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Terpenoids and Steroids from the Roots of *Salvia blepharochlaena*

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From the roots of *Salvia blepharochlaena* Hedge and Hub. Mor. 4 triterpenoids, 4 steroids, 6 diterpenoids, and an aromatic ester were isolated. The structures of these compounds were established by spectroscopic methods. Formosanolide was isolated for the first time from the genus *Salvia*.

Key Words: *Salvia blepharochlaena* Hedge and Hub. Mor., Lamiaceae, triterpenoids, steroids, diterpenoids, aromatic ester.

Introduction

There are 87 *Salvia* species growing naturally in Turkey, half of which are endemic[1]. *Salvia* species are used in folk medicine all around the world, and have exhibited various biological activities such as antioxidant[2], antibacterial[3], antiviral[4], cytotoxic[5] and hypoglycemic properties[6]. *Salvia miltiorrhiza* Bunge has been used in China in the treatment of coronary heart diseases (angina pectoris and myocardial infraction)[7].

In a continuation of our investigations of Turkish *Salvia* species, we have now studied the roots of *Salvia blepharochlaena* Hedge and Hub. Mor. (Lamiaceae). In a previous study on this plant, 2 new (blephaein and 12-methylpisiferic acid methyl ester) and 8 known diterpenoids (pisiferic acid, 12-methylpisiferic acid, multicaulin, multiorthoquinone, 2-demethylmultiorthoquinone, ferruginol, horminone, and 7-acetylhorminone) were obtained and their structures were elucidated by spectral methods[8]. The aromatized nor-abietane diterpenes, multicaulin, multiorthoquinone and 2-demethylmultiorthoquinone, which were found to be potential antituberculous agents showing high activity against *Mycobacterium tuberculosis*, were first isolated from the roots of *Salvia multicaulis* by our group[9], and then from *Salvia blepharochlaena*[8]. In the latter study, some of these diterpenoids were further investigated for their antibacterial activity, and horminone, 7-acetylhorminone, 12-methylpisiferic acid and ferruginol were found to be more or less active against several

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standard bacteria[8]. Ferruginol and oleanolic acid 3-acetate were tested against a panel of cell lines in our previous study and both compounds were found to be active against human colon cancer with IC_{50} values of 9.7 μg/mL and 10.4 μg/mL, respectively[10]. Ferruginol was also found to be active against a mouse leukemia cell line (IC_{50}16.3 μg/mL). Two of the constituents of *Salvia blepharochlaena*, pisiferic acid and its methyl ether, have been previously isolated from several *Salvia* species[11]. Pisiferic acid was found to possess cytotoxic activity while its 12-methyl ether was considered an important defensive compound. Their antifungal activities were also investigated[12,13].

In the present study, 4 triterpenoids, 24-methylenecycloartanol (**1**)[14], erythrodiol-3-acetate (**2**)[15], α-amyrin (**3**)[16] and oleanolic acid-3-acetate (**4**)[17], 4 steroids, 3β-hydroxystigmast-5-en-7-one (**5**)[18], stigmast-4-en-3-one (**6**)[18], β-sitosterol (**7**)[18] and stigmasterol (**8**)[19], 6 diterpenoids, 8-hydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid 8,20-lactone (**9**)[20], sugiol (**10**)[21], 7,20-epoxyroyleanone (**11**)[22], formosanolide (**12**)[23], royleanone (**13**)[24] and cryptanol (**14**)[25], and an aromatic ester, 4,4′-bisbenzoic acid heptyl ester (**15**)[26], were isolated and their structures were determined using UV, IR, ^{1}H and ^{13}C NMR and MS spectral methods.

Experimental

General Experimental Procedures. The UV spectra (λ_{max}) were recorded on a Shimadzu UV-1601 in MeOH, IR spectra (ν_{max}) on a Perkin-Elmer Model 983 in $CHCl_3$, NMR spectra on a Varian Mercury-Vx 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR (TMS as an internal standard), and EIMS spectra on a ZabSpec mass (Micromass) spectrometer. Melting points were recorded on a Kofler apparatus (Reichert) and are uncorrected. Si gel columns (E. Merck, Art. 7734), and ready-made (silica gel 60 $PF_{254+366}$, E. Merck, Art. 7748, 1 mm thick) and ready-to-use plates (silica gel 60 F_{254}, E. Merck, Art. 5554, 0.25 mm thick) were used for chromatographic separations.

