

Phenylpropanoids, Sesquiterpenoids and Flavonoids from *Pimpinella tragi* Vill. subsp. *lithophila* (Schischkin) Tutin

Hilal Özbek¹, Zühal Güvenalp^{1*}, Ayşe Kuruüzüm-Uz²,
Cavit Kazaz³ and L. Ömür Demirezer²

¹Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, Erzurum 25240, Türkiye

²Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara 06100, Türkiye

³Department of Chemistry, Faculty of Science, Atatürk University, Erzurum 25240, Türkiye

(Received December 03, 2014; Revised July 16, 2015; Accepted July 17, 2015)

Abstract: A new sesquiterpenoid named germacradiene-6-*O*-(6'-*O*-acetyl)- β -D-glucoside (**1**) and a new flavonol glycoside named rhamnetin-3-*O*-(2''-*O*- β -D-glucopyranosyl)- β -D-galactopyranoside (**2**), along with three known sesquiterpenoids dictamnol (**3**), radicol (**4**), germacradiene glucoside (**5**); three phenylpropanoids 4-methoxy-2-(3-methyloxiranyl)-phenyl 2-methylbutanoate (**6**), 4-methoxy-2-(3-methyloxiranyl)-phenyl angelate (**7**), thellungianin E (**8**); and a flavonol glycoside platanoside (**9**) were isolated from the aerial parts of *Pimpinella tragi* Vill. subsp. *lithophila* (Schischkin) Tutin. Their structures were elucidated by detailed analyses of 1D and 2D NMR, UV, IR and HR-ESI-MS data.

Keywords: *Pimpinella tragi*; Apiaceae; phenylpropanoid; sesquiterpenoid; flavonoid. © 2015 ACG Publications. All rights reserved.

1. Introduction

The genus *Pimpinella* L. belongs to the Apiaceae family and contains 25 species, 5 subspecies, 4 varieties in the flora of Turkey and 7 of them are endemic [1-2]. This genus is well known for its potential uses in traditional medicine such as carminative, digestive, sedative, expectorant and to increase lactation [3]. Earlier pharmacological studies have shown that some *Pimpinella* species possess antimicrobial, antifungal, antimalarial, estrogenic [4], antibacterial [5], antispasmodic [6], acaricide [7], anticonvulsant [8] and antioxidant [9] activities. Phenylpropanoids [10], flavonoids [11], coumarins [12], monoterpenes [13], diterpenes [14], sesquiterpenes [15], triterpene saponins and steroids [16] were reported in the previous phytochemical studies.

In a previous paper, we described the isolation and structure elucidation of a new trinorguanian-type sesquiterpene and two new flavonol derivatives along with six known flavonoid glycosides and three known sesquiterpenoids from the aerial parts of *Pimpinella cappadocica* Boiss. & Bal. [17]. In a continuation of the research of the secondary metabolites of *Pimpinella* species, we report here the isolation and structure elucidation of a new sesquiterpenoid named germacradiene-6-*O*-(6'-*O*-acetyl)- β -D-glucoside (**1**) and a new flavonol glycoside named rhamnetin-3-*O*-(2''-*O*- β -D-glucopyranosyl)- β -D-galactopyranoside (**2**), along with three known sesquiterpenoids dictamnol (**3**)

* Corresponding author: E-mail: guvenalp@atauni.edu.tr; Phone: 90-442-2315226 Fax: 90-442-2315201

[18], radical (4) [18], germacradiene glucoside (5) [15]; three phenylpropanoids 4-methoxy-2-(3-methyloxiranyl)-phenyl 2-methylbutanoate (6) [19], 4-methoxy-2-(3-methyloxiranyl)-phenyl angelate (7) [20], thellungianin E (8) [21]; and a flavonol glycoside platanoside (9) [22] (Figure 1) from *Pimpinella tragium* subsp. *lithophila*.

