Journal of Reports in Pharmaceutical Sciences 2018, 7(2), 221-226

Two Iridoid Structures from *Eremostachys macrophylla* Montbr. & Auch. Rhizomes

Abbas Delazar[a], Solmaz Asnaashari[b*]

[a]Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[b]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2018-03-02
Revised: 2018-04-27
Accepted: 2018-05-02
ePublished: 2018-05-29

***Keywords*:**
Eremostachys Macrophylla
6- Hydroxyl Loganin
Lamalbide
Iridoid

A B S T R A C T

The air- dried and ground rhizomes of *Eremostachys macrophylla* Montbr. & Auch., as wild-growing plant in East Azerbaijan province, of Iran, were extracted with n-hexane, dichloromethane (DCM) and methanol (MeOH) solvents using a soxhlet apparatus. The 10% MeOH in water Sep-Pak fractions of the MeOH extract was subjected to preparative reversed-phase high performance liquid chromatography (RP-HPLC) and the isolated pure compounds were identified by one- dimensional nuclear magnetic resonance (1D-NMR) spectroscopic technique. The obtained results showed the presence of two pure components, 6- Hydroxyl Loganin, (1) and Lamalbide (2) with iridoid structures. The results demonstrated the rhizomes of *E. macrophylla* could be a good source of iridoids.

[*]**Corresponding Author: Solmaz Asnaashari, E-mail:** asnaasharisolmaz@gmail.com

Introduction

Eremostachys macrophylla Montbr. & Auch. is one of the 15 distributed species of Eremostachys genus in Iran. This wild-growing plant have some medicinal uses in folk medicine, including wound healing, snake bites, rheumatism and joint pains [1, 2]. Moreover, our previous studies proposed *in vitro* antimalarial, antioxidant and antiproliferative effects of this species [3, 4].

Phytochemical evaluations on the extracts of a number of species of genus *Eremostachys* showed the presence of different chemical structures. Iridoid glycosides, flavonoids, phenylethanoid glycosides and phytostrols were reported from *E. laciniata* [5, 6]. Ferulic acid derivatives, furanolabdane-type diterpenoids, and phenylethanoid glycosides were identified from *E. glabra* [7- 9]. Additionally, iridoid glycoside, flavonoid, phenylethanoid, fatty acid and steroid structures from *E. azerbaijanica* [10- 13], iridoids and flavonoids from *E. loasifolia* [14- 16], iridoid glycosides from *E. moluccelloides* [17] and different flavonoid structures from *E. vicaryi* [18] were isolated previously.

According to earlier phytochemical studies on some essential oils of *Eremostachys* species, the presence of terpenoids, linear hydrocarbons and derivatives as main constituents in different stages of growth were revealed [19-22].

There are several studies about *E. macrophylla* essential oil GCMS analysis. According to Javidnia and et al. report spathulenol, hexadecanoic acid and caryophyllene oxide were the main compounds of this species [19]. Also in the other study by Nori-shargh and et al. the oil of *E. macrophylla* aerial parts consisted mainly of germacrene-D, germacrene-B and γ-elemene [23]. Rustaiyan and et al. evaluated different parts of *E. macrophylla* separately and according their reports the major structures in the flower oil were 1,8-cineol and germacrene D-4-ol, while the leaf oil contained α-pinene, 1,10-di-epi cubenol, elemol and bornyl acetate. The oil of the stem was dominated also by 1, 10-di-epi cubenol and elemol [20]. However, there is a little scientific research about phytochemicals of *E. macrophylla* extracts. The objective of this study is extraction, purification and identification of natural compounds from the rhizomes of *E. macrophylla* growing East Azerbaijan province, Iran.

Material and Methods

Plant material

The rhizomes of *E. macrophylla* Montbr. & Auch. were collected during July 2012 from Sahand mountains in East Azarbaijan province in Iran 37.759 (37° 45' 32.4" N) latitude 45.9783 (45° 58' 41.9" E) longitude and altitude 1950 m above sea level.