Plant Material. Dr. G. Kökdil and D. Karaca collected the roots of *Salvia blepharochlaena* in June, 1999, from Aktepe, Nevşehir at 1050 m altitude, and Prof. Dr. M. Vural (from Gazi University in Ankara) identified the plant. A voucher specimen was deposited in the Herbarium of the University of Ankara, Pharmacy Faculty (AEF 21128).

Extraction and Isolation. The extraction procedure for the plant material was given in our previous paper[8]. Fraction E (3.5 g), which was not investigated in the previous study and that showed antifungal activity against 3 yeasts (YCP galactose 51.8% , PRAD 52 galactose 64.6% and PRAD 52 glucose 48.1% at 100 μM), was applied on top of a chromatographic column (2.5 x 45 cm) and eluted with a CH_2Cl_2 gradient of EtOH followed up 100% to afford triterpenoids and steroids in addition to diterpenoids. Compounds were further purified using ready-made and ready-to-use plates. From Fraction E, 24-methylenecycloartanol (15 mg), erythrodiol-3-acetate (8 mg), α-amyrin (9 mg), oleanolic acid-3-acetate (7 mg), 3β-hydroxystigmast-5-en-7-one (5 mg), stigmast-4-en-3-one (13 mg), β-sitosterol (25 mg), stigmasterol (16 mg), 8-hydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid 8,20-lactone (20 mg), sugiol (15 mg), 7,20-epoxyroyleanone (3 mg), formosanolide (8 mg), royleanone (4 mg), cryptanol (5 mg), and 4,4′-bisbenzoic acid heptyl ester (15 mg) were isolated.

24-Methylenecycloartanol (1): UV (MeOH) λ_{max}: 204 nm; IR ($CHCl_3$) ν_{max}: 3445, 2916, 2848, 2360, 2341, 1653, 1472, 1462, 1215, 1061, 756, 668 cm^{-1}, EIMS (EI, 70 eV) m/z 440 $[M]^+$ (calc. for $C_{31}H_{52}O$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.29 (1H, m, H-3α), 0.33 (1H, d, J= 4.2 Hz, H-19), 0.55 (1H,

d, J= 4.2 Hz, H-19′), 4.66 (1H, br s, H-24), 4.72 (1H, br s, H-24′), 0.89 (3H, d, J= 6.9 Hz, Me-21), 1.02 (3H, d, J= 6.9 Hz, Me-26), 1.03 (3H, d, J= 6.9 Hz, Me-27), 0.90 (3H, s, Me-28), 0.97 (6H, s, Me-18 and Me-29), 0.81 (3H, s, Me-30). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 31.99 (C-1), 30.38 (C-2), 78.90 (C-3), 40.54 (C-4), 47.23 (C-5), 21.16 (C-6), 28.19 (C-7), 48.02 (C-8), 19.35 (C-9), 26.50 (C-10), 26.04 (C-11), 32.93 (C-12), 45.41 (C-13), 48.02 (C-14), 29.74 (C-15), 26.58 (C-16), 52.30 (C-17), 18.06 (C-18), 29.74 (C-19), 32.93 (C-20), 18.39 (C-21), 35.15 (C-22), 31.41 (C-23), 159.79 (C-24), 33.89 (C-25), 21.94 (C-26), 19.35 (C-27), 18.09 (C-28), 14.04 (C-29), 25.47 (C-30), 106.10 (C-31).

Erythrodiol-3-acetate (2): UV (MeOH) λ_{max}: 208 nm; IR ($CHCl_3$) ν_{max}: 3436, 1720, 1654, 1639, 1456, 1370, 1248 cm^{-1}, EIMS (EI, 70 eV) m/z 484 [M]$^+$ (calc. for $C_{32}H_{52}O_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.48 (1H, dd, J = 6.9 Hz, H-3α), 5.12 (1H, t, J = 3 Hz, H-12), 0.92 (3H, s, Me-23), 0.82 (3H, s, Me-24), 0.95 (3H, s, Me-25), 0.96 (3H, s, Me-26), 1.06 (3H, s, Me-27), 3.18 (1H, d, J = 11 Hz, H-28), 3.51 (1H, d, J = 11 Hz, H-28′), 0.97 (3H, s, Me-29), 1.12 (3H, s, Me-30), 2.02 (3H, s, 3-OAc).