2. Materials and Methods

2.1. General

¹H and ¹³C NMR spectra were recorded on a Varian Mercury Plus, 400 MHz for protons and 100 MHz for carbons NMR by using TMS as the internal standard. The solvents were CD₃OD and CDCl₃. HR-ESI-MS was performed on Bruker MicroTOF-Q 55. Optical rotations were obtained on Rudolph-Research Analytical Autopol IV automatic polarimeter. UV spectra were measured with Agilent 8453 spectrophotometer. IR spectra were run on a Perkin Elmer FT-IR Spectrum Bx. Silica gel 60 (0.063-0.200 mm, Merck) and Sephadex LH-20 (Fluka) were used for open column chromatographic separations. Lichroprep RP-18 (25-40 μm, Merck) material was used for vacuum liquid chromatography (VLC). TLC and preparative TLC were carried out on pre-coated Kieselgel 60 F₂₅₄ aluminum sheets (Merck) and compounds were detected under UV fluorescence and sprayed with 1% vanillin-H₂SO₄ reagent, followed by heating at 105 °C.

2.2. Plant Material

The aerial parts of *P. tragium* Vill. subsp. *lithophila* (Schischkin) Tutin were collected from Kayabaşı Bridge on the 20. km of Köprüköy-Hınıs road, Erzurum, Turkey, in July 2009 (1720 m) and identified by Dr. Ebru Dogan Guner from Gazi University, Turkey. A voucher specimen (HUEF 10006) has been deposited in the herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

2.3. Extraction and Isolation

The air-dried and powdered aerial parts (700 g) of *P. tragium* subsp. *lithophila* were extracted with methanol at 40 °C (4 × 2 L). The filtrate was evaporated *in vacuo* to yield 135.1 g residue which was later suspended in 75 mL water and successively fractionated by *n*-hexane (3 x 0.5 L), chloroform (7 x 0.5 L), ethyl acetate (4 x 0.5 L), and *n*-butanol (10 x 0.5 L) to gain 14.5 g, 16.9 g, 8.2 g and 59.7 g residues, respectively.

Chloroform extract was subjected to a silica gel column using *n*-hexane-ethyl acetate solvent system of increasing polarity (100 : 0 → 0 : 100, v/v) to give five subfractions (*Fr. A - E*). Repetitive silica gel column chromatography (CC) with *n*-hexane-ethyl acetate (100 : 0 → 30 : 70) on *Fr. A* gave compound 3 (11.2 mg) and a mixture of 6 (10 mg) and 7 (8 mg); *Fr. C* gave compound 8 (6.9 mg); *Fr. D* gave compound 4 (12.4 mg).

n-Butanol extract was loaded to a silica gel column and eluted with a mixture of CHCl₃/CH₃OH (100 : 0 → 0 : 100) to give four subfractions (*Fr. A - D*). *Fr. A* was submitted on a *Sephadex LH-20* column using CH₃OH as eluent to yield compound 9 (19.6 mg). *Fr. B* was applied to a *Sephadex LH-20* column using CH₃OH as eluent to get *Fr. B₁* which was subjected to preparative TLC with CHCl₃/CH₃OH/H₂O (61 : 32 : 7) to afford compound 1 (6.3 mg). *Fr. C* was submitted on a *Sephadex LH-20* column using CH₃OH and the resulting fraction was chromatographed on silica gel column using CHCl₃/CH₃OH (100 : 0 → 95 : 5) to give *Fr. C₁₋₂*. *Fr. C₁* was submitted on vacuum liquid chromatography (VLC) eluting with H₂O/CH₃OH (100 : 0 → 75 : 25) to give compound 5 (30 mg). *Fr. C₂* was purified by preparative TLC with CHCl₃/CH₃OH/H₂O (61 : 32 : 7) to obtain compound 2 (6.6 mg).

2.4. Germacradiene-6-*O*-(6'-*O*-acetyl)- β -*D*-glucoside (**1**)

White amorphous powder; $[\alpha]_D^{30}$ -73.9 (c 0.7, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ): 241 (1.83), 275 (2.60); IR (KBr) ν_{\max} : 3338, 1731 cm⁻¹; HR-ESI-MS m/z 481.2429 [$M + Na$]⁺ (calcd for C₂₃H₃₈O₉Na, 481.5306); ¹H and ¹³C NMR data, see Table 1.

