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Short Communication

Chemical Constituents of *Eremostachys macrophylla* Montbr. & Auch. Aerial Parts

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Abstract

Background: *Eremostachys macrophylla* Montbr. & Auch. is one of the wild growing species of herbs found in East Azerbaijan province of Iran. These species are used in folk medicine for the healing of wound, treatment of snake bites, rheumatism and joint pains. The primary aim of this study was to obtain natural pure compounds and this was done by subjecting the aerial parts of *Eremostachys macrophylla* Montbr. & Auch. to phytochemical analysis.

Methods: The air-dried and crushed aerial parts were respectively extracted with n-hexane, dichloromethane (DCM) and methanol (MeOH) solvents using a soxhlet apparatus. The 10%, 20% and 40% of MeOH in water Sep-Pak fractions of the MeOH extract were subjected to a preparative reversed- phase high performance liquid chromatography (RP-HPLC). Also, the isolated pure compounds were identified by one-dimensional nuclear magnetic resonance (1D NMR) spectroscopic technique

Results: The results obtained in this study showed the presence of seven pure components; (1) Lamalbide, (2) Sesamoside, (3) Phlomiol, (4) Verbascoside, (5) Luteolin-7-O- glucoside, (6) Apigenin-7-O- rutinoside and (7) Kaempferol-3-O- glucoside with iridoid, phenylethanoid and flavonoid structures.

Conclusion: The results from the study demonstrated that the aerial parts of *E. macrophylla* could be a good source of iridoids, phenylethanoids and flavonoids.

Introduction

The genus *Eremostachys* belongs to the Lamiaceae family. It is made up of about 60 species of perennial herbs, which originate generally in Central, Middle-East and South-West Asian countries.[1,2] This genus is presented by fifteen species in Iran flora.[3] Some plants of this genus are traditionally used in making local analgesia, for treating inflammation, allergy, for wound healing, for treating snake bites, rheumatism, joint pains, headache and liver diseases.[1,3,4] Moreover, previous studies have shown the wide range of biological activities from the genus *Eremostachys;* for example, according to published reports, *E. laciniata* has possessed analgesic, antinociceptive, antidepressant, antioxidant, antibacterial and anti-inflammatory effects.[2,3,5-8]

In pursuant to previous literatures, phytochemical studies about the essential oils of some *Eremostachys* species revealed the presence of terpenoids, linear and branched hydrocarbons and derivatives as the main constituents in different stages of growth. The analysis of the volatile oil of *E. adenatha* using Gas Chromatography Mass Spectroscopy (GCMS) demonstrated the presence of dodecanal, hexadecanoic acid and 6, 10, 14-trimethyl-2-pentadecanone as major components; whereas, 6, 10, 14-trimethyl-2-pentadecanone, 1, 8-cineole and α-pinene were the main compound of the essential oil of *E. labiosa* aerial parts and the chief constituents identified from the stem oil were α-phellandrene , β-phellandrene , α-pinene and tetradecane.[9,10] The analysis of the *E. laevigata* aerial parts essential oil represented dodecanal, germacrene-D, β- caryophyllene and caryophyllene oxide structures as the main components.[11] Although, the major constituent of *E. laciniata* aerial parts was dodecanol,[12] the chief components of the *E. azerbaijanica* oil were hexahydrofarnesyl acetone, 2-methyl-6-propyl-dodecane and tricosane.[13]

Eremostachys macrophylla Montbr. & Auch or "Sonbole biabani"is a species which is distributed in Turkey, Iraq, Iran and Central Asia. This wild-growing species have been used for treatment of snake bites, healing of wounds, rheumatism and joint paints.[14,15] There are several studies about phytochemical analysis of *E. macrophylla* essential oil. According to Javidnia et al. report, spathulenol, hexadecanoic acid and caryophyllene oxide were the main

