SHORT REPORT

A C G publications

Rec. Nat. Prod. 9:4 (2015) 609-613

records of natural products

Two New Flavone Glycosides from *Chenopodium ambrosioides* Growing Wildly in Egypt

Hala M. Hammoda[1*], Fathalla M. Harraz[1], Maged G. El Ghazouly[1,2], Mohamed M. Radwan[1,4], Mahmoud A. ElSohly[3,4], Amira S. Wanas[4,5] and Samar M. Bassam[2]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University 21521, Alexandria, Egypt*
[2] *Department of Pharmacognosy, Faculty of Pharmacy, Pharos University, Alexandria, Egypt*
[3] *Department of Pharmaceutics, School of Pharmacy, University of Mississippi, MS 38677, USA*
[4] *National Center for Natural Products Research, School of pharmacy, University of Mississippi, University MS 38655, USA*
[5] *Department of Pharmacognosy, Faculty of Pharmacy, University of Minia, Egypt*

(Received May 25, 2014; Revised August 24, 2014; Accepted August 31, 2014)

Abstract: *Chenopodium ambrosioides* (Chenopodiaceae) growing wildly in Egypt was subjected to antioxidant –guided phytochemical investigation and the EtOAc fraction afforded the two new flavone glycosides; scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside **(1)** and scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside **(2)**. In addition, the *in vitro* antioxidant activities of the plant alcohol extract, $CHCl_3$ fraction, EtOAc fraction and isolates were studied.

Keywords: *Chenopodium ambrosioides*; scutellarein-7-*O*-*α*-rhamnopyranosyl-(1→2)-*α*-rhamnopyranosyl-(1→2)-*α*- rhamnopyranoside; scutellarein-7-*O*-*α*-rhamnopyranosyl-(1→2)-*α*-rhamnopyranoside; flavone glycoside; Antioxidant..

1. Plant Source

Species of the family Chenopodiaceae are widely distributed in the East Mediterranean area. The genus *Chenopodium* is abundant in the tropics and adjacent warmer regions, especially in tropical America and Africa. *Chenopodium* consists of 120 species, 9 of which are found in Egypt. *Chenopodium ambrosioides* is distributed in Egypt in the Nile region, oases, Mediterranean region and Sinai [1]. In this study, *Chenopodium ambrosioides var. ambrosioides* L. was investigated and we reported on the structure elucidation of two new flavone glycosides; scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (**1**) and scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (**2**) (Figure 1).

Chenopodium ambrosioides was collected in June 2011 from Al Awayed, Alexandria, Egypt. The plant was identified by the Staff of the Botany Department, Faculty of Science, Alexandria University, Egypt. A voucher sample (UA-CA-110) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt.

* Corresponding author: E-mail: hala22hammoda@yahoo.com

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 06/01/2015 EISSN: 1307-6167

2. Previous Studies

The extracts of *C. ambrosioides* were analyzed in previous studies and were found to contain flavonoids [2, 3], organic acids [2], sterols [2, 4], monoterpenes [5]and carotenoid terpenes [2, 6].
In 1978, Harraz separated two kaempferol glycosides, triterpenes and steroids from the Egyptian *C. ambrosioides* [7]. In 1988, a study investigated the insecticidal activity of the Egyptian plant and found that the active fractions contained heptadecane, ethyl hexadecanoate and arachidonic acid [8].

3. Present Study

TLC Bioautographic screening was done to EtOH extracts of the aerial parts of *Chenopodium ambrosioides* and its fractions to detect the fraction with the highest antioxidant activity using DPPH spray. The most active fraction was found to be that of EtOAc, so it was subjected to further phytochemical investigation.