α-Amyrin (3): M.p.= 186 °C, UV (MeOH) λ_{max}: 210 nm; IR ($CHCl_3$) ν_{max}: 3400, 1650, 1455, 1380, 1360 cm^{-1}, EIMS (EI, 70 eV) m/z 426 [M]$^+$ (calc. for $C_{30}H_{50}O$). ^{1}H (400 MHz, $CDCl_3$): δ_H 3.24 (1H, dd, J = 5, 10 Hz, H-3), 5.18 (1H, t, J = 2.5 Hz, H-12), 0.87 (3H, s, Me-23), 0.79 (3H, s, Me-24), 0.85 (3H, s, Me-25), 1.04 (3H, s, Me-26), 1.06 (3H, s, Me-27), 0.96 (3H, s, Me-28), 1.00 (3H, d, J = 6.5 Hz, Me-29), 1.02 (3H, d, J = 6.5 Hz, Me-30).

Oleanolic acid-3-acetate (4): UV (MeOH) λ_{max}: 218 nm; IR ($CHCl_3$) ν_{max}: 2500, 1725, 1690, 1645, 1450, 1382, 1260, 780 cm^{-1}, EIMS (EI, 70 eV) m/z498 [M]$^+$ (calc. for $C_{30}H_{50}O_4$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.63 (1H, t, J = 2.6 Hz, H-3α), 5.33 (1H, t, J = 2.6 Hz, H-12), 2.83 (1H, dd, J= 4, 13 Hz, H-18) 0.75 (3H, s, Me-23), 0.78 (3H, s, Me-24), 0.91 (3H, s, Me-25), 1.04 (3H, s, Me-26), 1.06 (3H, s, Me-27), 0.96 (3H, s, Me-28), 1.00 (3H, s, Me-29), 1.02 (3H, s, Me-30). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 38.1 (C-1), 23.6 (C-2), 81.0 (C-3), 37.7 (C-4), 55.4 (C-5), 18.2 (C-6), 32.6 (C-7), 39.4 (C-8), 47.6 (C-9), 37.0 (C-10), 23.4 (C-11), 122.6 (C-12), 143.6 (C-13), 41.6 (C-14), 27.7 (C-15), 22.9 (C-16), 46.6 (C-17), 41.0 (C-18), 45.7 (C-19), 30.7 (C-20), 33.6 (C-21), 32.5 (C-22), 28.1 (C-23), 17.2 (C-24), 15.2 (C-25), 16.7 (C-26), 25.9 (C-27), 184.0 (C-28), 33.1 (C-29), 23.6 (C-30), 21.3 (Ac), 171.1 (CO).

3β-Hydroxystigmast-5-en-7-one (5): UV (MeOH) λ_{max}: 240 nm; IR ($CHCl_3$) ν_{max}: 3440, 2990, 2850, 1680, 1480, 1395, 1285, 1080 cm^{-1}, EIMS (EI, 70 eV) m/z 428 [M]$^+$ (calc. for $C_{29}H_{48}O_2$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H3.69 (1H, m, H-3α), 5.70 (1H, d, J = 1.7 Hz, H-6), 0.69 (3H, s, Me-18), 1.20 (3H, s, Me-19), 0.93 (3H, d, J = 6.5 Hz, Me-21), 0.84 (3H, d, J = 6.5 Hz, Me-26), 0.82 (3H, d, J = 6.7 Hz, Me-27), 0.85 (3H, t, J = 7.1 Hz, Me-29). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 36.62 (C-1), 31.18 (C-2), 70.52 (C-3), 41.79 (C-4), 169.35 (C-5), 126.13 (C-6), 204.21 (C-7), 45.39 (C-8), 50.02 (C-9), 38.24 (C-10), 21.20 (C-11), 39.68 (C-12), 41.79 (C-13), 49.93 (C-14), 26.30 (C-15), 28.53 (C-16), 54.69 (C-17), 11.87 (C-18), 17.28 (C-19), 36.06 (C-20), 18.90 (C-21), 33.93 (C-22), 26.07 (C-23), 45.80 (C-24), 29.11 (C-25), 19.77 (C-26), 19.01 (C-27), 23.04 (C-28), 11.95 (C-29).

