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Flavonoids from *Zosimia absinthifolia* (Vent.) Link

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Abstract: Flavonoids are secondary metabolites produced mainly in plants and fruits. Their biological activities have an impact on human health so that they serve as target molecules to develop new drugs. From methanolic extract of aerial parts of *Zosimia absinthifolia* (Vent.) Link. (Umbelliferae), two flavonoids namely 4′- ethoxy naringenin (compound 1), an acetylated chrysoeriol rhamnoside(compound 2) and a chalcone derivative(compound 3) have been isolated by column chromatography (CC) and preparative TLC. Those structures were elucidated by UV, ^{1}H- and ^{13}C- NMR, HMBC, EI-MS and IR spectra.

Key words: *Zosimia absinthifolia* · Umbelliferae · Methanolic extract · Flavonoid · 4′- Ethoxy naringenin · Acetylated chrysoeriol rhamnoside

INTRODUCTION

Zosimia absinthifolia (Vent.) Link. is a species of Umbelliferae herb which growing in many regions of Iran and Turkey. The genus *Zosimia* is represented in Iran by two species: *Z. radians* Boiss. and Hohen. and Z. *absinthifolia* (Vent.) Link, which *Z. radians* is endemic plant [1]. The composition of the oil of dried fruits of *Z. absinthifolia* from Turkey has been reported. Octyl acetate (38.1%) and octyl hexanoate (31.9%) were the main constituents [2]. In investigation on phytochemical analyses of *Z. absinthifolia* roots, two lactones compound (zosimin and deltoin) were identified in methanolic extract [3]. Our previous report on the essential oil of *Z. absinthifolia* from North-west Iran showed that its major components were octyl acetate (24.7%) and β-caryophyllene (22.2%) [4]. In addition, a report of the essential oil of *Z. absinthifolia* aerial parts from south Iran is found in the literature [5].

Octyl butyrate (19.2%), β- caryophyllene (13.9%), octanol (9.6%), geranyl valerate (9.6%) and caryophyllene oxide (5.7%) were the major constituents. Quantitative and qualitative changes of essential oil from *Zosimia absinthifolia* (Vent.) Link. in different phenological stages so has been reported from Iran. α- Pinene, *n*-octanol, germacrene-D, β-caryophyllene, octyl acetate, caryophyllene oxide and limonene were the main components of essential oil of different growth stages [6]. We now report on 2 flavonoids and a chalcon of the methanol extract from aerial parts of *Zosimia absinthifolia* (Vent.) Link. for first time.

MATERIALS AND METHODS

General Experimental: The IR spectra were determined on a Bruker Tensor 27 spectrometer. The ^{1}H-NMR and ^{13}C - NMR spectra were recorded on a Bruker AM 300 spectrometer. Column chromatography was performed over silica- gel (70-230 mesh, Merck,) using petroleum ether, AcOEt, methanol gradients as elution solvents. UV spectra were recorded on a Perkin- Elmer Lambda 12 spectrophotometer and Mass spectra were recorded on an AEI MS-50 spectrometer.

Plant Materials: The aerial parts of *Zosimia absinthifolia* was collected in June 2009 from Givi- Khalkhal road (Ardabil province) in northwest of Iran at an altitude of 1400m. A voucher specimen (No: 028) has been deposited at the Herbarium of the Agriculture Research Centre (A.R.C.) Ardabil, Iran.

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Extraction and Isolation: Dried and finely powdered *Zosimia absinthifolia* aerial parts (400g) were extracted with methanol in a Soxhlet apparatus during 2 days. The concentrated total extract (25g) was extracted with petroleum ether, CHCl3, EtOAc and *n*-BuOH, respectively. Part of the EtOAc portion (3 g) was subjected to silica gel column chromatography (70-230 mesh, Merck), eluted with an equivalent petroleum ether, AcOEt, methanol stepwise gradients to obtain 38 fractions (15mL each). Fractions 9-15 after solvent evaporate were in turn chromatographed over silica gel with $CHCl_3$: MeOH mixture to provide 18 subfractions. Subfraction 8 (120 mg) was rechromatographed on silica gel into 22 fractions (22×15 mL.) using as eluents an 8.25:1.75 $CHCl_3$: MeOH mixture. The combined fractions 5 to 8(23 mg) were further purified on a preparative TLC to give compound 1 (14 mg).