The dried aerial parts of *C. ambrosioides* (700 g) were extracted with 95% aq. EtOH by maceration. The extract was concentrated under reduced pressure, and then fractionated successively with petroleum ether (PE), $CHCl_3$, EtOAc and BuOH. The different fractions were subjected to antioxidant screening and the EtOAc fraction showed the highest activity. The EtOAc fraction (7 g) was further fractionated using column chromatography (SiO_2, $CHCl_3$, $CHCl_3$-MeOH and MeOH in order of increasing polarity) yielding 18 fractions. Fractions 8-12 were eluted with 30% MeOH in $CHCl_3$ and combined yielding two yellow spots when TLC screened. These spots were further purified by subjecting to prep. TLC (SiO_2; $CHCl_3$/MeOH 8:2) to afford **1** (Rf 0.413, 10 mg) and **2** (Rf, 0.565, 14 mg) (Figure 1).

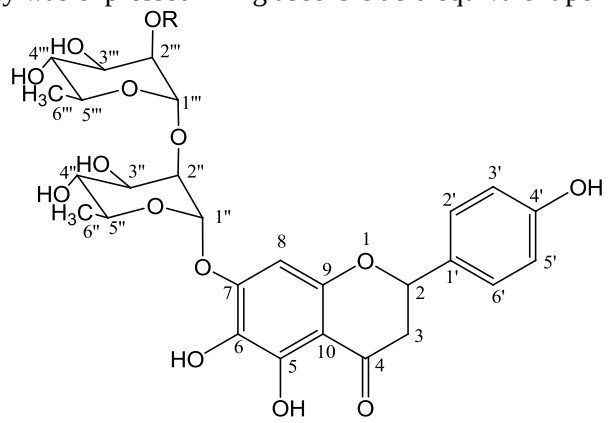
Scutellarein-7-O-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside: 5,6,7,4'-tetrahydroxyflavone-7-O-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside, yellowish white amorphous powder. UV (MeOH): λ_{max} (A) 349 (0.158), 265 (0.371). UV (MeOH/MeONa): 397 (0.232), 270 (0.399). UV (MeOH/$AlCl_3$): 389 (0.163), 339 (0.214), 272 (0.403). UV (MeOH/$AlCl_3$/HCl): 388 (0.219), 330 (0.228), 274 (0.410). UV (MeOH/AcONa): 352 (0.191), 265 (0.467). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 6.42 (1H, s, H-3); 6.74 (1H, s, H-8); 7.75 (2H, d, *J*=8.4 Hz, H-2', H-6'); 6.90 (2H, d, *J*=8.4 Hz, H-3', H-5'); 5.75 (1H, br.s, H-1"), 3.81 (1H, s, H-2"), 0.79 (3H, d, *J*=5.4, H-6"), 5.52 (1H, br.s, H-1'"), 3.96 (1H, s, H-2'"), 0.84 (3H, d, *J*= 5.2, H-6'"), 5.28 (1H, br.s, H-1""), 1.10 (3H, d, *J*= 5.2, H-6""), 3.15-4.35 (overlapped remaining protons of sugars). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 158.2 (C, C-2); 103.1 (CH, C-3); 172.9 (C, C-4); 158.2 (C, C-5); 130.1 (C, C-6); 162.1 (C, C-7); 94.9 (CH, C-8); 156.6 (C, C-9); 109.5 (C, C-10); 120.6 (C, C-1'); 131.1 (CH, C-2',C-6'); 115.9 (CH, C-3', C-5')), 162.1 (C, C-4'), 102.3 (CH, C-1"), 77.3 (CH, C-2"), 71.1 (CH, C-3"), 72.9 (CH, C-4"),70.3 (CH, C-5"), 18.0 (CH_3, C-6"), 99.9 (CH, C-1'"), 77.2 (CH, C-2'"), 71.6 (CH, C-3'"),73.0 (CH, C-4'"), 70.5 (CH, C-5'"), 18.4 (CH_3, C-6'"), 98.9 (CH, C-1""),70.8 (CH, C-2""), 72.1 (CH, C-3""), 73.8 (CH, C-4""), 70.7 (CH, C-5"")), 18.7 (CH_3, C-6""). HRESI-MS: positive ion mode: *m/z* 747. 224 [M+ Na] $^+$, negative ion mode: *m/z* 577.1666 [M-Rha]$^-$.