Stigmast-4-en-3-one (6): UV (MeOH) λ_{max}: 241 nm; IR ($CHCl_3$) ν_{max}: 2940, 1730, 1678, 1616, 1470, 1380, 1215, 1190, 863 cm^{-1}, EIMS (EI, 70 eV) m/z 412 [M]$^+$ (calc. for $C_{29}H_{48}O$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H5.74 (1H, d, J = 2.2 Hz, H-4), 0.72 (3H, s, Me-18), 1.19 (3H, s, Me-19), 0.93 (3H, d, J = 6.6 Hz, Me-21), 0.84 (3H, d, J = 6.8 Hz, Me-26), 0.82 (3H, d, J = 6.8 Hz, Me-27), 0.85 (3H, t, J = 7.2 Hz, Me-29). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 35.68 (C-1), 33.89 (C-2), 198.92 (C-3), 123.64 (C-4), 171.01

(C-5), 32.86 (C-6), 32.07 (C-7), 35.73 (C-8), 53.84 (C-9), 38.58 (C-10), 21.03 (C-11), 39.48 (C-12), 42.35 (C-13), 55.94 (C-14), 24.12 (C-15), 28.10 (C-16), 56.08 (C-17), 11.98 (C-18), 17.38 (C-19), 36.10 (C-20), 18.72 (C-21), 34.01 (C-22), 25.99 (C-23), 45.80 (C-24), 29.11 (C-25), 19.81 (C-26), 19.18 (C-27), 23.10 (C-28), 11.14 (C-29).

β-Sitosterol (7): M.p. = 138-139 °C, UV (MeOH) λ_{max}: 205 nm; IR ($CHCl_3$) ν_{max}: 3445, 3305, 2925, 2850, 1643, 1375, 1063, 955, 835 cm^{-1}, EIMS (EI, 70 eV) m/z 414 $[M]^+$ (calc. for $C_{29}H_{50}O$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 3.52 (1H, m, H-3), 5.35 (1H, m, H-6), 0.69 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.4 Hz, Me-21), 0.83 (3H, d, J = 6.8 Hz, Me-26), 0.81 (3H, d, J = 6.9 Hz, Me-27), 0.85 (3H, t, J = 7.8 Hz, Me-29). ^{13}C (100 MHz, $CDCl_3$): δ_C 37.33 (C-1), 31.63 (C-2), 71.73 (C-3), 42.20 (C-4), 140.71 (C-5), 121.63 (C-6), 31.96 (C-7), 31.81 (C-8), 51.13 (C-9), 36.43 (C-10), 21.09 (C-11), 39.79 (C-12), 42.37 (C-13), 56.75 (C-14), 24.15 (C-15), 28.25 (C-16), 56.02 (C-17), 11.84 (C-18), 19.46 (C-19), 36.07 (C-20), 18.68 (C-21), 33.95 (C-22), 26.10 (C-23), 45.82 (C-24), 29.15 (C-25), 19.77 (C-26), 19.21 (C-27), 23.13 (C-28), 11.04 (C-29).

Stigmasterol (8): UV (MeOH) λ_{max}: 208 nm; IR ($CHCl_3$) ν_{max}: 3422, 1654, 1455, 1380, 1360 cm^{-1}, EIMS (EI, 70 eV) m/z 412 $[M]^+$ (calc. for $C_{29}H_{48}O$). ^1H (400 MHz, CD_3COCD_3): δ_H3.40 (1H, m, H-3α), 5.30 (1H, d, J = 5 Hz, H-6), 0.71 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.95 (3H, d, J = 7 Hz, Me-21), 5.00 (1H, dd, J = 8, 14 Hz, H-22), 5.21 (1H, dd, J = 8, 14 Hz, H-23), 0.82 (3H, d, J = 7 Hz, Me-26), 0.83 (3H, d, J = 7 Hz, Me-27), 0.97 (3H, t, J = 7 Hz, Me-29).