A portion of the AcOEt (0.37 g of fractions 18- 26 after removal of solvent) was chromatographed over a small column (15cm × 1.5 cm) with AcOEt: MeOH (8.5: 1.8) as the eluents. A total of 13 fractions were collected. The combined fractions 9 to 13(65 mg) according to TLC analysis were further purified on a preparative TLC to give compound 2(19 mg).

Fractions 30- 36 (235 mg) of the main initial 38 fractions, was divided into six subfractions on a silica gel column using AcOEt: methanol as eluents. The subfraction 3,4and5 with medium polarity (83 mg) was further resolved on a silica gel column using a gradient of $CHCl_3$: MeOH as eluents to afford 9 subfractions. The third one (11 mg) was compound 3. The known flavonoids were readily identified as Naringenin 4′- ethoxy, Flavone- 5, 8- dihydroxy- 7- *O*- rhamnosyl- 3′- methoxy- 4′- *O*- acetyl and Chalcon- 2′, 3, 4′, 6′- tetrahydroxy- 4- O- angelyl, by comparing their physical and spectroscopic data with those reported in the literature [17- 20].

RESULTS AND DISCUSSION

The ^{1}H-NMR spectrum of compound 1 displayed the characteristic signals of the naringenin nucleus. Compound 1 (4′- ethoxy naringenin) was obtained in the form of yellow amorphous solid, mp 154-157°C. The molecular formula, $C_{17}H_{16}O_5$ was obtained on the basis of the ^{13}C-NMR and Mass spectra analysis. The identification of the compounds was supported by comparison with published data of related compounds [7-11].

The combination of ^{1}H-, ^{13}C -NMR and HMBC correlation spectral data of 1 indicated the presence of an ethoxyl group [δH 4.05(2H, q, H-1′′), 1.22(3H, t, H-2′′), with the corresponding δC 62.8 and 14.6, respectively], one oxygenated -CH group [δH 5.43(1H, dd, J= 13.5, 6.0Hz, H-2) with the correlated δC 78.4(C-2)], a coupling system with H-2 comprises the two protons on the α- position to carbonyl group, which because of stereocenter in the molecule are diastereotopic[δH 3.26(1H, dd, J=13.5, 17.5 Hz, H_a-3) and 2.68 (1H, dd, J=17.5,6.0 Hz, H_b-3) with the relevant δC 42.3].

The ^{13}C-NMR spectrum showed 17 signals corresponding to six aromatic -CH- and seven quaternary carbons [12]. The UV spectrum exhibited absorption maxima at 281 nm and 368 nm that are characteristic absorption bands of a flavanone skeleton. The absorption maximum 269 and 363 nm for compound 2(a flavone skeleton), 212 and 375 nm those are characteristic of a chalcone skeleton [16].

In the HMBC spectrum, correlation were observed between δH 4.05 and δC 157.7 (C-4′), confirming the locations of the ethoxyl group (fig.1). The above mentioned data suggested compound 1 to be an ethoxylated flavone.

