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Isolation and Structure Elucidation of Uncommon Secondary Metabolites from *Cistus salviifolius* L.

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Abstract: To our knowledge this is the first report on the isolation of a flavonoid glycoside: quercetin 3-*O*-*α*-arabinopyranoside **(5)**, two phenylbutanon glycosides: 4-(4'-*O*-[6"-*O*-galloyl-*β*-galactopyranosyl]-3'-hydroxyphenyl)-butan-2-on **(8)**, 4-(3'-*O*-*β*-glucopyranosyl-4'-hydroxyphenyl)-butan-2-on **(9)**, one phloroglucinol glycoside: 1-*O*-*β*-glucopyranosyl-3,5-dimethoxybenzene **(10)** and a steroid glycoside: sitosterol-3-*O*-(6"-*O*-butanoyl)-*β*-galactopyranoside **(14)** from the *Cistus* species (Cistaceae). Additional to these compounds three flavonol aglycones: kaempferol **(1)**, quercetin **(2)**, myricetin **(3)**; three flavonoid glycosides; kaempferol 3-*O*-*β*-(6"-*O*-*trans*-*p*-coumaroyl)-glucopyranoside **(4)**, quercetin 3-*O*-*β*-galactopyranoside **(6)**, myricetin 3-*O*-*β*-galactopyranoside **(7)**; one phloroglucinol glycoside: 1-*O*-*β*-glucopyranosyl-3,5-dimethoxybenzene **(11)**; one steroid aglycone: *β*-sitosterol **(12)**; one steroid glycoside: Sitosterol-3-O- *β*-glucopyranoside **(13)** were isolated from the aerial parts of the *Cistus salviifolius* L.. Their structures were identified using spectral methods (UV, IR, 1D- and 2D-NMR, and ESI-MS).

Keywords: *Cistus*; Cistaceae; flavonoid; phenylbutanon; chemotaxonomy.

1. Introduction

The genus *Cistus* (Cistaceae), which is a characteristic element of the macchias and garigues of the Mediterranean region, comprises 21 species worldwide and 5 species in the flora of Turkey [1,2]. *Cistus* species are used widely in Turkish folk medicine to cure some ailments such as rheumatism, stomach ache, hemorrhoids, sterility, urinary inflammations, peptic ulcer and diabetes mellitus [3,4]. Their homeostatic, antipyretic, expectorant and sedative properties have also been reported [4]. Pharmacological activities including the cytotoxic [5-7], anti-microbial [8-11], anti-viral [12-15], anti-inflammatory [6,16-18], antioxidant [19-21], analgesic [18,22,23], spasmolytic [24-27], anti-ulcerogenic and gastro protective [28-30], antihyperglycemic [31], and platelet aggregation inhibitory [32,33] activities of *Cistus* species have been previously reported. Simple phenols, flavonoids [34,35], flavan-3-ols [36,37], lignans [17] and phloroglucinol glycosides [37, 38], labdane type diterpenoids [5,38-41], triterpenoids and steroids [42,43], have been isolated from different *Cistus* species. In

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addition, essential oil studies on some of the species have been reported [44-46]. The aim of this study is to isolate and characterize the secondary metabolites of *Cistus salviifolius*.

2. Materials and Methods

2.1. General experimental procedures

The UV (MeOH) spectra were recorded on an M-Quant Biomolecular spectrophotometer (Bio-Tek Instruments). The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Mercury plus 400 MHz for proton and 100 MHz for carbon. Chemical shifts were given in ppm with Me_4Si (TMS) as internal standard. DMSO-d_6 , MeOH-d_4 and $CDCl_3$ were used for NMR analyses. IR spectra were recorded on a Perkin Elmer FT-IR Spectrum Bx. ESI-MS analyses were performed on a Waters 2695 Aliance Micromass ZQ spectrophotometer. Chromatographic separations were carried out on silica gel 60 (0.063-0.200 mm, Merck, Darmstadt), Sephadex LH-20 (Fluka) and polyamide (Polyamide SC6) by open column chromatography (CC). Lichroprep RP-18 (25-70 µm, Merck, Darmstadt) reversed phase material was used for middle pressure liquid chromatography (MPLC). TLC analyses were carried out on pre-coated Kieselgel 60 F_{254} aluminium sheets (Merck). Compounds were detected by UV fluorescence and spraying 1% vanillin-H_2SO_4 reagent, followed by heating at 105 °C for 1-2 min.

