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Isolation and structure elucidation of coumarin and cinamate derivatives from *Lycium ruthenicum*

Hassan Valizadeh[a,*], Fatemeh Mahmoodi Kordi[b], Reza Kouhkan[a], Mir Babak Bahadori[a], Mehdi Moridi Farimani[c]

[a]*Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*

[b]*Department of Biology, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*

[c]*Department of phytochemistry, Shahid Beheshti University, Tehran, Iran*

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Abstract

Lycium species is a popular medicinal plant in the traditional Chinese medicine and *Lycium ruthenicum* is a native medicinal plant of Iran. *Lycium* genus has several biologically important properties too. Investigation of chemical composition of ethyl acetate extract of this plant is the goal of this study. Two coumarins (Scopoletin and Sculetin) and Methyl-2-hydroxy-4-undecanoxy-trans-cinamate were isolated and characterized as the major constituents using ^{1}H NMR, ^{13}C NMR and FT-IR spectroscopic data, MS spectrometry, elemental analysis and by comparison with the literature values. Phytochemical investigation of *Lycium ruthenicum* demonstrated the presence of important biologically active compounds. This is the first phytochemical study of this species in Iran.

Keywords: Lycium ruthenicum; traditional medicine; phytochemistry; coumarine; Scopoletin; Esculetin.

*Corresponding author: Hassan Valizadeh
Tel: +98 (412) 4327541, Fax: +98 (412) 4327541
E-mail: h-valizadeh@azaruniv.edu

Introduction

The plant kingdom is a wide field to discovery new drugs and pharmacologically active compounds [1]. Production of pharmaceutically active oils and waxes were a major business in Mesopotamian and Egyptian times and the history of the extraction of natural products dates back to this time [2]. About half of the pharmaceuticals which are used today are derived from natural products [3]. *Lycium ruthenicum* (Solanaceae) is one of approximately 80 species distributed worldwide [4]. This plant particularly concentrated in South America, southwestern North America and southern Africa [5]. It is also distributed in salinized plains in eastern Azerbaijan province of Iran without any known utility. It can be a plant for preventing soil desertification and alleviating the degree of soil salinity–alkalinity because of its special physiological characteristics of drought-resistance and salt-resistance [6].

Lycium ruthenicum is known as a nutritional and medicinal food in China and is used in traditional Chinese medicine to nourish liver and kidney, and brighten the eye [7]. It is used as a seasoning to steamed rice. The dried fruit is a well-known traditional medicine in China, Korea, Japan, Vietnam, Thailand, and Tibet [8]. The antitumor activity of *Lycium chinensis* polysaccharides in rat liver cancer has been reported [9]. Phenolic amides isolated from the root bark of *Lycium chinense* showed anti-fungal effects [10]. Crude extracts from the fruit of this plant showed a high antioxidant capacity and antimicrobial activity [11]. Previous studies indicate that *Lycium ruthenicum* has antioxidant properties, immunomodulation, antitumor activity and cytoprotection effect [12]. Various chemical constituents are found in *Lycium* species such as β-sitosterol, p-coumaric acid, vitamins and minerals [13]. