyl-dammara-20,25-dien-3-one (2)

Viscous oil, ^1H ^{13}C NMR (Table 2), HRESIMS, m/z 477.3706, (calcd for $C_{31}H_{50}O_2$, 477.3709) $[M+Na]^+$.

3.3.3. 25-Methyldammara-20,24-diene-3-β-yl-acetate (3)

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Amorphous white solid, mp 120–122 °C, hexanes. ^{1}H ^{13}C NMR (Table 2), HRESIMS, m/z 519.4174, (calcd for $C_{34}H_{56}O_2$, 519.4178) $[M+Na]^+$.

3.3.4. Stigmast-4-en-3-one (4)

Amorphous solid, mp 77–80 °C (lit. mp 87–88 °C) [12], and for the ^{1}H ^{13}C NMR data, see Table S1 in the Supplementary Material. HREIMS mass spectrometry, showed the molecular ion peak at m/z 435.3592, (calcd for $C_{29}H_{48}O$, 435.3603) $[M+Na]^+$.

3.3.5. Quercetin-3-O-rutinoside, Rutin (7)

Compound 7 was isolated as a yellow solid with a melting point of 240 °C and presented a molecular formula of $C_{27}H_{30}O_{16}$, which was determined by high-resolution high-resolution high-efficiency high-efficiency high-efficiency high-energy high-energy mass spectrometry (HREIMS) mass spectrometry (HRMS), with a molecular ion peak at m/z 609.1460 (calcd for $C_{27}H_{29}O_{16}$, 609.1456) $[M-H]^+$. Peracetylation of **7** afforded the known rutin decaacetate **7a**; both were identified by MS and ^{1}H and ^{13}C NMR spectrometry (Kazuma et al., 2003; de Alcantara et al., 2023) [13,14].

3.3.6. Kaempherol-3-O-rutinosidenonaacetate, nicotiflorin acetate (8).

The molecular formula of $C_{45}H_{48}O_{24}$ was determined by high-resolution high-resolution high-energy ion mass spectrometry (HREIMS) mass spectrometry, which revealed a molecular ion peak at m/z 995.2460 (calcd for $C_{45}H_{48}O_{24}$+Na, 995.2433) $[M+Na]^+$. ^{1}H and ^{13}C NMR spectra were obtained (Jayasinghe et al., 2004) [15].

3.3.7. Trans-resveratrol (3,4′,5-trihydroxystilbene) (9).

Compound **9** was isolated as a yellow solid with a melting point of 254 °C (lit. mp 265–268 °C) [16]. The molecular formula $C_{14}H_{12}O_3$ was determined by high-resolution high-resolution high-energy ion mass spectrometry (HREIMS) mass spectrometry, which revealed a molecular ion peak at m/z 227.0711 (100%) (calcd for $C_{14}H_{11}O_3$, 227.0708) $[M-H]^+$. ^{1}H and ^{13}C NMR spectra were obtained (Šmidrkal et al., 2010) [16].

3.3.8. 2-O-p-Coumaroylglycerol triacetate (Juncusyl ester B triacetate) (10).

The molecular formula $C_{18}H_{20}O_8$ was determined by high-resolution high-resolution high-energy ion mass spectrometry (HREIMS) mass spectrometry, which revealed a molecular ion peak at m/z 387.1061 (100%) (calcd for $C_{18}H_{20}O_8$+Na, 387.1056) $[M+Na]^+$. ^{1}H NMR (C_6D_6, 500 MHz) δ: 4.33 (1H, dd, J = 12.06, 4.02 Hz, H_{1a}), 4.25 (1H, dd, J = 12.06, 4.16 Hz, H_{1b}), 5.38 (1H, dddd, J = 6.03, 5.89, 4.16 and 4.02 Hz, H_2), 4.20 (1H, dd, J = 11.92, 6.03 Hz, H_{3a}), 4.05 (1H, dd, J = 11.92, 5.89 Hz, H_{3b}), 6.39 (2H, d, J = 8.5 Hz, $H_{8,12}$), 6.11 (2H, d, J = 8.5 Hz, $H_{9,11}$), 1.70, 1.68, 1.62 (each 3H, s, Ac). ^{13}C NMR (C_6D_6, 125 MHz) δ: 62.97 C-1, 69.97 C-2, 62.82 C-3, 166.4 C-4, 118.11 C-5, 144.96 C-6, 132.29 C-7, 129.82 C-8, 12, 122.64 C-9, 11, 152.9 C-10, (20.81, 168.41; 20.81, 170.15; 20.49, 169.99) 3Ac. The signal assignments of H-1 and C-1 can be interchanged with those of H-3 and C-3.

3.3.9. Ethyl p-hydroxybenzoate (11).

The molecular formula $C_9H_9O_3$ was determined by high-resolution high-resolution high-energy ion mass spectrometry (HREIMS) mass spectrometry, which revealed a molecular ion peak at m/z 165.0548 (100%) (calcd for $C_9H_9O_3$, 165.0552) $[M]^+$. ^{1}H and ^{13}C NMR spectra were obtained (Fier et al., 2016) [17].

3.3.10. p-Hydroxybenzaldehyde (12).

Compound **12** was isolated as an amorphous solid with a melting point of 114–116 °C in MeOH (lit. mp 115–116 °C (from ethanol) [18]. The molecular formula $C_7H_5O_2$ was determined by high-

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resolution high-resolution high-energy ion mass spectrometry (HREIMS) mass spectrometry, which revealed a molecular ion peak at m/z 121.0293 (100%) (calcd for $C_7H_5O_2$, 121.0290) $[M-H]^+$. ^1H and ^{13}C NMR spectra were obtained (Kashparova et al., 2017) [18].

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