2.2. Plant material

The aerial parts of *Cistus salviifolius* L. were harvested from Mahmutlar, in Alanya province in April 2009. The voucher specimen was stored in the Herbarium at the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 09002).

2.3. Extraction and isolation

The air-dried aerial parts of *Cistus salviifolius* (1.4 kg) were first extracted with *n*-hexane at 37 °C (3 × 2.5 L) and then extracted with methanol at 37 °C (3 × 2.5 L). The combined extracts were evaporated under vacuum to give crude extracts (n-hexane: 18 g, MeOH: 35 g). The methanol extract was suspended in H_2O (0.15 L). The water soluble portion was partitioned between petroleum ether (5 × 0.15 L) and n-BuOH (5 × 0.15 L) respectively. The n-BuOH fraction (24 g) was subjected to polyamide (PA) column chromatography and eluted with H_2O, followed by increasing concentrations of MeOH to yield 5 main fractions (Frs. 1-5) [Fr. 1: (H_2O), 11 g; Fr. 2: (25% MeOH), 2.2 g; Fr. 3: (50% MeOH), 2.19 g; Fr. 4: (75% MeOH), 1.1 g; Fr. 5: (MeOH), 2.03g].

Fr. 1 (11 g) was subjected to silica gel column eluting with $CHCl_3$-MeOH-H_2O (80:20:2) to yield Fr. 1_D (450 mg) and Fr. 1_H (340 mg) from 9 sub fractions. Silica gel column chromatography (Sigel CC) of Fr. 1_D (450 mg) by eluting EtOAc-MeOH-H_2O (100:2.5:0 → 100:5:2) gave six sub fractions (Fr. 1_{Da}-Fr. 1_{Df}) and Sephadex LH-20 column chromatography of Fr. 1_{Da} and Fr. 1_{Dd} gave compound **11** (8 mg) and compound **9** (90 mg) in pure form. Fr. 1_H (340 mg) was subjected to silica gel column eluting with EtOAc-MeOH-H_2O (100:2.5:0 → 100:5:2) and purified by Sephadex LH 20 Column Chromatography (CC) using MeOH to yield compound **10** (20 mg).

Fr. 2 (2.2 g) was fractionated using MPLC by eluting with increasing concentrations of MeOH (20% → 100%) to give 14 sub fractions (Fr. 2_{A-N}). Compound **8** (12 mg) was isolated after eluting the Fr. 2_F (160 mg) with $CHCl_3$-MeOH-H_2O (99:1:0 → 70:30:3) using Sigel CC.

Fr. 3 (2.19 g) was subjected, on an MPLC system (Column: 3.6 × 46 cm; 5 mL/min), eluting with MeOH-H_2O (0% → 100%) to yield 10 sub fractions (Fr. 3A → Fr. 3J). Purification of Fr. 3J (40 mg) and Fr. 3I (55 mg) by Sephadex LH-20 CC using MeOH gave compounds **1** (11 mg) and **2** (31 mg) respectively. Fr. 3G (264 mg) was subjected to Sephadex LH-20 CC and eluted with MeOH. Four sub fractions (Fr. $3G_{1-4}$) were obtained. Compound **3** (17 mg) was isolated from Fr. $3G_4$. Fr. $3G_2$ (75 mg) was chromatographed using Sigel CC and eluted with $CHCl_3$-MeOH (85:15 → 80:20) mixtures to get 3 sub fractions. The first and third fraction gave compounds **5** (20 mg) and **6** (25 mg) respectively.