The identity of the plant was confirmed by anatomical examination in comparison with the herbarium specimens (voucher Nos. TBZ-fph-739) deposited in the Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Extraction, separation and identification of compounds

The air-dried and ground rhizomes of *E. macrophylla* (50 g) were extracted with n-hexane, dichloromethane (DCM) and MeOH (solvents were from Caledon, Canada), successively with a soxhlet apparatus (500 mL each).

The MeOH extract (2 g) was subjected to solid phase extraction (SPE) using a C_{18} Sep-Pak cartridge (Waters, USA) , eluting with a step gradient of MeOH/water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0). All these extracts and fractions were separately concentrated using a rotary evaporator at a maximum temperature of 45 °C [5]. The obtained fractions were analyzed by reversed-phase HPLC (analytic-HPLC, Adept, Cecil) with a C_{18} column (250 mm length, 4.6 mm i.d, 10 µm particle sizes, Dr. Maisch, Germany) system and the best analyzed fraction (10% faction) was selected for preparative reversed-phase HPLC (prep-HPLC) conducted on a Knauer HPLC (preparative pump 1800) fitted with a C_{18} column (250 mm length, 20mm i.d, 10 µm particle sizes, Dr. Maisch, Germany) system. The mobile phase which consisted of 0%-36% MeOH in water at a flow rate of 8 ml/min, in 75 min run time and a detector set at 220 nm was used. The isolated pure compounds were identified by a Bruker

Spectrospin 400 MHz NMR-spectrometer. The spectroscopic data of the known compounds were also compared with the respective published data [5, 10].

Results

Reversed-phase preparative HPLC analysis of 10% fraction of the MeOH extract of *E. macrophylla* rhizomes afforded two iridoid glycoside structures, which were identified unequivocally as 6- Hydroxyl Loganin (8 mg, Rt: 39.83 min) and Lamalbide (5 mg, Rt:43.00 min) on the extensive 1D ^{1}H-NMR and ^{13}C-NMR data analyses (Table.1). The spectroscopic data of the known compounds were also compared with the respective published data [24].

Table 1. ^{1}HNMR and ^{13}CNMR spectroscopic data for compounds 1 and 2. *Overlapped peaks; Spectra obtained in D_2O; 400 MHz

POSITION	CHEMICAL SHIFT δ IN PPM (1) UV Λ MAX (MEOH): 237 NM		CHEMICAL SHIFT δ IN PPM (2) UV Λ MAX (MEOH): 235 NM	
	δ_H	δ_C	δ_H	δ_C
1	5.40, d, 1H, J: 2.48 Hz	94.65	5.55, bs, 1H	92.28
2	-	-	-	-
3	7.36, s, 3H	151.23	7.37, s, 1H	150.16
4	-	109.38	-	108.76
5	2.69, dd, 1H, J: 8.45, 3.69 Hz	38.56	2.86, dd,1H, J: 10.92, 2.66 Hz	33.67
6	3.83, 1H*	81.99	3.97, t, 1H, J: 3.84 Hz	73.70
7	3.47, dd, 1H, J: 8.8, 6.1 Hz	83.46	3.57, d, 1H, J: 4.33 Hz	75.97
8	1.64, m, 1H	36.40	-	76.07
9	2.07, m, 1H	41.91	2.75, bd, 1H, J: 10.96 Hz	45.57
10	1.04, d, 3H, J: 6.51 Hz	14.82	1.11, s, 3H	19.06
11	-	169.24	-	167.80
OCH3	3.65, s, 3H	51.35	3.66, s, 3H	50.34
1'	4.67, 1H *	97.98	4.66, 1H*	96.59
2'	3.17, t, J: 8.7 Hz	72.04	3.17, t, J: 8.4 Hz	70.96
3'	3.25-3.40 *	75.04	3.24—3.41 *	74.29
4'	3.25-3.40 *	68.98	3.24—3.41 *	67.97
5'	3.25-3.40 *	75.75	3.24—3.41 *	74.70
6'(a)	3.83, bd, 1H※, J: 12.28 Hz	51.35	3.84, bd, 1H: 12.26 Hz	59.10
6'(b)	3.63, dd, 1H, J: 11.8, 5.6 Hz		3.64, dd, 1H, 12.53, 6.26	