2.5. Rhamnetin-3-*O*-(2''-*O*- β -*D*-glucopyranosyl)- β -*D*-galactopyranoside (**2**)

Yellowish amorphous powder; $[\alpha]_D^{30}$ -74.2 (c 0.6, MeOH); UV (MeOH) λ_{\max} (log ϵ): 256 (4.43), 357 (4.29); IR (KBr) ν_{\max} : 3301, 1651, 1592 cm⁻¹; HR-ESI-MS m/z 663.1546 [$M + Na$]⁺ (calcd for C₂₈H₃₂O₁₇Na, 663.5319); ¹H and ¹³C NMR data, see Table 2.

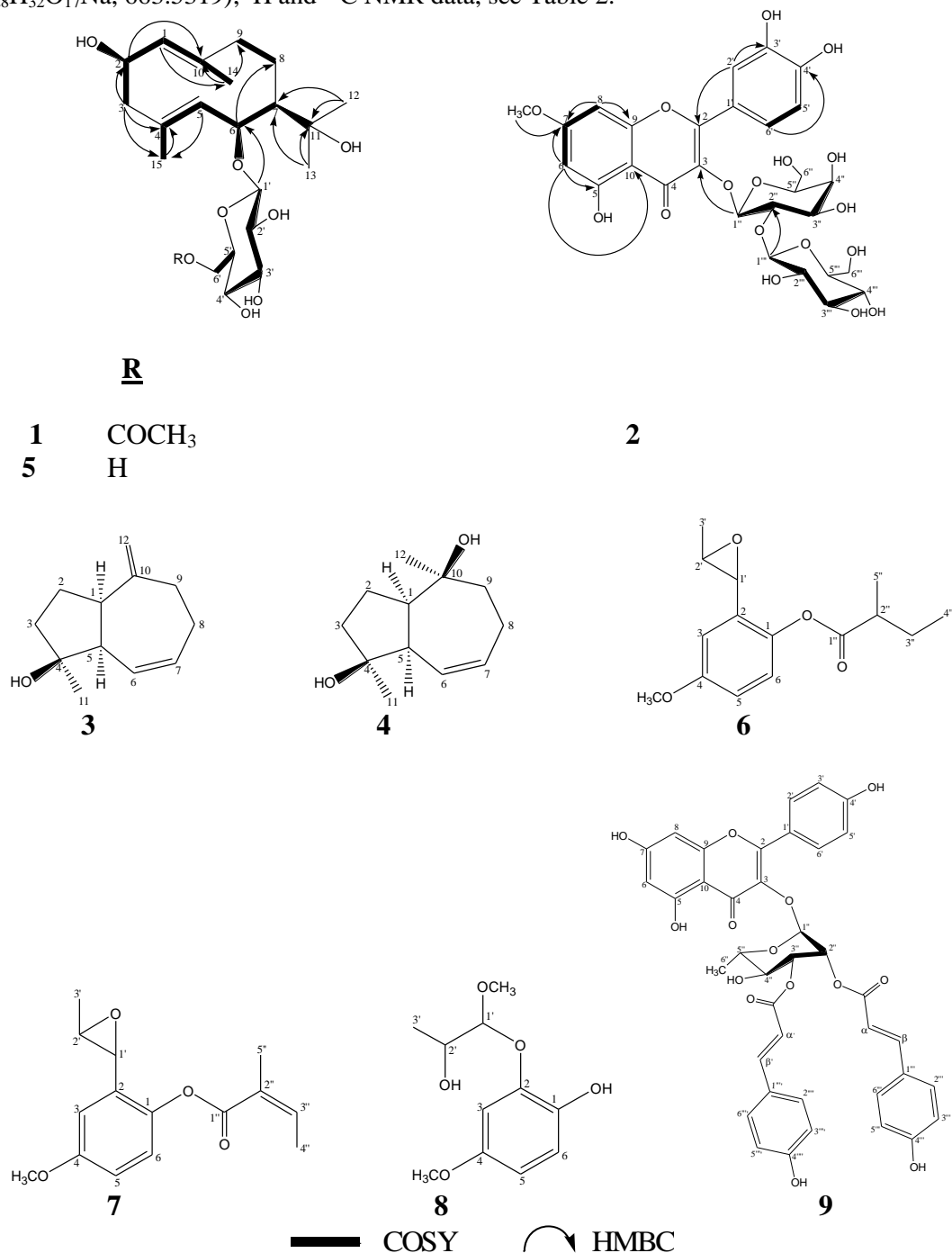


Figure 1. ¹H-¹H COSY correlations and the selected HMBC correlations of compounds **1-2** and the structures of compounds **1-9**.