Fr. 3E (128 mg) was subjected to Sephadex LH-20 CC and compound **7** (56 mg) was purified by eluting MeOH.

Fr. 5 (2.03 g) was subjected to Sephadex LH-20 CC. MeOH was used as eluting solvent and 4 fractions (Fr. 5A→ Fr. 5D) were obtained. Fr. 5A (170 mg) was subjected to silica gel CC and eluted with different proportions of $CHCl_3$: MeOH (97:3→ 90:10). Three fractions were obtained from this separation and the third fraction gave pure compound **4** (50 mg).

The n-hexane fraction (18 g) was subjected to silica gel (Sigel) column chromatography and eluted with n-hexane-$CHCl_3$ (90:10→0:100) to yield 9 main fractions (Frs. 1-9) [Fr. 1: 2.4 g, Fr. 2: 330 mg, Fr. 3: 3.8 g, Fr. 4: 7.5 g, Fr. 5: 1.5 g, Fr. 6: 600 mg, Fr. 7: 700 mg, Fr. 8: 300 mg, Fr. 9: 340 mg)].

Fr. 4 (7.5 g) was fractionated using Sigel CC by eluting with different concentrations of n-hexane- EtOAc (99:1→ 50:50) to give 8 sub fractions (Fr. 4A→ Fr. 4H). Fr. 4H (700 mg), was subjected to a Sigel CC eluting with n-hexane- EtOAc (90:10→ 60:40) and purified by Sephadex LH-20 CC using CH_2Cl_2: MeOH (95:5) to yield compound **12** (104 mg).

Fr. 7 (700 mg), was fractionated using silica gel column chromatography by eluting with different concentrations of CH_2Cl_2-MeOH (98:2→ 95:5) to yield 2 sub fractions. The second fraction was subjected to Sephadex LH-20 CC by eluting with CH_2Cl_2-MeOH (95:5) and purified by silica gel column using , n-hexane-EtOAc (50:50→45:55, 40:60) to yield compound **14** (11 mg).

Fr. 9 (340 mg) was subjected to Sephadex LH-20 column eluting with CH_2Cl_2-MeOH (95:5) and purified by Sigel CC using CH_2Cl_2:MeOH (90:10) to yield compound **13** (15 mg).

3. Results and Discussion

In this study, from the aerial parts of *Cistus salviifolius*, the following were isolated by fractionation of the n-butanol and n-hexane extracts through an open column chromatograph on polyamide, silica gel and Sephadex LH-20, followed by MPLC: three flavonoid aglycones: kaempferol (**1**), quercetin (**2**), myricetin (**3**); four flavonoid glycosides: kaempferol 3-*O*-*β*-(6"-*O*-*trans*-*p*-coumaroyl)-glucopyranoside (*Trans*-tirilosid) (**4**), quercetin 3-*O*-*α*-arabinopyranoside (Guaijaverin) (**5**), quercetin 3-*O*-*β*-galactopyranoside (**6**), myricetin 3-*O*-*β*-galactopyranoside (**7**); two phenylbutanon glycosides: 4-(4'-*O*-[6"-*O*-galloyl-*β*-galactopyranosyl]-3'-hydroxyphenyl)-butan-2-on **(8)**, 4-(3'-*O*-*β*-glucopyranosyl-4'-hydroxyphenyl)-butan-2-on **(9)**; two phloroglucinol glycosides: 1-*O*-*β*-glucopyranosyl-3-methoxy-5-hydroxybenzene **(10)**, 1-*O*-*β*-glucopyranosyl-3,5-dimethoxybenzene **(11)**; one steroid aglycone: *β*-sitosterol **(12)**; two steroid glycosides: Sitosterol-3-*O*- *β*-glucopyranoside **(13)** and Sitosterol-3-*O*-(6"-*O*-butanoyl)-*β*-galactopyranoside **(14)** (See Figure 1).