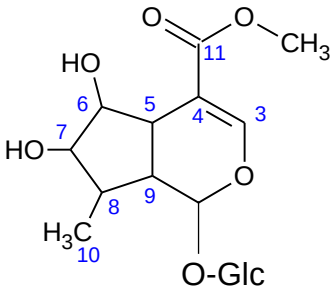
Discussion

Analysis of the MeOH extract of *E. macrophylla* rhizomes using reversed phase preparative HPLC, afforded two iridoid glycoside structures, which were identified as 6- Hydroxyl Loganin (1), Lamalbide (2) on the basis of comparison of ^{1}HNMR and ^{13}CNMR data with previous published data.

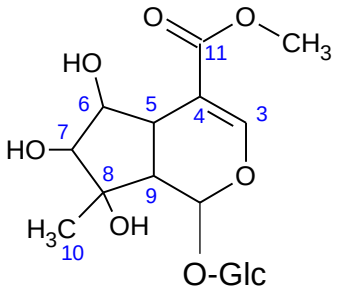
Both compounds (1) and (2) showed UV, ^{1}HNMR and ^{13}CNMR signals in agreement with iridoid glycoside skeletons [24].

The signals of compound (1) presented a methyl group at C_8 (δ_{H10}: 1.04 ppm, δ_{C10}: 14.82 ppm), a methoxy group ($\delta_{H(OCH3)}$: 3.65 ppm, $\delta_{C(OCH3)}$: 51.35 ppm), an olefinic methine at C_3 (δ_{H3}: 7.36 ppm, δ_{C3}: 151.23 ppm), oxymethines at C_1 (δ_{H1}: 5.40 ppm, δ_{C1}: 94.65 ppm) , C_6 (δ_{H6}: 3.83 ppm, δ_{C6}: 81.99 ppm) and C_7 (δ_{H7}: 3.47 ppm, δ_{C7}: 83.46 ppm), two methine at C5 (δ_{H5}: 2.69 ppm, δ_{C5}: 38.56 ppm) and C9 (δ_{H9}: 2.07 ppm, δ_{C9}: 41.91 ppm), a carbonyl group (δ_{C11}: 169.24 ppm) and a β-glucose unit ($\delta_{H1'}$: 4.67 ppm, $\delta_{C1'}$: 97.98 ppm).

Moreover, The ^{1}H-NMR and ^{13}C-NMR data of compound (2) showed the presence of a methyl group at C_8 (δ_{H10}: 1.11 ppm, δ_{C10}: 19.06 ppm), a methoxy group ($\delta_{H(OCH3)}$: 3.66 ppm, $\delta_{C(OCH3)}$: 50.34 ppm), an olefinic methine at C_3 (δ_{H3}: 7.37 ppm, δ_{C3}: 150.16 ppm), oxymethines at C_1 (δ_{H1}: 5.55 ppm, δ_{C1}: 92.28 ppm) , C_6 (δ_{H6}: 3.97 ppm, δ_{C6}: 73.70 ppm) and C_7 (δ_{H7}: 3.57 ppm, δ_{C7}: 75.97 ppm), two methine at C5 (δ_{H5}: 2.86 ppm, δ_{C5}: 33.67 ppm) and C9 (δ_{H9}: 2.75 ppm, δ_{C9}: 45.57 ppm), a carbonyl group (δ_{C11}: 167.80 ppm) and a β-glucose unit ($\delta_{H1'}$: 4.66 ppm, $\delta_{C1'}$: 96.59 ppm).



(1)



(2)

Fig. 1. Two suggested structures: 6- Hydroxyl Loganin (1) and Lamalbide (2).

Data were consistent with previously published data and the results identified the both structures (1) and (2) as two iridoid glycoside structures, 6-Hydroxyl Loganin (1), Lamalbide (2) [25, 26]. The two compounds have been isolated previously from *E. laciniata* and *E. moluccelloides* aerial parts as the other species of genus *Eremostachys* [17, 27].

