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FLAVONOIDS of CENTAUREA KILAEA and C. SALONITANA

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SUMMARY

In this study, the flavones of the whole plant extracts of *Centaurea kilaea* Boiss. and *C. salonitana* Vis. will be presented. From *C. kilaea* Boiss, five flavones salvigenin (scutellarein-6,7,4'-methyl ether), 6-hydroxyluteolin-6,7,3',4'-tetramethyl ether, luteolin-7,3',4'-trimethyl ether, jaceosidin (6-hydroxyluteolin-6,3'-dimethyl ether) and pectolinarigenin (6-hydroxyapigenin-6,4'-dimethyl ether) have been isolated, and *C. salonitana* Vis. afforded five flavones, pectolinarigenin, 6-hydroxyluteolin-4'-methyl ether, cirsiliol (6-methoxyluteolin-7-methyl ether), hispudilin (6-methoxyapigenin), and apigenin 7-*O*-glucoside. Their structures were identified by spectroscopic means.

ÖZET

Türkiye'de yetişen *C. kilaea* Boiss. ve *C. salonitana* Vis. türlerinin flavonoid bileşiklerini sunmak amacıyla yapılan bu çalışmada *C. kilaea*'dan bilinen beş flavon, salvigenin (scutellarein-6,7,4'-methyl ether), 6-hydroxyluteolin-6,7,3',4'-tetramethyl ether, luteolin-7,3',4'-trimethyl ether, jaceosidin (6-hydroxyluteolin-6,3'-dimethyl ether) and

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pectolinarigenin (6-hydroxy apigenin-6,4'-dimethyl ether), *C. salonitana*' dan ise biri pektolinarigenin olan beş flavon 6-hydroxyluteolin-4'-methyl ether, cirsiliol (6-methoxyluteolin-7-methyl ether), hispudilin (6-methoxyapigenin), ve apigenin 7-*O*-glucoside elde edilmiştir. Bileşiklerin yapıları spektroskopik yöntemlerle aydınlatılmıştır.

Key words: *Centaurea kilaea, C. salonitana, flavones*

INTRODUCTION

The genus *Centaurea* is represented by about 170 species as the largest genus of the family of Compositae in Turkey (1). Centaurea species are used as antipyretic, menstruating, appetizing, tonic and stomachic in traditional medicine in Turkey (2) and diuretic, astringent, antifebrile, antimalarial, cytostatic, cytotoxic, allergenic, stomachic, tonic, digestive and emmenagogue in the world (3-6).

From Turkish *Centaurea* species, various types of structures have been isolated being mainly flavonoids (6-10) and sesquiterpene lactones (11-12) besides some aromatics (13).

We recently presented isolation and structure elucidation of aromatic compounds syringin, 4-hydroxyphenyl-2-ethyl-β-D-glucose and 4-(β-D-glucopyranosyl)benzylalcohol, two cyclo-hexenones vomifoliol and dehydroxyvomifoliol-*O*-β-D-glucoside, sesquiterpene lactones dehydromelitensin, melitensin-8α-β-*O*-D-glucopyranoside, sinaicin, 11,13-dihydrodesacetyl-cynaropicrin, and stigmasterol from the whole plant of *Centaurea salonitana* (14).

In this study, we report on isolation of the five flavones from *C.kilaea* and five flavones from *C. salonitana,* only one flavone pectolinarigenin isolated from both plants, and all the isolated flavones were methoxylated, except apigenin 7-*O*-glucoside from *C. salonitana.* Their structures were identified based on ^{1}H and ^{13}C NMR, MS and UV spectra as well as comparison with authentic samples as salvigenin (scutellarein-6,7,4'-methylether) **(1)**, 6-hydroxyluteolin-6,7,3',4'-tetramethylether **(2)**, luteolin-7,3',4'-trimethylether **(3)**, jaceosidin (6-hydroxyluteolin-6,3'-dimethylether) **(4)** and pectolinarigenin (6-hydroxyapigenin-6,4'-dimethylether) **(5)**, from *C. kilaea* and pectolinarigenin (5), 6-hydroxyluteolin-4'-methylether **(6)**, cirsiliol (6-methoxyluteolin-7-methylether) **(7)**, hispudilin (6-methoxyapigenin) **(8)**, and apigenin 7-*O*-glucoside (9) from *C. salonitana.*

When we searched the flavonoid profiles of the *Centaurea* species, the abundance of 6- and/or 7-methoxylated flavones is clearly seen and, particularly as apigenin and luteolin derivatives (15-17). In our previous studies on the flavonoid constituents of *Centaurea* species, we have also isolated many 6-methoxylated flavones from several *Centaurea* species (6-9), and the later literature reports the activity studies of 6-methoxylated flavones as well as apigenin.