3. Results and Discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{30}$ -73.9 (c 0.7, CHCl₃); with the molecular formula C₂₃H₃₈O₉, which was deduced by HR-ESI-MS (m/z 481.2429 [$M + Na$]⁺, calcd. 481.5306). The IR spectrum showed absorption bands for hydroxyl groups (3338 cm⁻¹) and carbonyl ester (1731 cm⁻¹). Two allylic methyl groups at δ (H) 1.71 (3H, *s*, H-C(14)), 1.52 (3H, *s*, H-C(15)) and δ (C) 21.3 (C(14)), 16.2 (C(15)); two olefinic methines at δ (H) 5.05 (1H, *d*, $J = 10.2$ Hz, H-C(1)), 5.36 (1H, *d*, $J = 7.1$ Hz, H-C(5)) and δ (C) 132.6 (C(1)), 134.4 (C(5)) with two oxygenated methines at δ (H) 4.60 (1H, *ddd*, $J = 10.1/5.4/-$ Hz, H-C(2)), 4.82 (1H, *d*, $J = 7.1$ Hz, H-C(6)) and δ (C) 66.0 (C(2)), 80.4 (C(6)) were observed in ¹H and ¹³C NMR spectra (Table 1) of compound **1**. Two tertiary methyls at δ (H) 1.23 (3H, *s*, H-C(12)), 1.39 (3H, *s*, H-C(13)) and at δ (C) 27.1 (C(12)), 28.3 (C(13)) with the hydroxyl group and a quaternary carbon at δ (C) 73.3 composed a hydroxyisopropyl side chain of the aglycone. The signals of the ¹³C NMR and DEPT spectra containing four methyl groups, three methylenes, five methines and three quaternary carbons were in accordance with a germacradiene skeleton. An anomeric proton signal at δ (H) 4.30 (1H, *d*, $J = 7.8$ Hz, H-C(1')) with a large coupling constant together with other sugar signals revealed the existence of β -glucose unit by comparing with literature [24] and HMBC correlation between H-C(1') (δ (H) 4.30) and C(6) (δ (C) 80.4) suggested that it was attached to C(6) of the aglycone (Figure 1). In addition, an acetyl group was determined to be affixed at C(6') deduced from the HMBC correlation from the methyl proton (δ (H) 2.03, *s*, 3H) to C-6' (δ (C) 63.4) (Figure 1). Consequently, the structure of **1** was elucidated as germacradiene-6-*O*-(6'-*O*-acetyl)- β -D-glucoside.

Table 1. ¹H NMR (at 400 MHz in CD₃OD, δ in ppm, J in Hz) and ¹³C NMR (at 100 MHz in CD₃OD, δ in ppm) data for compound **1**.

Position	δ_H	δ_C
1	5.05 (1H, <i>d</i> , $J = 10.2$)	132.6 (CH)
2	4.60 (1H, <i>ddd</i> , $J = 10.1, 5.4, -$)	66.0 (CH)
3	2.10 (1H, <i>m</i>) 2.47 (1H, <i>dd</i> , $J = 11.1, 5.3$)	48.0 (CH ₂)
4		130.1 (C)
5	5.36 (1H, <i>d</i> , $J = 7.1$)	134.4 (CH)
6	4.82 (1H, <i>d</i> , $J = 7.1$)	80.4 (CH)
7	1.23 (1H, <i>m</i>)*	50.0 (CH)
8	1.71 (1H, <i>m</i>) 2.03 (1H, <i>m</i>)	25.8 (CH ₂)
9	1.70 (1H, <i>m</i>) 2.47 (1H, <i>m</i>)	35.6 (CH ₂)
10		136.2 (C)
11		73.3 (C)
12	1.23 (3H, <i>s</i>)	27.1 (CH ₃)
13	1.39 (3H, <i>s</i>)	28.3 (CH ₃)
14	1.71 (3H, <i>s</i>)	21.3 (CH ₃)
15	1.52 (3H, <i>s</i>)	16.2 (CH ₃)
1'	4.30 (1H, <i>d</i> , $J = 7.8$)	103.7 (CH)
2'	3.13 (1H, <i>t</i> , $J = 9.1$)	73.7 (CH)
3'	3.33 (1H, <i>m</i>)*	76.8 (CH)
4'	3.21 (1H, <i>t</i> , $J = 9.2$)	70.4 (CH)
5'	3.40 (1H, <i>m</i>)	74.0 (CH)
6'	4.20 (1H, <i>dd</i> , $J = 11.4, 6.8$) 4.28 (1H, <i>dd</i> , $J = 11.4, 2.1$)	63.4 (CH ₂)
OCOCH ₃		171.2 (C)
OCOCH ₃	2.03 (3H, <i>s</i>)	19.4 (CH ₃)