Iridoid glycosides as a class of natural structures, which isolated from different species of plants exhibit a wide range of pharmacological and biological effects. According to previous literatures, they are used in the preparation of anti-inflammatory, anti-rheumatic, anti-ulcer, bitter tonics, febrifuges, cough medicines, sedatives, hypo and hypertensive drug formulations. Other pharmacological and biological effects of these structures are anti-bacterial, anti-fungal, anti-protozoal, anti-viral, anti-oxidative, anti-cancer, anti-coagilant, anti-diabetic, anti-hyperlipidaemic, anti-nociceptive, anti-osteoporosis, human neutrophil elastase inhibitory, immunomodulatory, melanogenesis inhibitory, hepatoprotective, neuroprotective and neuritogenic activities [28- 30]

The presence of common phytochemicals in the plants can be a reason for their similar pharmacological and biological effects.

Conclusion

The present study has shown that the rhizomes of *E. macrophylla* are a source of iridoid glycosides. Since the several biological activities such as analgesic, anti-inflammatory and anti-arthritic properties of iridoid glycosides have previously been confirmed by several in vitro studies [28], thus it is reasonable to conclude that *E. macrophylla* can be useful in managing of some inflammation diseases.

Acknowledgments

The authors would like to thank the Drug Applied Research Center, Tabriz University of Medical sciences, Tabriz, Iran for financial support of this project.

Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

References

[1] Mozaffarian V. A dictionary of Iranian plant names. 4 th ed. Iran, Tehran.: Farhang Moaser; 1996 2007. 700 p.
[2] Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. J Ethnopharmacol. 2012;141:80-95.
[3] Asnaashari S, Afshar FH, Ebrahimi A, Moghadam SB, Delazar A. In vitro antimalarial activity of different extracts of *Eremostachys macrophylla* Montbr. & Auch. BioImpacts: BI. 2015;5:135.
[4] Asgharian P, Delazar A, Vatankhah A, Javadzadeh M, Asnaashari S. In vitro bioactivity and phytochemical evaluation of extracts from aerial parts of *Eremostachys macrophylla* Montbr. & Auch. growing in Iran. Res J Pharmacog. 2017;4:65-73.
[5] Delazar A, Sarker SD, Nahar L, Jalali SB, Modaresi M, Hamedeyazdan S, et al. Rhizomes of *Eremostachys laciniata*: isolation and structure elucidation of chemical constituents and a clinical trial on inflammatory diseases. Adv Pharm Bull. 2013;3:385.
[6] Khan S, Nisar M, Simjee SU, Rehman W, Khan R, Jan I, et al. Evaluation of micronutrients level and antinociceptive property of *Eremostachys laciniata* (L) Bunge. Afr J Biotechnol. 2010;9.
[7] Delazar A, Shoeb M, Kumarasamy Y, Byres M, Nahar L, Modarresi M, et al. Two bioactive ferulic acid derivatives from *Eremostachys glabra*. DARU J Pharm Sci. 2004;12:49-53.
[8] Delazar A, Modarresi M, Shoeb M, Nahar L, Reid RG, Kumarasamy Y, et al. Eremostachiin: a new furanolabdane diterpene glycoside from *Eremostachys glabra*. Nat Prod Res. 2006;20:167-172.
[9] Delazar A, Gibbons S, Kumarasamy Y, Nahar L, Shoeb M, Sarker SD. Antioxidant phenylethanoid glycosides from the rhizomes of *Eremostachys glabra* (Lamiaceae). Biochem Syst Ecol. 2005;33:87-90.
[10] Asnaashari S, Afshar FH, Ebrahimi A, Moghadam SB, Delazar A. Chemical composition and radical scavenging activity of essential oil and methanolic extract of *Eremostachys azerbaijanica* Rech. f. from Iran. Res Pharm Sci. 2016;11:113.
[11] Asnaashari S, Afshar FH, Moghadam SB, Delazar A. Evaluation of In Vitro Antimalarial Activity of Different Extracts of *Eremostachys azerbaijanica* Rech. f. Iran J Pharm Res. 2016;15:523.
[12] Asnaashari S, Delazar A, Asgharian P, Lotfipour F, Moghaddam SB, Afshar FH. In-vitro Bioactivity and Phytochemical Screening of Extracts from Rhizomes of *Eremostachys azerbaijanica* rech. f. Growing in Iran. Iran J Pharm Res. 2017;16:306.
[13] Fouladnia M, Modarresi M. Phenylethanoid glycosides from *Eremostachys azerbaijanica* Rech. F. Res Pharm Sci. 2012;7:760.
[14] Mughal UR, Fatima I, Malik A, Bakhsh Tareen R. Loasifolin, a new flavonoid from *Eremostachys loasifolia*: Note. J Asian Nat Prod Res. 2010;12:328-3230.
[15] Mughal UR, Fareed G, Zubair A, Malik A, Versiani MA, Afza N, et al. Loasins A and B, new flavonoids from *Eremostachys loasifolia*. Nat Prod Res. 2013;27:1906-1910.
[16] Ali B, Mehmood R, Mughal UR, Malik A, Safder M, Hussain R, et al. Eremosides A–C, new iridoid glucosides from *Eremostachys loasifolia*. Helv Chim Acta. 2012;95:586-593.
[17] Çalış İ, Güvenç A, Armağan M, Koyuncu M, Gotfredsen CH, Jensen SR. Iridoid Glucosides from *Eremostachys moluccelloides* Bunge. Helv Chim Acta. 2007;90:1461-1466.