8-Hydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid 8,20-lactone (9): UV (MeOH) λ_{max}: 245, 336 nm; IR (KBr) ν_{max}: 2920, 1785, 1690, 1640, 1390, 1370 cm^{-1}, EIMS (EI, 70 eV) m/z 315 $[M+1]^+$ (calc. for $C_{20}H_{26}O_3$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

Sugiol (10): M.p. = 265-269 °C, UV (MeOH) λ_{max}: 220, 233, 285 nm; IR ($CHCl_3$) ν_{max}: 3223, 2928, 2865, 2361, 2337, 1648, 1590, 1502, 1460, 1373, 1342, 1304, 1268, 1177, 1089, 906, 867 cm^{-1}, EIMS (EI, 70 eV) m/z 300 $[M]^+$ (calc. for $C_{20}H_{28}O_2$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

7,20-Epoxyroyleanone (11): UV (MeOH) λ_{max}: 216, 270, 400 nm; IR ($CHCl_3$) ν_{max}: 3380, 2960, 2880, 1665, 1657, 1640, 1600, 1580, 1460, 1390, 1380, 1250, 1160, 1130, 1100, 1080 cm^{-1}, EIMS (EI, 70 eV) m/z 330 $[M]^+$ (calc. for $C_{20}H_{26}O_4$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

Formosanolide (12): UV (MeOH) λ_{max}: 210, 281 nm; IR ($CHCl_3$) ν_{max}: 2954, 1742, 1582, 1491, 1459, 1282, 1203, 1106, 1091, 988, 734 cm^{-1}, EIMS (EI, 70 eV) m/z 328 $[M]^+$ (calc. for $C_{21}H_{28}O_3$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

Royleanone (13): M.p. = 180-182 °C, UV (MeOH) λ_{max}: 276, 405 nm; IR ($CHCl_3$) ν_{max}: 3380, 2970, 2880, 2860, 1683, 1640, 1610, 1466, 1402, 1384, 1368, 1318, 1300, 1253, 1168, 1132, 1108, 1019, 964, 909 cm^{-1}, EIMS (EI, 70 eV) m/z 316 $[M]^+$ (calc. for $C_{20}H_{28}O_3$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

Cryptanol (14): M.p. = 138-140 °C, UV (MeOH) λ_{max}: 274, 332 nm; IR ($CHCl_3$) ν_{max}: 3350, 2970, 2920, 2840, 1635, 1600, 1550, 1535, 1456, 1435, 1407, 1388, 1376, 1328, 1272, 1252, 1163, 1128, 1105, 958, 902, 879, 802 cm^{-1}, EIMS (EI, 70 eV) m/z 316 $[M]^+$ (calc. for $C_{20}H_{28}O_3$). ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR (see Tables 1 and 2).

Table 1. ^{1}H NMR data of isolated diterpenoids (**9-14**) (in $CDCl_3$, J in Hz).

H	**9**	**10**	**11**	**12**	**13**	**14**
1β	1.86 dd (12,4)	2.25 brdt (3,13)	2.68 dt (2,13)		2.76 brdt (3.5,14)	
1α	1.57 ddd (13,12,4)	1.56 m			1.14 dt (3.5,14)	
2β	2.16 ddddd (13,13,12,4,3)				1.73 dt (3,13.5)	
2α	1.60 ddd (13,4,3)				1.51 dq (3,13.5)	
3β	1.54 dd (12,3)				1.45 dt (3.5,14)	
3α	1.23 ddd (12,12,3)			1.20 m		
5	1.50 dd (12,5)			1.48 dd (10,6)	1.11 dd (1.5,12)	2.15 t (3)
6β	1.69 dddd (12,12,11,5)	2.52 dd (13,5,17.5)		2.25 ddd (13,6,4)	1.87 dd (7,13.5)	6.45 dd (3,10)
6α	1.93 ddd (11,6,5)	2.64 dd (3.5,17.5)		1.90 m	1.38 ddd (5.5, 12,13.5)	
7β	2.42 dd (13,5)	–		–	2.72 dd (5.5,21)	6.80 dd (3,10)
7α	1.46 ddd (13,12,6)	–	4.42 dd (1.5,4)	5.48 dd (4,1)	2.34 dd (21,7)	
11	5.92 s	6.70 s	–	6.73 s	–	
14	6.70 s	7.87 s	–	7.05 s	–	
15	2.97 sept. (7)	3.20 sept. (7)	3.16 sept. (7)	3.26 sept. (7)	3.15 sept. (7)	3.15 sept. (7)
16	1.03 d (7)	0.95 d (7)	1.22 d (7)	1.13 d (7)	1.23 d (7)	0.99 d (7)
17	1.07 d (7)	0.95 d (7)	1.18 d (7)	1.17 d (7)	1.20 d (7)	1.01 d (7)
18	0.98 s	1.22 s	0.92 s	0.83 s	0.91 s	0.95 s
19	0.92 s	1.22 s	0.88 s	0.91 s	0.94 s	0.92 s
20	–	1.22 s	3.73 d (7) 3.65 d (7)	–	1.24 s	1.02 s
OMe				3.81 s		

Table 2. ^{13}C NMR data of isolated diterpenoids (**9-14**) (in $CDCl_3$)*.