*Overlapped signals.

Compound **2** was obtained as yellow amorphous powder, $[\alpha]_D^{30}$ -74.2 (c 0.6, MeOH); with the molecular formula $C_{28}H_{32}O_{17}$, which was deduced by HR-ESI-MS (m/z 663.1546 $[M + Na]^+$, calcd. 663.5319). The UV spectrum displayed maximum absorption bands of a 3-OH substituted flavonol skeleton at λ_{max} 256 and 357 nm. [25]. The IR spectrum of **2** showed absorption bands for hydroxyl groups (3301 cm^{-1}), α , β -unsaturated carbonyl (1651 cm^{-1}), and aromatic C=C (1592 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra (Table 2) displayed a total of 28 carbon signals arising from 10 quaternary, 15 methines, 2 methylenes, and 1 methoxyl carbons corresponding to a flavonoid aglycone and two sugar moieties. In ^1H NMR spectrum, an ABX system was observed at $\delta(\text{H})$ 7.77 (1H, *d*, $J = 2.2$ Hz, H-C(2')), 7.58 (1H, *dd*, $J = 8.4/2.2$ Hz, H-C(6')) and 6.89 (1H, *d*, $J = 8.4$ Hz, H-C(5')), confirming the 3',4'-disubstituted B ring of a flavonoid. ^1H NMR spectrum displayed two characteristic meta coupled protons at $\delta(\text{H})$ 6.31 (1H, *bs*, H-C(6)) and 6.57 (1H, *bs*, H-C(8)) for 5,7-disubstituted A ring. In addition, the methoxyl signal at $\delta(\text{H})$ 3.87 was correlated with C(7) ($\delta(\text{C})$ 165.8) in the HMBC spectrum, showing the attachment of this methoxyl group to C(7) of ring A. Therefore, the aglycone part of **2** was identified as rhamnetin [26]. Two anomeric proton signals at $\delta(\text{H})$ 5.29 (1H, *d*, $J = 7.5$ Hz, H-C(1'')) and 4.76 (1H, *d*, $J = 7.4$ Hz, H-C(1''')) confirmed the presence of two sugar units which were deduced to be galactose and glucose by comparing their spectroscopic data with those given in literature [24]. The H-C(4) signal of galactose observed at $\delta(\text{H})$ 3.87 (1H, *m*) confirmed the presence of galactose unit. The HMBC correlation between the anomeric proton of galactose ($\delta(\text{H})$ 5.29) and C(3) ($\delta(\text{C})$ 133.8) in addition to anomeric proton of glucose ($\delta(\text{H})$ 4.76) and C(2'') ($\delta(\text{C})$ 79.2) showed the linkage of galactose unit to C(3) of the aglycone and glucose unit to down field signal of C(2'') (Figure 1). Therefore, the structure of compound **2** was determined as rhamnetin-3-*O*-(2''-*O*- β -D-glucopyranosyl)- β -D-galactopyranoside.

Table 2. ^1H NMR (at 400 MHz in CD_3OD , δ in ppm, J in Hz) and ^{13}C NMR (at 100 MHz, in CD_3OD , δ in ppm) data for compound **2**.