Kaempferol **(1):** Amorphous, yellow powder. ESI-MS *m/z* ($[M + H]^+$ $C_{15}H_{11}O_6$; calc. 287), UV λ_{max} (MeOH) nm: 254, 366, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1660 (C=O), 3300 (C-OH), ^1H (400 MHz, DMSO-d_6), and ^{13}C (100 MHz, DMSO-d_6 NMR data were identical to those reported in the literature [47,48].

Quercetin **(2):** Amorphous, yellow powder. ESI-MS *m/z* ($[M + H]^+$ $C_{15}H_{11}O_7$; calc. 303), UV λ_{max} (MeOH) nm: 250, 368, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1660 (C=O), 3300 (C-OH), ^1H (400 MHz, DMSO-d_6), and ^{13}C (100 MHz, DMSO-d_6 NMR data were identical to those reported in the literature [47,48].

Myricetin **(3):** Amorphous, yellow powder. ESI-MS *m/z* ($[M+H]^+$ $C_{15}H_{11}O_8$; calc. 319), UV λ_{max} (MeOH) nm: 254, 373, IR υ_{max} (1% KBr) cm^{-1}: 1623 (C=C), 1655 (C=O), 3330 (C-OH), ^1H (400 MHz, DMSO-d_6), and ^{13}C (100 MHz, DMSO-d_6 NMR data were identical to those reported in the literature [47,48].

Kaempferol 3-O-β-(6"-O-trans-p-coumaroyl)-glucopyranoside (Trans-tirilosid) **(4):** Amorphous, yellow powder. ESI-MS *m/z* ($[M + Na]^+$ $C_{30}H_{26}O_{13}Na$; calc. 617), UV λ_{max} (MeOH) nm: 230, 244, 252, 315, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1654 (C=O), 3370 (C- OH), ^1H-NMR (400 MHz,

DMSO-d_6), and ^{13}C-NMR (100 MHz, DMSO-d_6) data's were identical to those reported in the literature [49,50].

Quercetin 3-O-α-arabinopyranoside (Guaijaverin) **(5):** Amorphous, yellow powder. ESI-MS *m/z* ([*M* + Na]$^+$ $C_{20}H_{18}O_{11}Na$; calc. 457), UV λ_{max} (MeOH) nm: 229, 258, 357, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1650 (C=O), 3300 (C-OH). ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) data's are given in Table 1, 2.

Quercetin 3-O-β-galactopyranoside **(6):** Amorphous, yellow powder. ESI-MS *m/z* ([*M* + Na]$^+$ $C_{21}H_{20}O_{12}Na$; calc. 487), UV λ_{max} (MeOH) nm: 228, 260, 357, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1650 (C=O), 3290 (C-OH). ^{1}H (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) data's are given in Table 1, 2.

Myricetin 3-O-β-galactopyranoside **(7):** Amorphous, yellow powder. ESI-MS *m/z* ([*M* + Na]$^+$ $C_{21}H_{21}O_{13}Na$; calc. 481), UV λ_{max} (MeOH) nm: 228, 260, 357, IR υ_{max} (1% KBr) cm^{-1}: 1600 (C=C), 1650 (C=O), 3300 (C-OH). ^{1}H (400 MHz, CH_3OH-d_4) and ^{13}C-NMR (100 MHz, CH_3OH-d_4) data's are given in Table 1, 2.

Table 1. ^{1}H NMR data for compounds **5-7** (¢ in ppm, *J* in Hz).