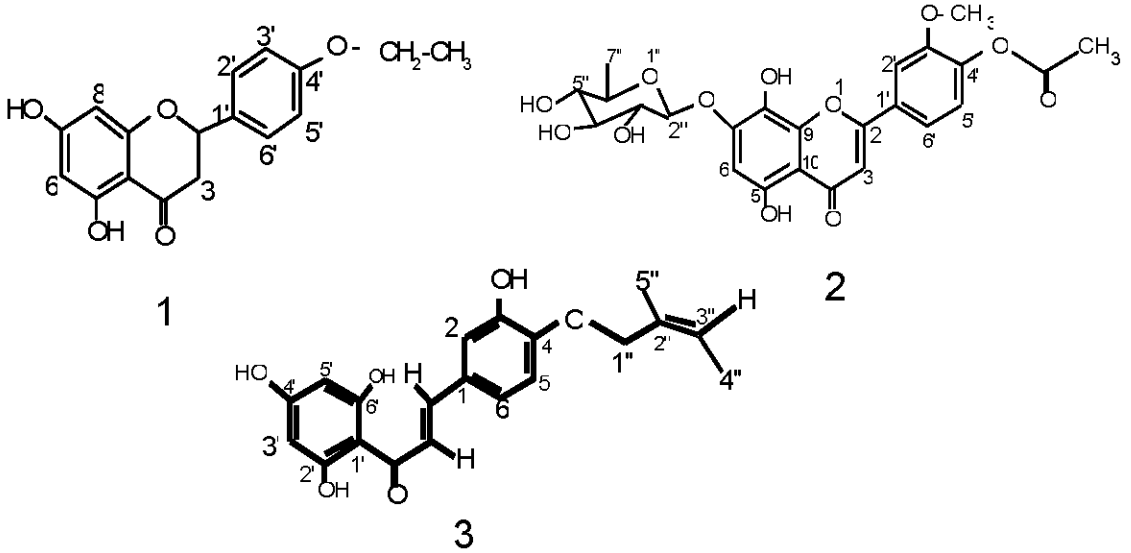
In compound 2 three methyl groups were observed on the spectrum which assignment to –O-Methyl [δH 3.96(3H, s)], sugar methyl [δH 1.12(3H, d, J=11.3Hz, H-7′′)] and acetyl group [δH 2.08(3H, s)] with the corresponded δC 55.7, 16.8 and 19.7 respectively. From the above described spectral evidence, compound 2 was identified conclusively as acetylated chrysoeriol rhamnoside namely 5, 8-dihydroxy-7-*O*-rhamnosyl-3′-methoxy-4′-*O*-acetylflavone.

^{1}H-NMR spectrum of compound 3 showed three ABX type phenyl protons at δH 7.49 (1H, d, *J* = 8.2 Hz, H-5), 6.88 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.85 (1H, d, *J* = 1.9 Hz, H-2)], two doublet signals at δH 7.21 (1H, d, J= 1.9Hz, H-3′) and 7.81 (1H, d, J= 1.9Hz, H-5′), two other doublet signals at δH 6.23 (1H, d, J= 15.6Hz, H- α) and 7.88 (1H, d, J= 15.6Hz, H- β). Furthermore, the ^{1}H- and ^{13}C-NMR spectral data (Table 1) indicated the presence of an angelyl moiety [δH 4.61(2H, s, H-1″), 4.11(1H, m, H-3″), 1.92(3H, s, H-5″)and 1.31(3H, d, J= 1.5Hz, H-4″)], which was in accordance with peaks observed at δC 101.4, 112.9, 25.1 and 13.5 ppm respectively. In addition to five angelyl carbons and the flavonoid group signals, 20 skeletal carbon resonances appeared in the ^{13}C-NMR spectrum (Table 1).

Table 1: ^{1}H, ^{13}C-NMR and HMBC data of compounds 1, 2 and 3(CD3OD)[a].