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compounds of this species.[9] Likewise, in the other study by Nori-shargh et al., the oil of *E. macrophylla* aerial parts consisted essentially of germacrene-D, germacrene-B and γ-elemene. Rustaiyan et al. assessed different parts of *E. macrophylla* separately and their reports demonstrated that 1,8-cineol and germacrene D-4-ol were the major structures in the flower oil, 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.[10]

No study has ever been performed on the non-volatile phyto-constituents of *E. macrophylla*, except for our previous evaluations on the phyto-constituents of *E. macrophylla* rhizomes, which represented two iridoid structures (Lamalbide and 6- Hydroxyl Loganin) in 2018.[16] Thus, the aim of this study was to extract, purify and identify natural constituents of *E. macrophylla* aerial parts grown in East Azerbaijan province, Iran.

Materials and Methods

Materials

All the solvents that were used for extraction and fractionation were bought from Caledon (Canada), D2O and d6-DMSO for NMR spectroscopy were purchased from Sigma (USA).

Plant material

The aerial parts of *E. macrophylla* Montbr. & Auch. were collected in the month of July, 2012 from Sahand mountains in East Azerbaijan province of Iran on 37.759 (37° 45' 32.4" N) latitude, 45.9783 (45° 58' 41.9" E) longitude and an altitude of 1950m above sea level. It was taxonomically identified and deposited by Dr. Hossein Nazemiyeh and Dr. Atefeh Ebrahimi in the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran (Voucher Nos. TBZ-fph-739).

Extraction, separation and identification of compounds

The air-dried and ground aerial parts of *E. macrophylla* (50g) were extracted with n-hexane, dichloromethane (DCM) and MeOH, with a soxhlet apparatus (500mL, throughout 8 hours for each solvent), sequentially (solvents were from Caledon, Canada).

The MeOH extract (2g) 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. The 10%, 20% and 40% MeOH in water Sep-Pak fraction were also subjected to preparative reversed-phase HPLC (prep-HPLC) conducted on a Knauer HPLC (preparative pump 1800) fitted with a C_{18} column (250mm length, 20mm i.d, 10µm particle size, Dr. Maisch, Germany) system.

The mobile phase time program for each fraction was set as below:

The 10% fraction: linear gradient of 0-36% MeOH in water during 0-50 min, isocratic gradient of 36% MeOH in water during 50-62.5 min, linear gradient of 36-0% during 62.5-67.5 min, isocratic gradient of 0% MeOH in water during 67.5-75 min to yield compound (1) (8.4mg, 51.1% of 10% fraction) and compound (2) (4.3mg, 21.8% of 10% fraction).

The 20% fraction: linear gradient of 20-40% MeOH in water during 0-50 min, isocratic gradient of 40% MeOH during 50-62.5 min, linear gradient of 40-20% MeOH in water during 62.5-67.5 min, isocratic gradient of 20% MeOH in water during 67.5-75 min to isolate compound (3) (3mg, 3.3% of 20% fraction).

The 40% fraction: linear gradient of 30-60% MeOH in water during 0-50 min, isocratic gradient of 60% MeOH during 50-62.5 min, linear gradient of 60-30% MeOH in water during 62.5-67.5 min, isocratic gradient of 30% MeOH in water during 67.5-75 min to isolate compound (4) (4.7mg, 6.1% of 40% fraction), compound (5) (9.1mg, 46.5% of 40% fraction), compound (6) (4.5mg, 1.8% of 40% fraction) and compound (7) (3.1mg, 1.7% of 40% fraction).