	5	**6**	**7**
Aglycone			
6	6.22 (1H, *d*, *J* = 2.0)	6.20 (1H, *d*, *J* = 2.1)	6.20 (1H, *d*, *J* = 2.12)
8	6.22 (1H, *d*, *J* = 2.0)	6.41 (1H, *d*, *J* = 2.1)	6.40 (1H, *d*, *J* = 2.08)
2'	7.53 (1H, *d*, *J* = 2.2)	7.54 (1H, *d*, *J* = 2.2)	7.40 (1H, *s*)
5'	6.86 (1H, *d*, *J* = 8.7)	6.82 (2H, *d*, *J* = 8.4)	
6'	7.67 (1H, *dd*, *J* = 2.2/8.4)	7.65 (1H, *dd*, *J* = 2.2/8.4)	7.40 (1H, *s*)
Sugar			
1"	5.29 (1H, *d*, *J* = 5.2)	5.37 (1H, *d*, *J* = 7.6)	5.21 (1H, *d*, *J* = 7.8)
2"	3.76 (1H, *dd*, *J* = 5.0/7.0)	3.57 (1H, *m*)	3.84 (1H, *dd J* = 7.8/9.6)
3"	3.52 (1H, *dd*, *J* = 3.2/7.1)	3.37 (1H, *m*)	3.60 (1H, *dd J* = 3.2/9.5)
4"	3.66 (1H, *d*, *J* = 2.5)	3.65 (1H, *d*, *J* = 2.8)	3.89 (1H, *d*, *J* = 3.1)
5"	3.23 (1H, *dd*, *J* = 2.0/11.4)	3.33 (1H, *m*)	3.54 (1H, *d*, *J* = 5.8)
	3.61 (1H, *dd*, *J* = 5.5/11.3)		
6"		3.45-3.50 (1H)*	3.67 (1H, *dd*, *J* = 6.0/11.1)
		3.30 (1H, *m*)	3.63 (1H, *m*)

* Signal patterns are unclear due to overlapping.

4-(4'-O-[6"-O-galloyl-β-galactopyranosyl]-3'-hydroxyphenyl)-butan-2-on **(8)**: Amorphous, white powder. ESI-MS *m/z* ([*M* + Na]$^+$ $C_{23}H_{26}O_{12}Na$; calc. 517), UV λ_{max} (MeOH) nm: 271, 291 IR υ_{max} (1% KBr) cm^{-1}: 1699 (C=O), 3360 (C-OH). ^{1}H (400 MHz, DMSO-d_6): δ_H 2.00 (3H, s, H-1), 2,52 (2H, d, *J*=5.4 Hz, H-3), 2.54, (2H, d, *J*=5.4 Hz, H-4), 6.84 (1H, d, *J*=1.8 Hz, H-2'), 6.66 (1H, d, *J*=8.3 Hz, H-5'), 6.64 (1H, d, *J*=8.1 Hz, H-6'), 4.73 (1H, d, *J*=5.6 Hz, H-1"), 3.30-3.40 (1H, H-2"), 3.75 (1H, m, H-3"), 3.30-3.40 (1H, H-4"), 3.30-3.40 (1H, H-5"), 4.33 (1H, dd, *J*=5.5/11.9 Hz, H-6$_a$"), 4.49 (1H, d, *J*=10.3 Hz, H-6$_b$"), 6.99 (6H, s, H-2"', H-6"'). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 30.01 (C-1), 208.23 (C-2), 44.68 (C-3), 28.91 (C-4), 132.67 (C-1'), 116.17 (C-2'), 145.27 (C-3'), 145.44 (C-4'), 116.93 (C-5'), 122.97 (C-6'), 102.61 (C-1"), 73.72 (C-2"), 74.40 (C-3"), 70.22 (C-4"), 76.04 (C-5"), 63.81 (C-6"), 119.80 (C-1"'), 109.10 (2C, C-2"', C-6"'), 146.04 (C-3"'), 139.01 (C-4"'), 146.04 (C-5"'), 166.26 (C-7"').