C	9	10	11	12	13	14
1	26.4	35.4	35.6	27.4	36.3	37.2
2	17.6	18.7	18.5	18.5	18.5	18.8
3	41.2	41.9	40.9	41.0	41.2	41.1
4	34.3	32.2	32.9	34.3	33.8	34.3
5	52.9	58.0	45.4	45.4	44.4	57.0
6	20.2	42.5	22.9	29.9	21.7	139.6
7	37.9	207.2	69.2	77.3	38.0	121.1
8	78.7	135.8	134.2	130.4	133.7	138.4
9	165.9	149.3	147.8	139.3	148.4	137.1
10	50.3	41.4	39.1	47.0	38.7	41.6
11	115.5	118.9	184.2	104.7	184.1	136.9
12	184.4	151.2	150.6	156.9	151.6	158.1
13	148.0	132.8	124.6	135.5	124.1	114.3
14	135.2	126.5	182.4	120.6	182.4	152.4
15	26.5	26.7	24.2	26.8	25.7	25.9
16	21.4	21.6	19.6	22.6	20.0	21.7
17	21.8	21.1	19.8	22.7	20.3	21.3
18	32.3	32.3	33.1	31.5	33.4	32.8
19	20.1	22.8	22.9	19.3	22.8	22.0
20	175.5	22.5	65.4	176.1	22.4	18.2
OMe				55.7		

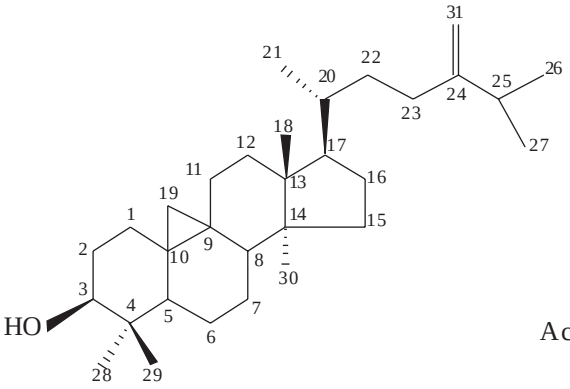
*Assignments were made based on APT experiments.

4,4′-Bisbenzoic acid heptyl ester (15): IR ($CHCl_3$) ν_{max}: 3050, 2960, 2925, 1735, 1610, 1600, 1520, 1480, 1390, 1260, 1180, 1110, 830, 720 cm^{-1}, EIMS (EI, 70 eV) m/z 438 $[M]^+$ (calc. for $C_{28}H_{38}O_4$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H7.08 (4H, d, J = 9 Hz, H-2, H-6 and H-2′, H-6′), 6.77 (4H, d, J = 9 Hz, H-3, H-5 and H-3′, H-5′), 4.22 (4H, t, J = 7 Hz, CH_2-8, CH_2-8′), 2.86 (4H, t, J = 7 Hz, CH_2-9, CH_2-9′), 2.28 (4H, t, J = 7 Hz, CH_2-10, CH_2-10′), 1.70 (4H, m, CH_2-11, CH_2-11′), 1.60 (4H, m, CH_2-12, CH_2-12′), 1.26 (4H, m, CH_2-13, CH_2-13′), 0.89 (6H, t, J = 7 Hz, Me-14, Me-14′).