Scutellarein-7-O-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside: 5,6,7,4'-tetrahydroxyflavone -7-O-α- rhamnopyranosyl-(1→2)-α-rhamnopyranoside, yellowish white amorphous powder. UV (MeOH): λ_{max} (A) 344 (0.055), 267 (0.181). UV (MeOH/ MeONa): 389 (0.060), 268 (0.280). UV (MeOH/ AlCl3): 399 (0.060), 352 (0.109), 276 (0.246). UV (MeOH/ $AlCl_3$/HCl): 395 (0.079), 347 (0.142), 276 (0.285). UV (MeOH/ AcONa): 347 (0.068), 266 (0.263). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 6.41 (1H, s, H-3); 6.74 (1H, s, H-8); 7.75 (2H, d, *J*=8.4, H-2', H-6'); 6.68 (2H, d, *J*=8.4, H-3', H-5'); 5.52 (1H, br.s, H-1"), 1.15 (6H, d, *J*=5.2, H-6",H-6'")), 5.28 (1H, br.s, H-1'"), 3.00-4.00 (overlapped remaining protons of sugars). ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 162.1 (C, C-2); 102.2 (CH, C-3); 178.2 (C, C-4); 158.2 (C, C-5); 134.8 (C, C-6); 161.4 (C, C-7); 94.8 (CH, C-8); 156.5 (C, C-9); 106.3 (C, C-10); 120.2 (C, C-1'); 131.1 (CH, C-2',C-6')); 116.0 (CH, C-3', C-5'), 161.5 (C, C-4'), 99.9 (CH, C-1"), 79.5 (CH, C-2"), 70.7 (CH, C-3"), 72.0 (CH, C-4"), 70.5 (CH, C-5"), 17.9 (CH_3, C-6"), 98.8 (CH, C-1'"), 71.1 (CH, C-2'"), 71.6 (CH, C-3'"), 72.8 (CH, C-4'"), 70.6 (CH, C-5'"), 18.4 (CH_3, C-6'"). HRESI-MS: positive ion mode: *m/z* 579.1692 $[M+H]^{p+}$, negative ion mode: *m/z* 577.1608 [M-H]$^-$.

In vitro antioxidant activity: 400 µl of each of the standard (ascorbic acid) and sample solutions was mixed with 2 ml of DPPH solution. The mixtures were kept in darkness for 15 min at room temperature and then the absorbance was measured at λ 517 nm. The absorbance of a control sample containing the same amount of solvent and DPPH solution was freshly prepared and measured. The percentage of absorbance inhibition at λ 517 nm was calculated using the following equation:

% inhibition = [(A control – A sample) / A control]*100

The extent of decolorisation was calculated as % reduction of absorbance, and this was determined as a function of concentration and calculated relatively to the equivalent ascorbic acid concentration. The radical scavenging activity was expressed in mg ascorbic acid equivalent per gram of sample.



(**1**) R= Rhamnopyranose
(**2**) R= H

Figure 1. Structure of Scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (**1**) and Scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (**2**) isolated from *C. ambrosioides.*