4-(3'-O-β-glucopyranosyl-4'-hydroxyphenyl)-butan-2-on **(9)**: Amorphous, white-grey powder. ESI-MS *m/z* ([*M* + Na]$^+$ $C_{16}H_{22}O_8Na$; calc. 365), UV λ_{max} (MeOH) nm: 271, 291, IR υ_{max} (1% KBr) cm^{-1}: 1699 (C=O), 3360 (C-OH). ^{1}H (400 MHz, DMSO-d_6): δ_H 2.09 (3H, s, H-1), 2.71 (2H, dd, *J*=6.1/13.2 Hz, H-3), 2.66 (2H, dd, *J*=6.0/13.2 Hz, H-4), 6.98 (1H, s, H-2'), 6.70 (2H, s, H-5', H-6'), 4.64 (1H, d, *J*=7.2 Hz, H-1"), 3.30 (1H, H-2"), 3.30 (1H, H-3"), 3.15 (1H, H-4"), 3.29 (1H, H-5"), 3.40-3.50 (1H, H-6$_a$"), 3.70-3.80 (1H, m, H-6$_b$"). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 30.20 (C-1), 208.49 (C-2),

44.74 (C-3), 29.00 (C-4), 132.42 (C-1'), 117.21 (C-2'), 145.58 (C-3'), 145.36 (C-4'), 116.07 (C-5'), 122.89 (C-6'), 102.80 (C-1"), 73.80 (C-2"), 76.43 (C-3"), 70.41 (C-4"), 77.68 (C-5"), 61.26 (C-6").

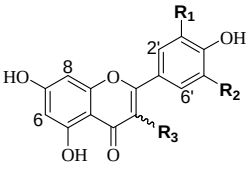
1-O-β-glucopyranosyl-3-methoxy-5-hydroxybenzene **(10):** Amorphous, white-grey powder. ESI-MS *m/z* ([*M* + H]$^+$ $C_{13}H_{19}O_8$; calc. 303), UV λ_{max} (MeOH) nm: 273, 291, IR υ_{max} (1% KBr) cm^{-1}: 2975 (C=H), 3360 (C-OH). ^{1}H (400 MHz, DMSO-d_6), and ^{13}C-NMR (100 MHz, DMSO-d_6) data's were identical to those reported in the literature [37].

1-O-β-glucopyranosyl-3,5-dimethoxybenzene (Taxicatin) **(11):** Amorphous, white powder. ESI-MS *m/z* ([*M* + H]$^+$ $C_{14}H_{21}O_8$; calc. 317), UV λ_{max} (MeOH) nm: 290, IR υ_{max} (1% KBr) cm^{-1}: 2970 (C=H), 3350 (C-OH). ^{1}H (400 MHz, DMSO-d_6): δ_H 6.22 (H, d, *J*=2.2 Hz, H-2), 6.31 (1H, d, *J*=2.4 Hz, H-4), 6.22 (1H, d, *J*=2.2 Hz, H-6), 3.76 (3H, s, OC$\underline{H}_3$), 3.68 (3H, s, OC$\underline{H}_3$), 4.86 (1H, d, *J*=7.6 Hz , H-1'), 3.26 (1H, H-2'), 3.28 (1H, H-3'), 3.27 (1H, H-4'), 3.28 (1H, H-5'), 3.78 (1H, H-6$_a$'), 3.48 (1H, H-6$_b$'). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 154.57 (C-1), 94.39 (C-2), 156.34 (C-3), 96.20 (C-4), 157.62 (C-5), 94.39 (C-6), 56.38 (O$\underline{C}$H$_3$), 55.59 (O$\underline{C}$H$_3$), 100.68 (C-1'), 73.57 (C-2'), 77.19 (C-3'), 69.83 (C-4'), 77.65 (C-5'), 60.92 (C-6').