Flow rate was 8 ml/min, volume of each injection was 1 ml and the detector set at 220 nm was used to monitor chromatogram. Elucidation of structures was performed using ^{1}HNMR and ^{13}CNMR (Bruker, Germany) and the spectroscopic data of the known compounds were also compared with the respective published data.[13]

Results and Discussion

The reversed-phase preparative HPLC analysis of 10%, 20% and 40% fraction of the MeOH extract of *E. macrophylla* aerial parts afforded three iridoid glycoside, one phenylethanoid glycoside and three flavonoid structures, which were identified unequivocally as (1) Lamalbide (White amorphous, 8.4mg, 51.1%, Rt: 51.35min), (2) Sesamoside (White amorphous, 4.3mg, 21.8%, Rt: 53.13min), (3) Phlomiol (White amorphous, 3mg, 3.3%, Rt: 53.65min, (4) Verbascoside (Yellow amorphous, 4.7mg, 6.1%, 23.38min), (5) Luteolin-7-O- glucoside (Brown amorphous, 9.1mg, 46.5%, 32.22min), (6) Apigenin-7- O-rutinoside (Brown amorphous, 4.5mg, 1.8%, Rt.36.50min) and (7) Kaempferol-3-O- glucoside (Brown amorphous, 3.1mg, 1.7%, Rt: 39.81min) on the extensive 1D H-NMR and C-NMR data analyses. The spectroscopic data of the known compounds were also compared with the respective published data.

All three compounds (1), (2) and (3) showed that UV, ^{1}HNMR and ^{13}CNMR signals were consistent with iridoid glycoside skeletons.[17]

Compound (1):

λ_{max}: 237 nm

^{1}H-NMR and ^{13}C-NMR signals of compound (1) presented a methyl group at C_8 (σ_{H10}: 1.11ppm, σ_{C10}: 19.09ppm), a methoxy group ($\sigma_{H(OCH3)}$: 3.66ppm, $\sigma_{C(OCH3)}$: 50.35ppm), an olefinic methine at C_3 (σ_{H3}: 7.36ppm, σ_{C3}: 150.18ppm), oxymethines at C_1 (σ_{H1}: 5.54ppm, σ_{C1}: 92.28ppm) , C_6 (σ_{H6}: 3.84ppm, σ_{C6}: 75.96ppm) and C_7 (σ_{H7}: 3.56 ppm, σ

$_{C7}$: 73.97ppm), two methine at C5 (σ $_{H5}$: 2.85ppm, σ $_{C5}$: 33.70ppm) and C9 (σ $_{H9}$: 2.74ppm, σ $_{C9}$: 45.55ppm) and a β-glucose unit (σ $_{H1'}$: 4.67ppm, σ $_{C1'}$: 96.60ppm).

Compound (2):
λ_{max}: 239 nm
The ^{1}H-NMR and ^{13}C-NMR data of compound (2) showed the presence of a methyl group at C_8 (σ $_{H10}$: 1.39ppm, σ $_{C10}$: 14.36ppm), a methoxy group (σ $_{H(OCH3)}$: 3.67ppm, σ $_{C(OCH3)}$: 50.61ppm), an olefinic methine at C_3 (σ $_{H3}$: 7.60ppm, σ $_{C3}$: 153.73ppm), oxymethines at C_1 (σ $_{H1}$: 5.62ppm, σ $_{C1}$: 93.31ppm) , C_6 (σ $_{H6}$: 4.30ppm, σ $_{C6}$: 74.73ppm) and C_7 (σ $_{H7}$: 3.53ppm, σ $_{C7}$: 70.88ppm), a methine at C_9 (σ $_{H9}$: 2.56ppm, σ $_{C9}$: 50.42ppm) and a β-glucose unit (σ $_{H1'}$: 4.70ppm, σ $_{C1'}$: 96.99ppm).