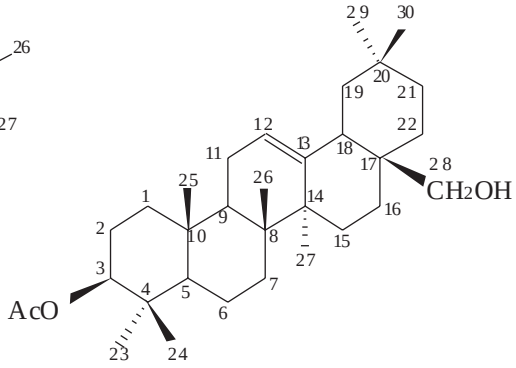
Results and Discussion

In this study, from the roots of *Salvia blepharochlaena* 24-methylenecycloartanol (**1**)[14], erythrodiol-3-acetate (**2**)[15], α-amyrin (**3**)[16], oleanolic acid-3-acetate (**4**)[17], 3β-hydroxystigmast-5-en-7-one (**5**)[18], stigmast-4-en-3-one (**6**)[18], β-sitosterol (**7**)[18], stigmasterol (**8**)[19] (see Figure 1), 8-hydroxy-12-oxo-abieta-9(11),13-dien-20-oic acid 8,20-lactone (**9**)[20], sugiol (**10**)[21], 7,20-epoxyroyleanone (**11**)[22], formosanolide (**12**)[23], royleanone (**13**)[24], cryptanol (**14**)[25], and 4,4′-bisbenzoic acid heptyl ester (**15**)[26] (see Figure 2) were obtained.

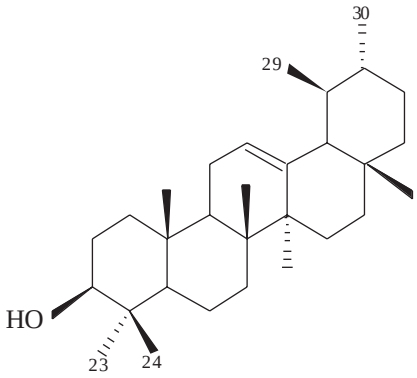
Spectroscopic methods (UV, IR, ^{1}H and ^{13}C NMR, and MS) were used for structure determination of these compounds and their spectral data were compared with those given in the literature [14–26]. TLC comparisons with authentic samples were also carried out. ^{1}H and ^{13}C NMR data of compounds **9-14** are given in Tables 1 and 2.



1



2



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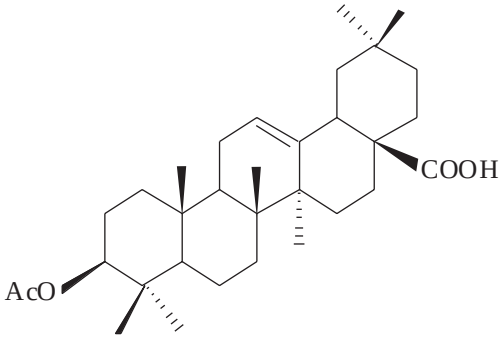

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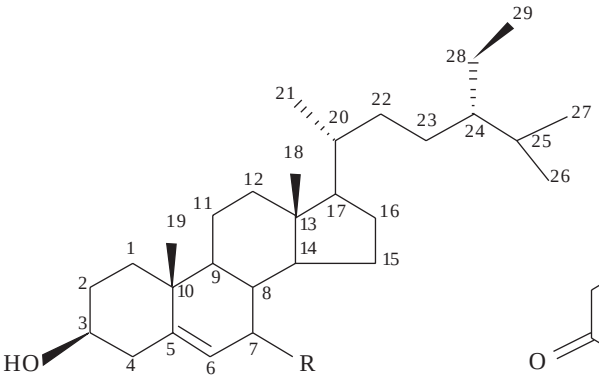
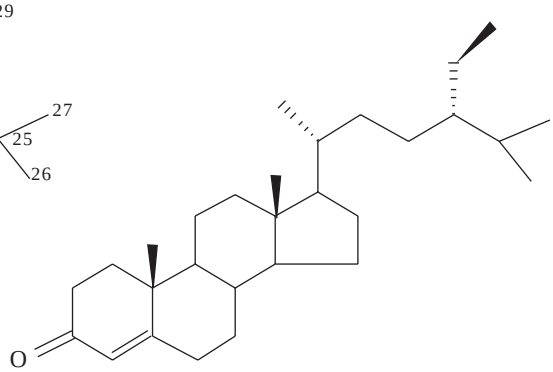


	R
5	=O
7	H
8	H, Δ^{22}



6

Figure 1. Isolated triterpenoids and steroids.