Position	δ_{H}	δ_{C}
2		157.7 (C)
3		133.8 (C)
4		178.5 (C)
5		161.4 (C)
6	6.31 (1H, <i>bs</i>)	97.6 (CH)
7		165.8 (C)
8	6.57 (1H, <i>bs</i>)	91.6 (CH)
9		156.9 (C)
10		105.1 (C)
1'		121.2 (C)
2'	7.77 (1H, <i>d</i> , $J = 2.2$)	116.4 (CH)
3'		144.6 (C)
4'		148.8 (C)
5'	6.89 (1H, <i>d</i> , $J = 8.4$)	114.8 (CH)
6'	7.58 (1H, <i>dd</i> , $J = 8.4, 2.2$)	121.7 (CH)
OCH ₃	3.87 (3H, <i>s</i>)	55.1 (CH ₃)
1''	5.29 (1H, <i>d</i> , $J = 7.5$)	100.2 (CH)
2''	4.07 (1H, <i>t</i> , $J = 8.7$)	79.2 (CH)
3''	3.73 (1H, <i>m</i>)	73.4 (CH)
4''	3.87 (1H, <i>m</i>)	68.6 (CH)
5''	3.46 (1H, <i>m</i>)	75.5 (CH)
6''	3.53 (1H, <i>dd</i> , $J = 11.0, 6.0$)	60.4 (CH ₂)
	3.61 (1H, <i>dd</i> , $J = 11.0, 4.5$)	
1'''	4.76 (1H, <i>d</i> , $J = 7.4$)	103.6 (CH)
2'''	3.43 (1H, <i>m</i>)	74.0 (CH)
3'''	3.33 (1H, <i>m</i>)	76.6 (CH)
4'''	3.44 (1H, <i>m</i>)	69.6 (CH)
5'''	3.45 (1H, <i>m</i>)	76.4 (CH)
6'''	3.72 (1H, <i>m</i>)	60.8 (CH ₂)
	3.79 (1H, <i>m</i>)	

In this study, radicol (**4**), germacradiene glucoside (**5**), thellungianin E (**8**) were isolated from *P. tragium* Vill. subsp. *lithophila* (Schischkin) Tutin and platanoside (**9**) from the genus *Pimpinella* for the first time.

Acknowledgments

This work was mainly supported by the financial support of Atatürk University, Turkey (2013/270).