Compound (3):
λ_{max}: 229 nm
The ^{1}H-NMR and ^{13}C-NMR data of compound (3) indicated the existence of a methyl group at C_8 (σ $_{H10}$: 1.06ppm, σ $_{C10}$: 18.99ppm), a methoxy group (σ $_{H(OCH3)}$: 3.67ppm, σ $_{C(OCH3)}$: 53.94ppm), an olefinic methine at C_3 (σ $_{H3}$: 7.51ppm, σ $_{C3}$: 155.25ppm), oxymethines at C_1 (σ $_{H1}$: 5.79ppm, σ $_{C1}$: 94.20ppm) , C_6 (σ $_{H6}$: 4.03ppm, σ $_{C6}$: 80.46ppm) and C_7 (σ $_{H7}$: 3.65ppm, σ $_{C7}$: 74.34ppm), a methine at C9 (σ $_{H9}$: 2.51ppm, σ $_{C9}$: 58.10ppm) and a β-glucose unit (σ $_{H1'}$: 4.66ppm, σ $_{C1'}$: 100.40ppm).
These three structures spectroscopic data were compared with the former published data and the presence of Lamalbide (1), Sesamoside (2) and Phlomiol (3) were established.[17-19]
Lamalbide and Sesamoside were reported previously from *E. azerbaijanica* rhizomes, E. *moluccelloides* and *E. laciniata* aerial parts. In addition, Phlomiol was isolated from *E. laciniata* rhizomes.[3,20-22] In addition, our earlier study on *E. macrophylla* rhizomes showed the presence of Lamalbide as an iridoid structure.[16] Previous literature revealed a broad spectrum of biological activities of the compounds with iridoid structure, such as neuroprotective, hypoglycemic, anti-inflammatory, hypolipidemic, immunomodulator, choleretic, hepatoprotective, cardioprotective, anti-cancer, anti-oxidant, anti-microbial, anti-spasmodic and purgative effects.[23] Moreover, several studies have been mentioned to antioxidant effect of Lamalbide and anti-tumor effect of Phlomiol.[24,25]

Compound (4):
UV λmax (MeOH): 220, 290, 330nm.
The ^{1}H-NMR spectrum of structure (4) indicated the presence of a tri-substituted phenyl moiety characterized by signals appearing at σ 7.02(bs, 1H, H-2'''), 6.96 (d, 1H, *J* = 8.22Hz, H-6''') and 6.76 (d, 1H, *J* = 8.08, H-5'''), a 3,4 di-hydroxyphenethyl alcohol moiety with proton resonances at σ 6.64 (s, 1H, H-2), 6.62 (bs,1H, H-5), 6.50 (d, 1H, *J* = 6.62Hz, H6), 3.72 (m, 2H, H-α) and 2.70 (bs, 2H, H-β) and olefinic protons of caffeic acid derivative at 6.21 (d,1H, H-α') and 7.47 (d, 1H, H-β'). Anomeric proton signals were presented at σ 4.36ppm (d, 1H, *J* = 7.82Hz, H-1') and σ 5.02ppm (bs, 1H, H-1") that were consistent with the β-glucopyranose unit and α-rhamnopyranosyl moiety, respectively. The results identified the compound (4) as phenylethanoid glycoside, acteoside or verbascoside. The data were in agreement with published spectral data.[13] This phenylethanoid structure had been previously reported from other species of the genus *Eremostachys* such as the aerial parts and rhizomes of *E. azerbaijanica* and *E. laciniata*.[7,13,22,26] Early reports described several pharmacological benefits for human health of Verbascoside, including anti-inflammatory, anti-oxidant, anti-neoplastic, wound healing and neuroprotective effects.[27,28]
The compounds (5), (6) and (7) from 40% MeOH fraction with flavonoid structures were confirmed with the UV and ^{1}H-NMR spectrum.

Compound (5):
UV λmax (MeOH): 207, 255, 350 nm
The ^{1}H-NMR data of compound (5) showed characteristic signals of 1, 2, 4- trisubstituted phenyl unit at σ 7.42ppm (bs, 1H, H-2'), 6.90ppm (d, 1H, J = 8.26Hz, H-5'), 7.45ppm (d, 1H, J = 8.39Hz, H-6'). Other signals at σ 6.75, 6.44 and 6.78ppm mentioned to the H-3, H-6 and H-8 in flavone, respectively. In addition, the anomeric proton signal appeared at σ 5.09ppm (d, 1H, J = 7.25Hz, H-1").