	1			2			3		
NO	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	-	-	-	-	-	-	-	119.3	-
2	5.43,dd(13.5, 6.0)	78.4	C-4, C-9	-	171.2	-	6.85,d(1.9)	123.8	C-4,C-β
3a	3.26,dd(17.5,13.5)	42.3	-	6.42,s	103.7	-	-	144.8	-
3b	2.68, dd(6, 17.5)	42.3	-	-	-	-	-	-	-
4	-	196.1	-	-	198.2	-	-	156.1	-
5	9.6, s(OH)	163.5	C-6, C-10	-	165.1	C-6, C-10	7.49,d(8.2)	105.5	C-1,C-3
6	5.9, d(1.9)	95.8	C-8, C-10	6.22,s	101.4	C-8, C-10	6.88,dd(8.2,1.9)	140.5	C-4,C-β
7	-	166.6	-	-	143.3	-	-	-	-
8	5.9, d(1.9)	94.9	C-6, C-10	8.02,s(OH)	127.9	-	-	-	-
9	-	162.8	-	-	157.5	-	-	-	-
10	-	101.8	-	-	105.2	-	-	-	-
1′	-	128.8	-	-	122.6	-	-	136.9	-
2′	7.32, d(8.2)	128.1	C-2, C-4′	7.31,d(1.9)	115.1	C-2, C-4′	-	162.4	-
3′	6.81,d(8.2)	115.2	C-1′, C-5′	-	134.6	-	7.21,d(1.9)	113.4	C-1′,C-5′
4′	-	157.7	-	-	149.3	-	-	162.8	-
5′	6.81,d(8.2)	115.2	C-1′, C-3′	6.91, d(8.2)	116.7	C-1′, C-3′	7.81,d(1.9)	113.4	C-1′,C-3′
6′	7.32, d(8.2)	128.1	C-2′, C-4′	7.66,dd(1.9,8.2)	122.1	C-2′, C-4′	-	172.1	-
1″	4.05	62.8	C-4′	-	-	-	4.61,s	101.4	C-4, 3″,5″
2″	1.22	14.6	-	5.56, d(13.5)	98.9	C-7	-	129.4	-
3″	-	-	-	3.29, m(sugar)	70.3	-	4.11,m	112.9	C-1″,5″
4″	-	-	-	3.32, m(sugar)	71.2	-	1.31,d(1.5)	13.5	-
5″	-	-	-	3.49, m(sugar)	73.6	-	1.92, s	25.1	C-1″,3″
6″	-		-	3.85, m(sugar)	77.3	-	-	-	-
7″	-	-	-	1.12, d(11.3)	16.8	C-5″	-	-	-
O-CH_3	-	-	-	3.96, s	55.7	C-3′	-	-	-
$COCH_3$	-	-	-	2.08, s	19.7	-	-	-	-
$COCH_3$	-	-	-	-	162.1	-	-	-	-
α	-	-	-	-	-	-	6.23,d(15.6)	122.1	C-1,C-1′
β	-	-	-	-	-	-	7.88,d(15.6)	141.2	CO,C-2,6

[a] Coupling pattern and coupling constants (*J* in Hz) are in parentheses.



Significant HMBC correlations were observed between H-1" and C-4 and between H-1" with C-3"and C-5", confirming the location of the –O angelyl group. The ^{1}H-, ^{13}C -NMR and HMBC spectral data of 3 indicated the presence of an α, β- unsaturated ketone system [δH 6.23(1H, d, J=15.6Hz, H-α), 7.88(1H, d, J= 15.6Hz, H- β), with the corresponding δC 122.1 and 141.2, respectively] (fig. 1.). The coupling constant between H-α and H-β