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

References

- [1] V. A. Matthews (1972). *Pimpinella* L., In: Flora of Turkey and the East Aegean Islands, ed: P. H. Davis, Univ. Press., Edinburgh, pp. 352-364.
- [2] A. Güner, S. Aslan, T. Ekim, M. Vural and M. T. Babaç (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul.
- [3] T. Baytop (1999). Türkiye’de Bitkiler ile Tedavi (Geçmişte ve Bugün). Nobel Tıp Kitabevleri, İstanbul.
- [4] K. H. C. Başer, N. Tabanca, N. Kırimer, E. Bedir, I. A. Khan and D. E. Wedge (2007). Recent advances in the chemistry and biological activities of the *Pimpinella* species of Turkey, *Pure Appl. Chem.* **79**, 539-556.
- [5] D. A. Ateş and O. T. Erdoğan (2003). Antimicrobial activities of various medicinal and commercial plant extracts, *Turk. J. Biol.* **27**, 157-162.
- [6] C. R. Tirapelli, C. R. De Andrade, A. O. Cassano, F. A. De Souza, S. R. Ambrosio, F. B. Da Costa and A. M. De Oliveira (2007). Antispasmodic and relaxant effects of the hidroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeus smooth muscle, *J. Ethnopharmacol.* **110**, 23-29.
- [7] H. S. Lee (2004). *p*-Anisaldehyde: Acaricidal component of *Pimpinella anisum* seed oil against the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, *Planta Med.* **70**, 279-281.
- [8] F. Karimzadeh, M. Hosseini, D. Mangeng, H. Alavi, G. R. Hassanzadeh, M. Bayat, M. Jafarian, H. Kazemi and A. Gorji (2012). Anticonvulsant and neuroprotective effects of *Pimpinella anisum* in rat brain, *BMC Complem. Altern. M.* **12**, 76.
- [9] Z. Güvenalp, H. Özbek, M. Yüzbaşıoğlu, A. Kuruüzüm-Uz and L. Ö. Demirezer (2010). Flavonoid quantification and antioxidant activities of some *Pimpinella* species, *Rev. Anal. Chem.* **29**, 233-240.
- [10] N. Tabanca, E. Bedir, D. Ferreira, D. Slade, D. E. Wedge, M. R. Jacob, I. S. Khan, N. Kırimer, K. H. C. Baser and I. A. Khan (2005). Bioactive constituents from Turkish *Pimpinella* species, *Chem. Biodivers.* **2**, 221-232.
- [11] A. Kuruüzüm-Uz, Z. Güvenalp, M. Yüzbaşıoğlu, H. Özbek, C. Kazaz and L. Demirezer (2010). Flavonoids from *Pimpinella kotschyana*, *Planta Med.* **76**, 1261.
- [12] B. Qiao, C. Wang, H. Shi, C. Mi and F. Li (1996). Studies on the chemical constituents of the root of *Pimpinella thellungiana*, *Zhongcaoyao* **27**, 136-138.
- [13] E. Fujimatu, T. Ishikawa and J. Kitajima (2003). Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise, *Phytochemistry* **63**, 609-616.
- [14] G. Burkhardt, J. Reichling, R. Martin and H. Becker (1986). Terpene hydrocarbons in *Pimpinella anisum* L., *Pharm. Weekblad.* **8**, 190-193.
- [15] W. Kisiel, Z. Janeczko and M. Zgud-Walaszek (1998). A germacradiene glycoside from roots of *Pimpinella saxifraga*, *Phytochemistry* **49**, 2031-2033.
- [16] L. Ö. Demirezer, A. Kuruüzüm-Uz, Z. Güvenalp, A. Simon and T. Patocs (2012). Further secondary metabolites from *Pimpinella kotschyana*, *Planta Med.* **78**, 1235.
- [17] H. Özbek, Z. Güvenalp, A. Kuruüzüm-Uz, C. Kazaz and L. Ö. Demirezer (2015). Trinorguaian and germacradiene type sesquiterpenes along with flavonoids from the herbs of *Pimpinella cappadocica* Boiss. & Bal., *Phytochem. Lett.* **11**, 74-79.
- [18] P. H. Zhao, X. P. Yang and C. S. Yuan (2008). A novel trinorguaian-type sesquiterpene from *Dictamnus radices*, *Nat. Prod. Res.* **22**, 208-211.
- [19] R. Martin, J. Reichling and H. Becker (1985). Reinvestigation of the phenylpropanoids from the roots of *Pimpinella* species, *Planta Med.* **51**, 198-202.

- [20] M. J. Macias, V. Martin, M. Grande and K. H. Kubeczka (1994). Phenylpropanoids from *Pimpinella villosa*, *Phytochemistry* **37**, 539-542.
- [21] F. X. Li, C. D. Wang, C. F. Mi, H. L. Shi and B. L. Qiao (1998). Chemical constituents of the root of *Pimpinella thellungiana*, *Zhiwu Xuebao* **40**, 88-90.
- [22] M. Kaouadji (1990). Acylated and non-acylated kaempferol monoglycosides from *Platanus acerifolia* buds, *Phytochemistry* **29**, 2295-2297.
- [23] A. A. Basma, A. Balboul, A. A. Ahmed, H. Otsuka and A. De Adams (1996). 4-Hydroxyphenylpropan-7,8-diols and derivatives from *Narvalina domingensis*, *Phytochemistry* **42**, 1191-1193.
- [24] S. Bendaikha, M. Gadaut, D. Harakat and A. Magid (2014). Acylated flavonol glycosides from the flower of *Elaeagnus angustifolia* L., *Phytochemistry* **103**, 129-136.
- [25] T. R. Markham (1982). Techniques of flavonoid identification. Academic Press, London.
- [26] A. J. Merina, D. Kesavan and D. Sulochana (2011). Isolation and antihyperglycemic activity of flavonoid from flower petals of *Opuntia stricta*, *Pharm. Chem. J.* **45**, 317-321.

ACG
publications

© 2015 ACG Publications