Compound (6):
UV λmax (MeOH): 205, 265, 345 nm
In the same manner, The ^{1}H-NMR data of compound (6) presented the 1, 4- disubstituted phenyl unit at σ 7.94ppm (d, 2H, J = 8.82Hz, H-2'and H-6'), 6.92ppm (d, 2H, J = 8.60Hz, H-3' and H-5'). The signals of H-3, H-6 and H-8 were displayed at 6.78ppm, 6.44ppm and 6.84ppm, respectively. Two anomeric protons were observed at 4.86ppm and 5.04ppm, which related to rutinoside group.

Compound (7):
UV λmax (MeOH): 205 265, 345 nm
The ^{1}H-NMR spectrum of compound (7) showed two doublet signals at σ 7.92ppm (J = 8.62 Hz) and 6.87ppm (J = 8.87 Hz) with orientation of two ortho situation protons for H-2', 6' and H-3', 5'. Two aromatic protons at σ 6.42ppm and 6.81ppm referring to H-6 and H-8 and a doublet signal at σ 5.07ppm (J = 7.13 Hz) were related to an anomeric proton.
These spectral data revealed the existence of (5) Luteolin-7-O- glucoside, (6) Apigenin-7- o- rutinoside and (7) Kaempferol 3-O-glucoside structures. (25-27) According to previous literatures, Luteolin-7-O- glucoside was reported from the aerial parts of *E. laciniata*.[22]
Flavonoids as a group of natural compounds with variable phenolic structures exhibit a wide range of bioactivities including anti-oxidant, anti-tumor, anti-inflammatory, anti-viral, anti-fungal, anti-bacterial and platelet aggregation inhibitory actions.[29] According to Ozcan and Matthaus, Luteolin-7-glucoside and Apigenin-7-glucoside

showed significant anti-oxidant activities as the phenolic compounds of olive leaves.[30] Moreover, anti-inflammatory effects of these two flavonoid structures were established previously.[31,32] In other previous study, Parveen et al. presented significant anti-inflammatiry and analgesic effects of Kaempferol 3-O-glucoside.[33]

The focus on the phytochemical studies of a number of species of genus *Eremostachys* showed the presence of various chemical structures. In some studies, iridoid glycosides, flavonoids, phenylethanoid glycosides and phytostrols were reported from the rhizomes or aerial parts of *E. laciniata*.[6,7] Ferulic acid derivatives, furanolabdane-type diterpenoids, and phenylethanoid glycosides were identified from the rhizomes of *E. glabra* in other studies.[1,34,35] Furthermore, several other studies reported iridoid glycoside, flavonoid, phenylethanoid, fatty acid