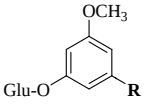
β-Sitosterol **(12)**: Amorphous, white powder. ESI-MS *m/z* ([*M* + H]$^+$ $C_{29}H_{51}O$; calc. 415), UV λ_{max} (MeOH) nm: 213, 229, 321, IR υ_{max} (1% KBr) cm^{-1}: 2935 (C-H), 3450 (C-OH). ^{1}H NMR (400 MHz, $CDCl_3$), and ^{13}C NMR (100 MHz, $CDCl_3$) data's were identical to those reported in the literature [51].

Sitosterol-3-O- β-glucopyranoside (Daucosterol) **(13):** Amorphous, white powder. ESI-MS *m/z* ([*M* + H]$^+$ $C_{35}H_{61}O_6$; calc. 577), UV λ_{max} (MeOH) nm: 229, IR υ_{max} (1% KBr) cm^{-1}: 2930 (C-H), 3420 (C-OH), 1639. ^{1}H NMR (400 MHz, $CDCl_3$), and ^{13}C NMR (100 MHz, $CDCl_3$) data's were identical to those reported in the literature [52].

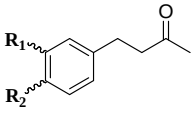
Sitosterol-3-O-(6'-O-butanoyl)-β-galactopyranoside **(14):** Amorphous, white powder. ESI-MS *m/z* ([*M* + H]$^+$ $C_{40}H_{69}O_7$; calc. 661), UV λ_{max} (MeOH) nm: 229, IR υ_{max} (1% KBr) cm^{-1}: 2930 (C-H), 3420 (C-OH), 1700. ^{1}H NMR (400 MHz, $CDCl_3$), and ^{13}C NMR (100 MHz, $CDCl_3$) data's were identical to those reported in the literature [53].



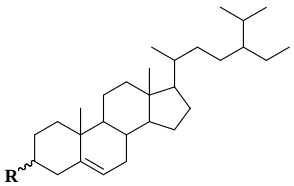
	R_1	R_2	R_3
1	H	H	OH
2	OH	H	OH
3	OH	OH	OH
4	OH	H	*O*- -(6"-*O*-*trans*-p-coumaroyl)-glucopyranose
5	OH	H	*O*-~-arabinopyranose
6	OH	H	*O*- -galactopyranoside
7	OH	OH	*O*- -galactopyranoside



	R
10	OH
11	OCH_3



	R_1	R_2
8	OH	*O*-(6"-*O*-galloyl- -galactopyranose)
9	*O*- -glucopyranose	OH



	R
12	OH
13	*O*- -glucopyranose
14	*O*-(6'-*O*-butanoyl)- -galactopyranose

Figure 1. The structures of compounds 1-14.

Chemical structures of compounds **1-14** were identified by comparing their spectral (UV, ^{1}H and ^{13}C NMR) data with those reported in previous studies as follows: kaempferol (**1**) [47,48] quercetin (**2**) [47,48], myricetin (**3**) [47,48], kaempferol 3-*O*-*β*-(6”-*O*-*trans*-*p*-coumaroyl)-glucopyranoside (**4**) [49,50], quercetin 3-*O*-*α*-arabinopyranoside (**5**) [47,54,55], quercetin 3-*O*-*β*-galactopyranoside (**6**) [47,48,54], myricetin 3-*O*-*β*-galactopyranoside (**7**) [47, 48, 54], 4-(4’-*O*-[6”-*O*-galloyl-*β*-galactopyranosyl]-3’-hydroxyphenyl)-butan-2-on **(8)** [54,56,57], 4-(3’-*O*-*β*-glucopyranosyl-4’-hydroxyphenyl)-butan-2-on **(9)** [57], 1-*O*-*β*-glucopyranosyl-3-methoxy-5-hydroxybenzene **(10)** [37], 1-*O*-*β*-glucopyranosyl-3,5-dimethoxybenzene **(11)** [58] , *β*-sitosterol **(12)** [59], Sitosterol-3-*O*- *β*-glucopyranoside **(13)** [52] and Sitosterol-3-*O*-(6”-*O*-butanoyl)-*β*-galactopyranoside **(14)** [53].