Compound **1**, scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside was isolated as yellowish amorphous powder. The occurrence of λ max below 350 nm in MeOH also indicated the presence of a flavone skeleton. The addition of NaOMe caused a 48 nm bathochromic shift in band I, thus indicated the presence of a free 4'-OH group. The unchanged band II upon the addition of NaOAc indicated a substituted 7-OH group and the absence of a peak at 320-330 nm suggested the presence of a 7-*O*- glycoside. The stable bathochromic shift in band I (40 nm) upon addition of $AlCl_3$ / HCl indicated the presence of a hydroxyl group at the 5- position. The effect of $AlCl_3$/HCl on compound **1** could not confirm if it was a 6- or 8-hydroxy due to a Wessely-Moser rearrangement that occurs in acidic medium, where the hydroxyl group on position 6 may switch to the 8 position or vice versa [9, 10]. For this reason, ^{13}C NMR analysis was the tool that confirmed the presence of the hydroxyl at the 6-position and the presence of a free proton at C-8 whose carbon appeared at less than 96 ppm [10-12]. ^{13}C NMR spectrum illustrated the presence of 33 carbons. ^{1}H NMR spectrum showed the presence of two ortho- coupled aromatic doublets at δ = 7.75 and 6.90 assigned to H-2', H-6' and H-3', H-5', respectively. This indicated a monosubstitued B ring at position 4'. The appearance of two singlets at δ= 6.42 and 6.74 were assigned to H-3 and H-8, respectively. The presence of C-6 at δ = 130.1 and C-8 at δ = 94.9 indicated hydroxylation at the 6-position. Together with UV, this finding proved that the genin is scutellarein [13]. The NMR spectra showed the presence of three anomeric protons indicating the presence of three sugar moieties. The anomeric protons appeared as broad singlets at δ = 5.75, 5.52 and 5.28 along with their carbon signals at δ = 102.3, 99.9 and 98.9 suggesting **1** was a triglycoside. The presence of three doublets in ^{1}H NMR spectrum at δ = 0.79 (3H), 0.84 (3H), 1.10 (3H) and three quartets in ^{13}C NMR spectrum at δ = 18.0, 18.4 and 18.7 were typical for three rhamnopyranose methyl groups. The appearance of the anomeric protons at downfield shift as broad singlets proved the α- nature of the rhamnopyranoses [14]. The 7-*O*-glycosylation was deduced from the UV data and confirmed by the presence of a signal C -7 at δ = 162.1 [15-17]. The (1→2) interglycosidic linkages were deduced from the downfield shift of carbon at position 2 (C-2" at δ = 77.3 and C-2''' at δ = 77.2) and upfield shift of carbons at position 3 (C-3" at δ

= 71.1 and C-3''' at δ = 71.6) of the two non-terminal rhamnopyranoses [18]. This was ascertained from the NOESY spectrum which illustrated a relation between H-1''' at δ = 5.52 and H-2'' at δ = 3.81 and another relation between H-1'''' at δ = 5.28 and H-2''' at δ = 3.96.

HMQC spectrum confirmed the assignment of protons and carbons at positions; 6'', 6''' and 6'''', ring protons and carbons at positions; 2', 3', 5', 6', 8 and 3, and also anomeric protons 1'', 1''' and 1''''. Sugar protons 3'', 5''', 5'', 2'''', 5'''' and 3''' were also deduced from the HMQC spectrum.

2D-NMR data allowed full assignment and correlations between protons and carbons and confirmed the suggested structure.

HRESI-MS spectrum of compound **1** showed an ion peak at *m/z* 747. 224 corresponding to $[M+ Na]^+$ ($C_{33}H_{40}O_{18}Na^+$) and an ion peak at *m/z* 577.1666 corresponding to $[M-Rha]^-$ ($C_{27}H_{29}O_{14}^-$).

These results indicated compound **1** is scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (5, 6, 7, 4'-tetrahydroxyflavone-7-*O*-α-trirhamnopyranoside).