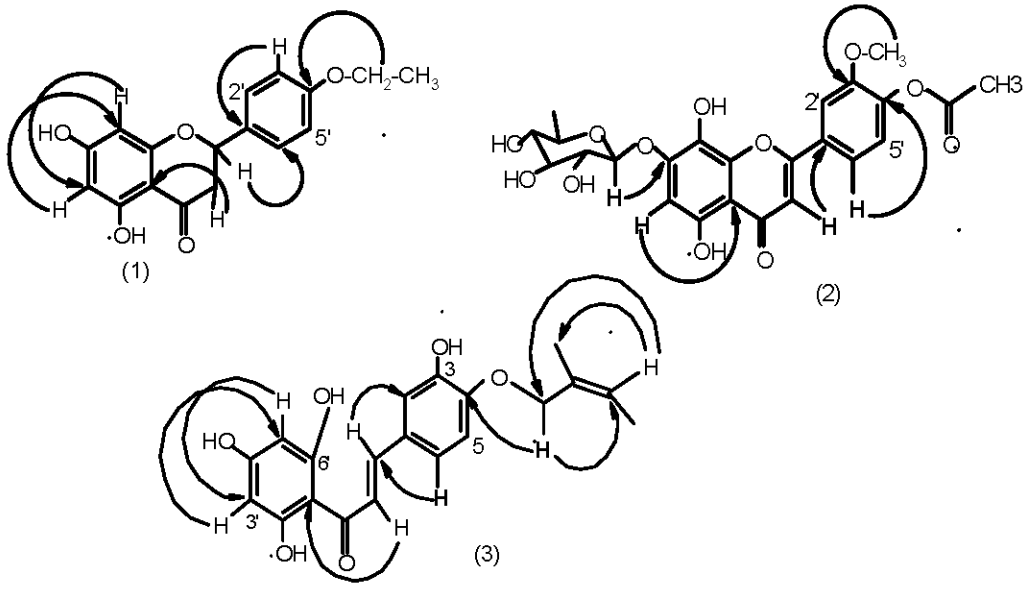


Fig. 1: The selected HMBC correlation of compounds 1, 2 and 3

(15.6Hz) show a trans status between those two protons (Table1). The compound was readily identified by comparing their physical and spectroscopic data with those reported in the literature [13-15]. From this results, compound 3 was identified as 2′, 3, 4′, 6′- tetrahydroxy- 4-O-angelylchalcon.

CONCLUSION

Compound 1 (Naringenin 4′- ethoxy): This compound was suggested to be a flavonoid based on the physico-chemical properties and chromatography performance. The IR absorptions displayed a hydroxyl at (3227 cm^{-1}) and ketone (1628 cm^{-1}) groups, yellow amorphous powder with MP. 154- 157°C.
$[\alpha]_D$: -12(*c* 0.05 MeOH).
UV λ_{max}(MeOH) 281 and 368 nm.
^{1}H-NMR and ^{13}C–NMR (CD_3OD): see Table 1.
ESIMS: *m/z* 300.82 [M-H] - ion.
Molecular formula $C_{17}H_{16}O_5$.
The mass, HRFABMS m/z $(M+H)^+$, calculated for $C_{17}H_{16}O_5$ was 300.8163; the mass found was 300.8176.

Compound 2 (Flavone- 5, 8- dihydroxy- 7- *O*- rhamnosyl- 3′- methoxy- 4′- *O*- acetyl):
It was obtained in the form of yellow amorphous crystals. The NMR spectrum of 2 has signals for the flavonoid with the sugar unit (rhamnoside). The identification of the monosaccharide chain was determined by analysis of a combination of 2D- COSY and HMBC NMR spectra. In the HMBC spectrum of 2, correlations were observed between δH 5.56 and δC 143.3 (C-7) and between δH 3.96 and δC 134.6 (C-3′), confirming the locations of the rhamnosyl and methoxyl groups, respectively (fig.1.).

UV λ_{max}(MeOH) 269 and 363 nm.
^{1}H-NMR and ^{13}C–NMR (CD_3OD): see Table 1.
HMBC Fig. 1.
ESIMS: *m/z* 456.82 [M-H] - ion.
Molecular formula $C_{24}H_{24}O_{12}$.
The mass, HRFABMS m/z $(M+H)^+$, calculated for $C_{24}H_{24}O_{12}$ was 456.8213; the mass found was 456.8221.

Compound 3(Chalcon- 2′, 3, 4′, 6′-Tetrahydroxy- 4-O-Angelyl): A pale yellow amorphous powder,
UV λ_{max}(MeOH) 212 and 375 nm.
^{1}H-NMR and ^{13}C–NMR (CD_3OD): see Table 1.
HMBC Fig. 1.
ESIMS: *m/z* 356.204 [M-H] - ion.
Molecular formula $C_{20}H_{20}O_6$.
The mass, HRFABMS m/z $(M+H)^+$, calculated for $C_{20}H_{20}O_6$ was 356.2041; the mass found was 356.2064.