EXPERIMENTAL

General: The spectra were recorded with the following instruments; UV: Shimadzu 1601, ^{1}H NMR: Bruker AC-250 and AC-200 MHz. MS: VG ZabSpec high resolution Mass Spectrometer. For the isolation and purification of the compounds TLC: Kieselgel 60F254 (E. Merck) precoated plates. CC: Silicagel 60 and Sephadex LH-20 were used.

Plant material: *Centaurea kliea* Boiss. was collected from Terkos- İstanbul (Turkey) in September 1997 and identified by Dr. A. Çırpıcı (Marmara University). A voucher specimen was deposited in the Herbarium of the Atatürk Faculty of Education, Marmara University, İstanbul (MARA 5630).

Centaurea salonitana Vis. was collected from Gelibolu-Çanakkale (Turkey) in June 1996 and identified by Prof. Dr. A. Çırpıcı (Marmara University). A voucher specimen was deposited in the Herbarium of the Atatürk Faculty of Education, Marmara University, İstanbul (MARA 5635).

Extraction and Isolation:

C. kilaea Boiss.- The air dried and powdered whole plant (1016 g) was extracted with petroleum ether-ether-ethanol (1:1:1) at room temperature for 24 hr. After filtration, the extract was evaporated *in vacuo* to a small volume. This extract was treated with MeOH and kept in a refrigerator for 2 hours to remove the long chain saturated hydrocarbons. After elimination of precipitate, the extract evaporated in *vacuo,* and the residue (60 g) was prefractionated on a silica gel column. The extract was first eluted with petroleum ether and gradients ethers and methanol, respectively. The similar fractions were combined and further chromatographed on small columns when necessary.The yields from *C. kilaea* were obtained as follows: salvigenin **(1)** (192 mg), 6-hydroxyluteolin-6,7,3',4'-tetramethyl ether **(2)** (30 mg), luteolin-7,3',4'-trimethylether **(3)** (8 mg), jaceosidin **(4)** (6 mg), pectolinarigenin **(5)** (4 mg).

C. salonitana Vis.- The air-dried and powdered whole plant (650 g) was extracted by following the above procedure and finally 38 g residue was obtained and fractionated on a silica gel column. Elution was started with petroleum ether and gradients chloroform and methanol were used, respectively. The similar fractions were combined and further separation carried out on small columns when necessary. The flavonoids were purified on a Sephadex LH-20 column eluting with MeOH.. The obtained compounds from *C. salonitana* were as follows: pectolinarigenin **(5)** (12 mg), 6-hydroxyluteolin-4'-methyl ether **(6)** (6 mg), hispudilin **(7)** (5 mg), cirsiliol **(8)** (8 mg) and apigenin 7-*O*-glucoside **(9)** (7 mg).

Salvigenin (scutellarein-6,7,4'-trimethylether) 1- UV λ_{max}^{MeOH} nm: 328, 278; (+NaOMe): 382, 295 ($AlCl_3$): 351, 292; ($AlCl_3$+HCl): 348, 285; (NaOAc): 330, 277; (NaOAc+H_3BO_3): 332, 279. 1H NMR (250 MHz, $CDCl_3$) : δ 3.89, 3.93 3.97 (each 3H, s, 3 x OCH_3), 6.55 (1H, s, H-3), 6.59 (1H, s, H-8), 7.01 (2H, brd, *J*= 8.6 Hz, H-3' and H-5'), 7.84 (2H, brd, *J*=8.7 Hz, H-2' and H-6'), 12.78 (s, 5-OH). ^{13}C NMR (62.90 MHz, $CDCl_3$) : δ 55.3 (4'-OCH_3), 56.3 (7-OCH_3), 60.8 (6-OCH_3), 90.6 (C-8), 104.1 (C-3), 106.1 (C-10), 114.5 (C-3' and C-5'), 123.5 (C-1'), 128.0(C-2' and C-6'), 132.2 (C-6),153.1 (C-5), 153.1 (C-9), 158.7 (C-7), 162.6 (C-4'), 164.0(C-2), 182.6 (C=O). EIMS *m/z*: 328 [M^+] (100), 313 [M-Me]$^+$ (83), 298 [313-Me](67), 283 [298-Me] (34), 269(45), 250 (28), 181 (89), 153 (78), 133 (56), 89 (45), 69 (88), 53 (95).