Compound **2**, scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside, showed similar spectroscopic data to **1** except that it had 2 sugar moieties. Study of the UV spectra of compound **2** in different shift reagents indicated that it is also a 5, 6 or 8, 7, 4'-tetrahydroxyflavone -7-*O*-glycoside [15]. UV spectra of compound **2** could not confirm if it was a 6- or 8-hydroxy due to a Wessely-Moser rearrangement that occurs in acidic medium. However, ^{13}C NMR confirmed the presence of the hydroxyl at the 6-position (δ = 134.8) and the presence of a free proton at C-8 (δ = 94.8). ^1H NMR and ^{13}C NMR spectra showed some characteristic peaks for the suggested structure. ^1H NMR showed 7 rather distinct peaks in addition to a number of overlapped peaks in the region extending from 3 to 4.5 nm. ^{13}C NMR spectra showed 27 carbon types. ^1H NMR spectrum showed the presence of two ortho- coupled aromatic doublets at δ = 7.75 and 6.68 assigned to H-2', H-6' and H-3', H-5', respectively. This indicated a monosubstitued B ring at 4'. The appearance of two singlets at δ = 6.41 and 6.74 were assigned to H-3 and H-8, respectively. These data suggested the flavone glycoside genin part was scutllarein [13]. The presence of two anomeric protons at δ = 5.52 and 5.28 indicated compound **2** was a diglycoside. The presence of a doublet at δ = 1.15 integarting for 6 protons, along with 2 carbon signals at δ = 17.9 and 18.4 were typical for two rhamnopyranose moieties. The appearance of the anomeric protons at downfield shifts and as broad singlets proved the α nature of the sugar linkage. Moreover, chemical shifts of the sugar carbons were characteristic for the rhamnopyranose form [19, 20]. The 7-*O*-glycosylation was deduced from the UV data and confirmed by the presence of a signal C-7 at δ = 161.4. The downfield shift of C-2'' of the internal rhamnopyranose moiety at δ = 79.5 indicated that the interglycosidic linkage between the two rhamnopyranose moieties is a 1→2 linkage [18].

HRESI-MS spectrum showed an ion peak at *m/z* 579.1692 corresponding to $[M+H]^+$($C_{27}H_{31}O_{14}^+$) , an ion peak at 601.2316 corresponding to $[M+Na]^+$ ($C_{27}H_{30}O_{14}^+Na$), an ion peak at 1179.2659 corresponding to $[2M+Na]^+$ ($C_{54}H_{60}O_{28}Na^+$) and an ion peak at 577.1608 corresponding to $[M-H]^-$ ($C_{27}H_{29}O_{14}^-$).

These results indicated that compound **2** can be identified as scutellarein-7-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranoside (5, 6, 7, 4'-tetrahydroxyflavone -7-*O*-α-dirhamnopyranoside).

According to literature survey, this is the first report for isolation of these two flavone glycosides from a natural source.

The flavone glycosides **1** and **2** isolated from *C. ambrosioides* showed significant antioxidant activities (11.88 and 38.89 mg AAe/g sample, respectively). The antioxidant activity of both glycosides may be traced back to the presence of many of the structural features that define the classical antioxidant potential of flavonoids; the presence of an unsaturation and a 4-oxo in ring C. The presence of a 5-OH also helps in chelation [21]. Flavone glycoside **1** was less active as an antioxidant than **2**, which may be due to steric hindrance offered by the third sugar molecule on the phenolic hydroxyl groups involved in the antioxidant action [22, 23].

References

[1] V. Takholm (1977). Students Flora of Egypt, Cairo Univ., Cairo, Egypt. 888.

[2] Z. Kokanova-Nedialkova, P. Nedialkov and S. Nikolov (2009).The genus *Chenopodium*: Phytochemistry, ethnopharmacology and pharmacology, *Phcogn. Rev*. **3**, 280-306.

[3] N. Jain, M. Sarwar Alam, M. Kamil, M. Ilyas, M. Niwa and A. Sakae (1990). Two flavonol glycosides from *Chenopodium ambrosioides*, *Phytochemistry* **29**, 3988-3991.

[4] T. A. Salt and J. H. Adler (1985). Diversity of sterol composition in the family Chenopodiaceae, *Lipids.* **20**, 594-601.

[5] A. A. Ahmed (2000). Highly Oxygenated Monoterpenes from *Chenopodium ambrosioides*, *Journal of natural products.* **63**, 989-991.