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REFERENCES

1. Mozaffarian, V., 2007. A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran, Iran, pp: 227.
2. Baser, K.H.C., T. Ozek, B. Demirci, M. Kurkeuoglu, Z. Aytac and H. Duman, 2000. Composition of the essential oil of *Zosimia absinthifolia* (Vent.) Link and *Ferula elaeochytris* Korovin from Turkey. Flavour and Fragrance J., 15(6): 371-372.
3. Nikonov, G.K. and D.I. Baranauskaite, 1965. Lactones of *Zosimia absinthifolia* (vent) link. Chemistry of Natural Compounds, 1: 169-172.
4. Shafaghat, A., 2007. Chemical Constituents of the Essential Oil of *Zosimia absinthifolia* (vent) Link. from Iran. J. Medicinal Plants, 6(22): 34-38.
5. Javidnia, K., R. Miri, M. Soltani and A.R. Khosravi, 2008. Constituents of the volatile oil of *Zosimia absinthifolia* (Vent.) Link. from Iran. J. Essential Oil Res., 20(2): 114-116.
6. Amiri, H., 2008. Quantitative and Qualitative Changes of Essential Oil of *Zosimia Absinthifolia* (Vent.) Link. in Different Phenological Stages. Iranian Journal of Medicinal and Aromatic Plants, 24(2): 217- 224.
7. Fathy, M.S., H.S. Afaf, E.K. Amal and M.E. Shahera, 2002. An Acylated Kaempferol Glycoside from Flowers of *Foeniculum vulgare* and F. Dulce. Molecules, 7: 245- 251.
8. Singh, V.P., Y. Bineeta and V.B. Pandey, 1999. Flavanone glycosides from *Alhagi pseudalhagi.* Phytochemistry, 51: 587-590.
9. Kang, T.H., S.J. Jeong, W.G. Ko, N.Y. Kim, B.H. Lee, M. Inagaki, T. Miyamoto, R. Higuchi and K.Y. Chul, 2000. Cytotoxic lavandulyl flavanones from *Sophora flavescens*. J. Natural Products, 63: 680-681.
10. Demole, E. and P. Enggist, 1974. Novel synthesis of 3,5,5-trimethyl-4-(2-butenylidene)-cyclohex-2-en-1-one, a major constituent of Burley Tobacco flavour. Helvetica Chemica Acta, 7: 2087-2091.
11. Andersson, R. and R. Lundgren, 1988. Monoaryl and cyclohexenone glycosides from needles of *Pinus sylverstris*. Phytochemistry, 27: 559-562.
12. Almahy, H.A., M. Rahmani, M.A. Sukari and A.M. Ali, 2003. The Chemical Constituents of *Ficus benjamina* Linn.and Their Biological Activities. Pertanika J. Sciences and Technol., 11(1): 73-81.
13. Yoshimura, M., A. Sano, J.I. Kamei and A. Obata, 2009. Identification and Quantification of Metabolites of Orally Administered Naringenin Chalcone in Rats, J. Agriculture and Food Chemistry, 57(14): 6432- 6437.
14. Giuseppina, C., M.E. Luis, B. Alessandra and T. Nunziatina, 2003. Antioxidant chalcone glycosides and flavanones from Maclura (Chlorophora) tinctoria. J. Natural Products, 66(8): 1061-1064.
15. Fuendjiep, V., J. Wandji, F. Tillequin, D.A. Mulholland, H. Budzikiewicz, Z.T. Fomum, A.M. Nyemba and M. Koch, 2002. Chalconoid and stilbenoid glycosides from *Guibourtia tessmanii*. Phytochemistry, 60(8): 803-806.
16. Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, London, pp: 17-265.
17. Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systematic Identification of Flavonoids. Springer, Berlin, pp: 15-250.
18. Markham, K.R., B. Ternai, R. Stanly, H. Geiger and T.J. Mabry, 1978. Carbon-13 NMR studies of flavonoids- III. Tetrahedron, 34: 1389-1397.
19. Harbone, J.B., 1986. In the Flavonoids. Advances in Research, Jay M. Ed. Chapman and Hall, London, New York, pp: 27-97.
20. Jorge, F.S.F., L.L. Devanand, S. Tomikazu and H. Arne, 2010. Flavonoids from *Artemisia annua* L. as Antioxidants and Their Potential Synergism with Artemisinin against Malaria and Cancer. Molecules, 15: 3135-3170.