[18] Imran M, Mehmood R, Mughal UR, Ali B, Malik A. Vicarin, a new isoflavone from *Eremostachys vicaryi*. J Asian Nat Prod Res. 2012;14:293-296.
[19] Javidnia K, Miri R, Soltani M, Khosravi A. Essential oil composition of two species of *Eremostachys* from Iran (*E. adenantha* Jaub. et Spach and *E. macrophylla* Montbr. et Auch.). J Essent Oil Res. 2008;20:226-228.
[20] Rustaiyan A, Masoudi S, Ezzatzadeh E, Akhlaghi H, Aboli J. Composition of the Aerial Part, Flower, Leaf and Stem Oils of *Eremostachys macrophylla* Montbr. & Auch. and *Eremostachys labiosa* Bunge. from Iran. J Essent Oil-Bear Plants. 2011;14:84-88.
[21] Amiri H, Meshkat Al Sadat M. Chemical composition of the essential oil of *Eremostachys Laevigata* bung. DARU J Pharm Sci. 2007;15:34-40.
[22] Navaei MN, Mirza M. Chemical composition of the oil of *Eremostachys laciniata* (L.) Bunge from Iran. Flavour Fragrance J. 2006;21:645-646.
[23] Nori-Shargh D, Kiaei S, Deyhimi F. The volatile constituents analysis of *Eremostachys macrophylla* Montbr. & Auch. from Iran. Nat Prod Res. 2007;21:733-735.
[24] Boros CA, Stermitz FR. Iridoids. An updated review, part II. J Nat Prod. 1991;54:1173-246.
[25] Damtoft S, Franzyk H, Jensen SR. Iridoid glucosides from *Picconia excelsa*. Phytochemistry. 1997;45:743-750.
[26] Modarresi M, Foladnia M, Rafiee Z, Jafari A, Zarzasangan K. Iridoid Glycosides from *Eremostachys azerbaijanica* Rech. f. Root. J Med Plants. 2013;2:66-77.
[27] Calis I, Güvenc A, Armagan M, Koyuncu M, Gotfredsen CH, Jensen SR. Secondary metabolites from *Eremostachys laciniata*. Nat Prod Commun. 2008;3:117-124.
[28] Dinda B, Debnath S, Harigaya Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chem Pharm Bull. 2007;55:689-728.
[29] Dinda B, Chowdhury DR, Mohanta BC. Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, part 3. Chem Pharm Bull. 2009;57: 765-796.
[30] Dinda B, Debnath S, Bank R, Naturally occurring iridoids, secoiridoids. An updated review, part 4. Chem Pharm Bull. 2011;59: 803-833.