[6] L. S. W. S. Hall, Valley Stream, NY, US) (2005) *Chenopodium ambrosioides* extract for treating uterine fibroids.

[7] F. M. H. Harraz (1978). Chemical Investigation of *Chenopodium ambrosioides* L. and Macro and Micromorphological Study of *Chenopodium murale* L. [Msc], Univ**e**rsity of Alexandria.

[8] M. A. K. Glenn, S. Peterson, Mounir D. Abdallah and Abdel-Aal A. Farag (1989). Isolation and Characterisation of Biologically-Active Compounds from Some Plant Extracts, *J. Pestic. Sci.* **25**, 337-342.

[9] J. J. Li (2004). Name reactions in heterocyclic chemistry II.Wiley-Interscience.

[10] V. M. Chari, R. J. Grayer-Barkmeijer, J. B. Harborne and B.-G. Österdahl (1981). An acylated allose-containing 8-hydroxyflavone glycoside from *Veronica filiformis*, *Phytochemistry* **20**, 1977-1979.

[11] I. G. Collado, F. Macias, G. Massanet and F. R. Luis (1985). Flavonoids from *Centaurea clementei*, *J. Nat. Prod.* **48**, 819-822.

[12] D. C. Albach, R. J. Grayer, S. R. Jensen, F. Özgökce and N. C. Veitch (2003). Acylated flavone glycosides from *Veronica*, *Phytochemistry* **64**, 1295-1301.

[13] M. Gao, W. Huang and C.-Z. Liu (2007). Separation of scutellarin from crude extracts of *Erigeron breviscapus* (vant.) Hand. Mazz. by macroporous resins, *J. Chromatogr B* **858**, 22-26.

[14] M. Kucukislamoglu, N. Yayli, H. B. Senturk, H. Genc and S. Ozden (2000). Flavonol glycosides from *Consolida armeniaca*, *Turk J. Chem*. **24**, 191-197.

[15] T. J. Mabry, K. R. Markham and M. B. Thomas (1970). The systematic identification of flavonoids.Springer-Verlag.

[16] L. Qin, L. Ming, T. J. Mabry and R. A. Dixon (1994). Flavonol glycosides from *Cephalocereus senilis*, *Phytochemistry* **36**, 229-231.

[17] K. Chiung-Sheue Liu, Y. Shi-Lin, M. F. Roberts and J. D. Phillipson (1989). Flavonol glycosides with acetyl substitution from *Kalanchoe gracilis*, *Phytochemistry* **28**, 2813-2818.

[18] G. G. S. Dutton, E. H. Merrifield, C. Laffite, F. Pratviel, Sosa and R. Wylde (1982). Comparative NMR study of rhamnobioses applications, *Org. Magn. Resonan.* **20**, 154-158.

[19] N. Mulinacci, F. Vincieri, A. Baldi, M. Bambagiotti-Alberti, A. Sendl and H. Wagner (1995). Flavonol glycosides from *Sedum telephium* subspecies Maximum leaves, *Phytochemistry* **38**, 531-533.

[20] P. K. Agrawal (1989). Carbon-13 NMR of flavonoids. Elsevier Science Ltd, Amsterdam.

[21] R. J. Williams, J. P. E. Spencer and C. Rice-Evans (2004). Flavonoids: antioxidants or signalling molecules?, *Free Radical Biol. Med.* **36**, 838-849.

[22] M. R. Cholbi, M. Paya and M. J. Alcaraz (1991). Inhibitory effects of phenolic compounds on CCl4-induced microsomal lipid peroxidation, *Experientia* **47**, 195-199.

[23] J. T. Han, M.H. Bang, O.K. Chun, D.O. Kim, C.Y. Lee and N.I. Baek (2004). Flavonol glycosides from the aerial parts of *Aceriphyllum rossii* and their antioxidant activities, *Arch. Pharmaceut. Res.* **27**, 390-395.