Table 2. ^{13}C NMR data for compounds **5-7** (¢ in ppm).

	5	**6**	**7**
Agylcone			
2	156.75 (C)	156.70 (C)	157.33 (C)
3	134.20 (C)	133.93 (C)	134.62 (C)
4	177.97 (C)	177.94 (C)	178.00 (C)
5	161.67 (C)	161.66 (C)	161.53 (C)
6	99.15 (CH)	99.15 (CH)	98.51 (CH)
7	164.70 (C)	164.63 (C)	164.62 (C)
8	93.99 (CH)	93.99 (CH)	93.30 (CH)
9	156.75 (C)	156.76 (C)	156.96 (C)
10	104.36 (C)	104.35 (C)	104.23 (C)
1’	121.36 (C)	121.53 (C)	120.33 (C)
2’	116.23 (CH)	116.42 (CH)	108.63 (CH)
3’	145.44 (C)	145.28 (C)	145.01 (C)
4’	149.06 (C)	148.93 (C)	136.74 (C)
5’	115.84 (CH)	115.66 (CH)	145.01 (C)
6’	122.49 (CH)	122.40 (CH)	108.63 (CH)
Sugar			
1”	101.88 (CH)	102.26 (CH)	104.23 (CH)
2”	71.19 (CH)	71.66 (CH)	71.91 (CH)
3”	72.11 (CH)	73.63 (CH)	73.74 (CH)
4”	66.53 (CH)	68.37 (CH)	68.66 (CH)
5”	64.75 (CH_2)	76.27 (CH)	75.82 (CH)
6”		60.58 (CH_2)	60.59 (CH_2)

4. Chemotaxonomic significance

To our knowledge, this is the first report on the isolation of Guaijaverin, 4-(4’-*O*-[6”-*O*-galloyl-*β*-galactopyranosyl]-3’-hydroxyphenyl)-butan-2-on,4-(3’-*O*-*β*-glucopyranosyl-4’-hydroxy-phenyl)-butan-2-on, 1-*O*-*β*-glucopyranosyl-3,5-dimethoxybenzene and Sitosterol-3-*O*-(6”-*O*-butanoyl)-*β*-galactopyranoside from *Cistus* species. For this reason these compounds have a chemotaxonomic importance for *Cistus* species and for Cistaceae. Guaijaverin which is isolated from *Psidium guajava* is told to be a potential antiplaque agent by inhibiting the growth of the *Streptococcus mutans* and has antioxidant properties [60, 61]. Also binding mechanism of Guaijaverin to human serum albumin were showed in recent studies [62]. According to our ongoing HPLC studies the total amount of this compound is 0,079±0,001 µg/g extract and the existence of this metabolite in *Cistus salviifolius* which grown in Turkey is important. Because *Cistus salviifolius* has a large distribution in Mediterranean region of the Turkey and extracting and isolation of large scales of the plant can lead to satisfactory amounts of this pharmacologically important flavonoid. The major compound of the n-butanol soluble part of the plant is 4-(3’-*O*-*β*-glucopyranosyl-4’-hydroxyphenyl)-butan-2-on. This compound has a limited distribution in the plant kingdom and has a structural similarity with raspberry ketone. Raspberry ketone (4-(4-hydroxyphenyl) butan-2-on; RK), one of the major aromatic compound of raspberry, is widely used as a fragrance in cosmetics and as a flavoring agent in foodstuffs [63]. The preventive and improving effects of RK against obesity and fatty liver activities have been shown in recent studies [64]. For this reason 4-(3’-*O*-*β*-glucopyranosyl-4’-

hydroxyphenyl)-butan-2-on is thought to have raspberry ketone like activities and this marker compound of *C. salviifolius* should be studied in detail.

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