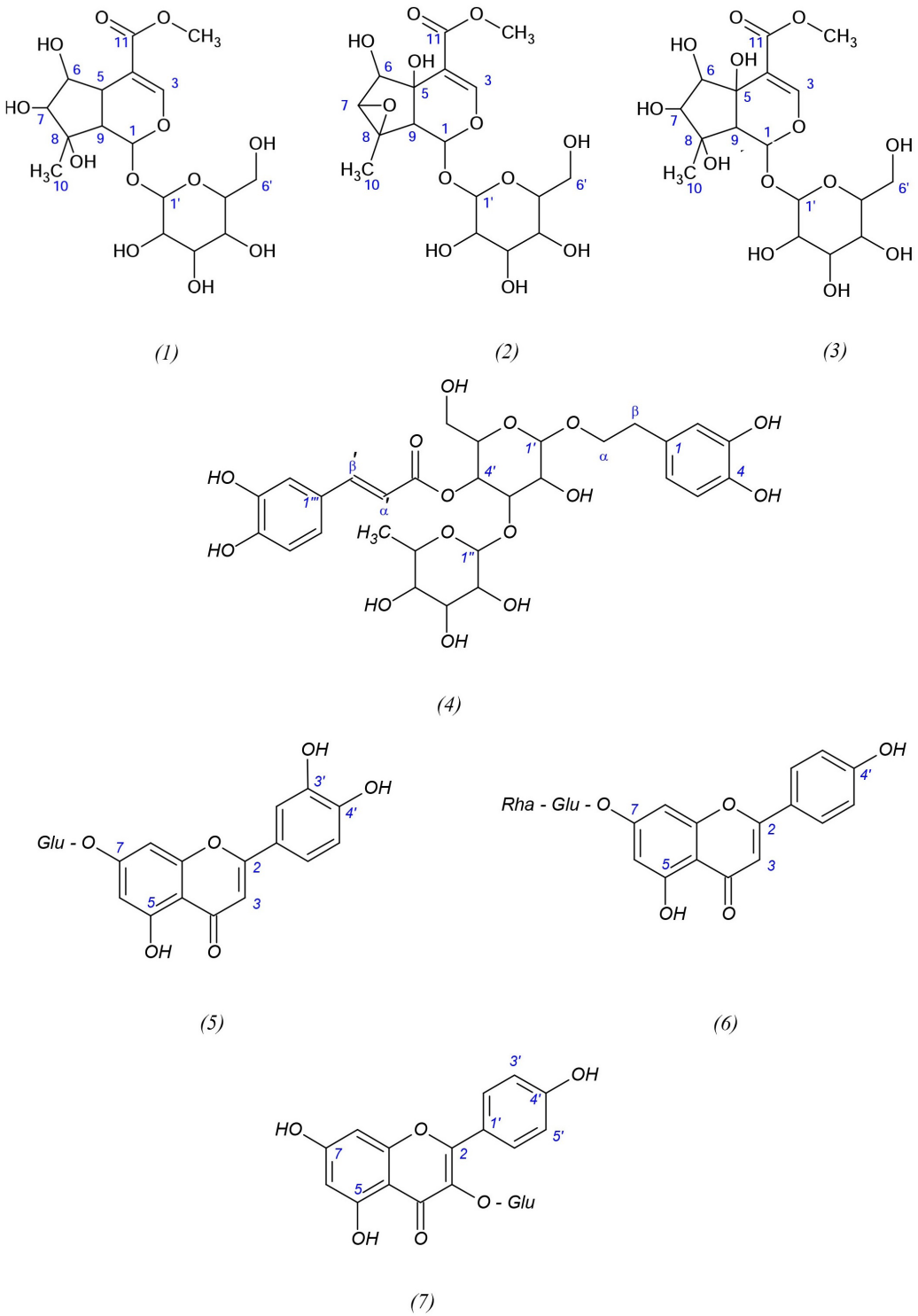

Figure 1. The suggested structures of seven pure compounds from *E. macrophylla* aerial parts: (1) Lamalbide, (2) Sesamoside, (3) Phlomiol, (4) Verbascoside, (5) Luteolin-7-O- glucoside, (6) Apigenin-7-O- rutinoside and (7) Kaempferol-3-O- glucoside.

and steroid structures from the rhizome or aerial parts of *E. azerbaijanica*,[13,36,37] iridoids and flavonoids from *E. loasifolia*,[38-40] iridoid glycosides from the aerial parts of *E. moluccelloides*[21] and various flavonoid derivatives from *E. vicaryi*[41] were isolated previously.

This is the first report on the purification and identification of iridoid, phenylethanoid and flavonoid structure from the aerial parts of *E. macrophylla*.

Conclusion

The present study has shown that the aerial parts of *E. macrophylla* are a source of iridoid, phenylethanoids and flavonoids. Since the anti-oxidant and anti-inflammatory effects of phenylethanoids and flavonoids,[42-46] analgesic, anti-inflammatory and anti-arthritic properties of iridoid glycosides have previously been confirmed by several *in vitro* studies,[47] it is therefore reasonable to conclude that *E. macrophylla* can be a good candidate for the studies on inflammatory diseases.

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Conflict of Interests

The authors claim that there is no conflict of interest.

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