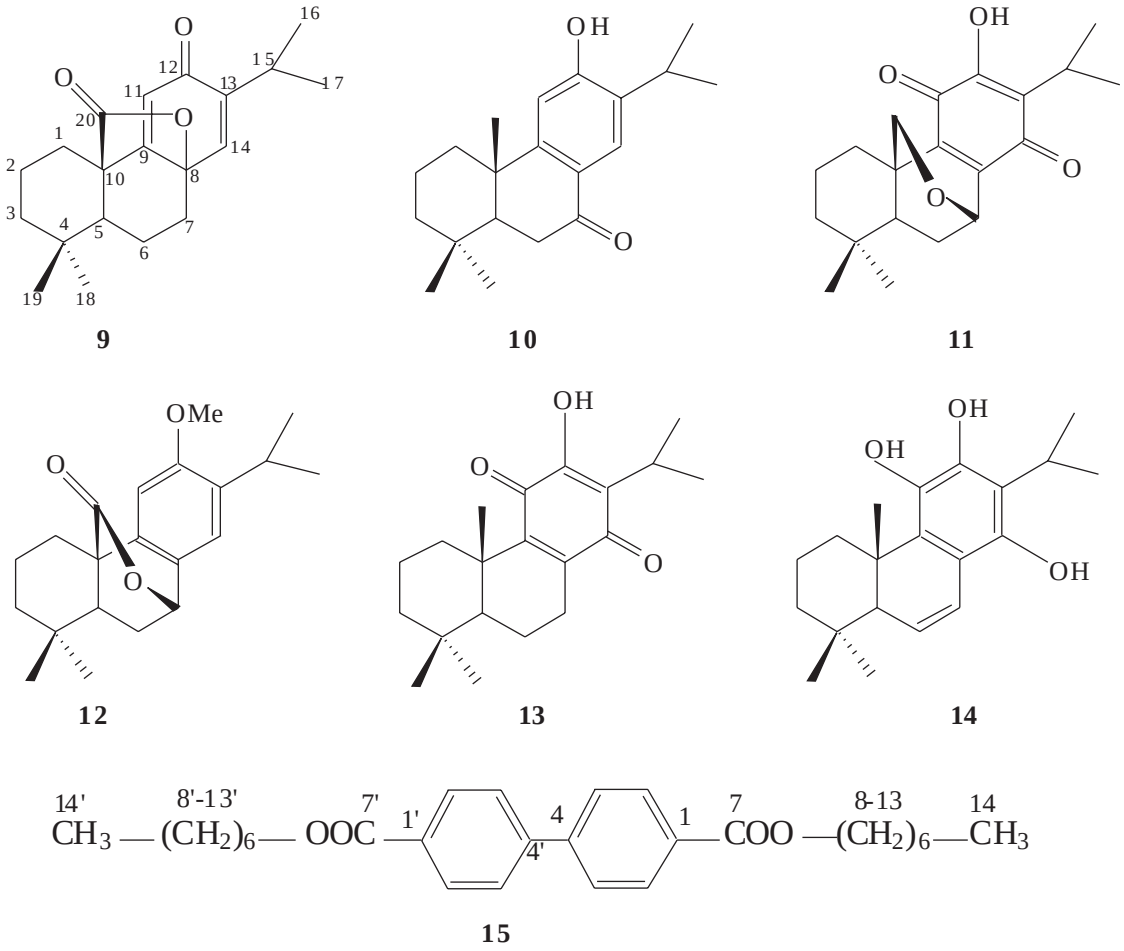


Figure 2. Isolated diterpenoids and an aromatic ester.

Formosanolide was isolated for the first time from the genus *Salvia*. Triterpenoid 24-methylenecycloartanol and diterpenoid 7,20-epoxyroyleanone have rarely been reported from *Salvia* species. The former was isolated from *Salvia nemorosa*[27] and the latter from *Salvia napifolia* among the more than 45 *Salvia* species we studied[28].

All the isolated compounds in this study, except for 7,20-epoxyroyleanone, were tested against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi*, *Shigella flexneri*, *Proteus mirabilis* ATCC 14153 and the yeast *Candida albicans* ATCC 10231 as per the literature [29−31]. These compounds were found to be inactive or fairly weakly active against the above bacteria and fungi.

Conclusion

Fifteen known compounds were isolated from the roots of *Salvia blepharochlaena* Hedge and Hub. Mor. However, almost no antimicrobial activity was determined against the above mentioned bacteria and fungi.

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