6-Hydroxyluteolin-6,7,3',4'-tetramethylether 2- UV λ_{max}^{MeOH}nm:275, 339 (NaOMe)275, 339; ($AlCl_3$) 270, 280 , 348 ($AlCl_3$ + HCl): 280, 348: (NaOAc): 269, 275, 340; (NaOAc+H_3BO_3): 269, 276, 338. ^1H NMR (250 MHz, $CDCl_3$) : δ 3.68, 3.93, 3.98, 3.99 (each 3H, s, 4 x OCH_3), 6.56 (^1H, s, H-3), 6.60 (1H, s, H-8), 6.98 (^1H, d, J= 8.5 Hz, H-5'),7.34 (^1H, d, J=2.2 Hz, H-2') 7.53 (1H, dd, J= 2.2 and 8.5 Hz, H-6'). ^{13}C NMR (62.90 MHz, $CDCl_3$) : δ 56.16 (3'- and 4'-OCH_3), 56.39 (7-OCH_3), 60.89 (6-OCH_3), 90.56 (C-8), 104.50 (C-3), 105.62 (C-10), 108.86 (C-2'), 111.23 (C-5'), 120.12 (C-6'), 123.81 (C-1'), 133.12 (C-6), 149.82 (C-3'), 152.43 (C-9) 153.08 (C-4'), 153.25 (C-5), 158.78 (C-7), 163.99 (C-2), 182.65 (C=O). EIMS *m/z* : 328 [M]$^+$ (100), 313 [M-Me]$^+$ (80), 282 (60), 251 (55), 180 [$C_9H_4O_8$]$^+$(40), 165 [180-Me]$^+$ (35), 162 [$C_{10}H_{10}O_2$]$^+$ (29), 150 [165-Me]$^+$ (47), 147 [162-Me]$^+$ (63), 132 [147-Me]$^+$ (56).

Luteolin-7, 3',4'-trimethylether (3): ^1H NMR (250 MHz, $CDCl_3$) : δ 3.88, 3.86, 3.92 (each 3H, s, 3 x OCH_3), 6.48 (1H, brs, H-8), 6.51 (1H, s, H-3), 6.88 (1H, d, *J*= 8.5 Hz, H-5'), 6.19 (1H, brs, H-6), 7.37 (1H, dd, *J*= 2 and 8.5 Hz, H-6'), 7.43 (d, *J*= 2 Hz, H-2'), (12.68, 5-OH) ;^{13}C NMR (62.90 MHz, $CDCl_3$) : δ 56.19, 56.35, 60.89 (3 x OCH_3), 90.63 (C-6 and C-8), 104.55 (C-3), 105.82 (C-10), 110.75 (C-2'), 112.39 (C-

5'), 119.14 (C-6'), 125.1 (C-1'), 147.23 (C-3'), 151.05 (C-4'), 153.29 (C-9), 163.80 (C-2), 165.19 (C-7), 182.49 (C=O). HRMS *m/z* : 328.0940 (calcd. 328.0946) for $C_{18}H_{16}O_6$.

Jaceosidin (4)- UV λ_{max}^{MeOH}nm: 276, 342 ; (NaOMe) 275, 331, 400; ($AlCl_3$) 275, 348 ($AlCl_3$ + HCl): 280, 354: (NaOAc): 275, 340; (NaOAc+H_3BO_3): 275, 342. ^1H NMR (250 MHz, $CDCl_3$) : δ 4.00 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 6.57 (1H, s, H-3), 6.60 (^1H, s, H-8), 7.03 (^1H, d, *J*=8.0 Hz, H-6'), 7.34 (^1H, d, *J*= 2 Hz, H-2'), 7.47 (^1H, dd, *J*= 2 and 8.0 Hz, H-6'), 13.09 (s, 5-OH).

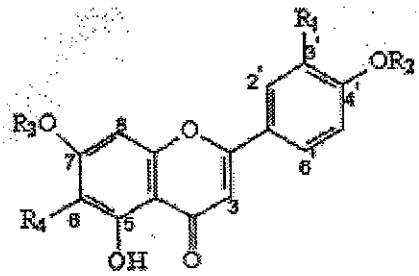
Pectolinarigenin (5)- UV λ_{max}^{MeOH} nm: 276.5, 334 ; (NaOMe): 281, 364.5; ($AlCl_3$): 300.5, 358; ($AlCl_3$ + HCl): 299.5, 353.5; (NaOAc): 275.5, 335.5 ; (NaOAc+H_3BO_3): 270, 334. ^1H NMR : δ 3.80 (3H, s, OCH_3), 4.20 (3H, s, OCH_3), 6.70 (1H, s, H-3), 6.88 (1H, s, H-8), 7.03 (2H, dd, *J*=8.2 and 1.7 Hz, H-3', H-5'), 7.97 (2H, dd, *J*=8.1 and 1.8 Hz, H-2', H-6'); HRMS *m/z*: 314.0777 calcd. 314.0790) for $C_{17}H_{14}O_6$.

6-Hydroxyluteolin-4'-methyl ether (6)- UV λ_{max}^{MeOH} nm: 254, 272.5, 347.5 ; (NaOMe) 276.5, 411.5; (+$AlCl_3$): 275, 378.5; ($AlCl_3$+HCl): 261, 284, 363 ; (NaOAc): 273, 349; (NaOAc+ H_3BO_3): 262.5, 371.5.

Cirsiliol (6-Methoxyluteolin-7-methyl ether) (7)- UV λ_{max}^{MeOH}nm: 274, 343; (NaOMe) : 275, 404.5; ($AlCl_3$): 277, 424.5; ($AlCl_3$+HCl) : 260, 281, 360; (NaOAc): 272.5, 347.5 ; (NaOAc+H_3BO_3): 263.5 , 372 . ^1H NMR (200 MHz, $CDCl_3$): δ 3.83 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 6.54 (1H, s, H-3), 6.62 (1H, s, H-8), 6.91 (1H, d, *J*=8.2 Hz, H-5'), 7.37 (1H, d, *J*=2.1 Hz, H-2'), 7.33 (1H, dd, *J*=2.1 and 8.2 Hz, H-6'), 12.96 (5-OH). HRMS *m/z* : 330.0728 (calcd. 330.0739) for $C_{17}H_{14}O_7$.

Hispudilin (6-Methoxyapigenin) (8)- UV λ_{max}^{MeOH} nm: 269.5, 330; (NaOMe): 275, 387.5; ($AlCl_3$): 277, 298, 343, 370.5; ($AlCl_3$+HCl) : 277, 297.5, 339.5; (NaOAc): 269.5, 334; (NaOAc+H_3BO_3): 269.5, 333.

Apigenin 7-*O*-glucoside (9)- UV λ_{max}^{MeOH}nm: 266 , 337; (NaOMe): 242 (sh), 270, 302 (sh), 350 (sh), 387; ($AlCl_3$): 274, 299, 345, 384 (sh); ($AlCl_3$+HCl): 275.5, 298, 340.5, 382 (sh); (NaOAc): 254 (sh), 268.5, 340, 390 (sh);(NaOAc + H_3BO_3): 268.5, 337.5. ^1H NMR (200 MHz, $CDCl_3$+ CD_3OD): δ 3.4-4.2 (7H, m, sugar protons), 4.91(1H, d, *J*=7 Hz, anomeric proton), 6.37 (1H, d, *J*=2 Hz, H-6), 6.45 (1H, s, H-3), 6.55 (1H, d, *J*=2Hz, H-8), 6.80 (2H, dd, *J*=8.5 and 2 Hz, H-3' and H-5'), 7.66 (2H, dd, *J*=8.5 and 2 Hz, H-2' and H-6'), 12.75 (s, 5-OH).



	R_1	R_2	R_3	R_4
1	H	CH_3	CH_3	OCH_3
2	OCH_3	CH_3	CH_3	OCH_3
3	OCH_3	CH_3	CH_3	H
4	OCH_3	H	H	OCH_3
5	H	CH_3	H	OCH_3
6	OH	CH_3	H	OH
7	H	H	H	OCH_3
8	OH	H	CH_3	OCH_3
9	H	H	H	O-glucose

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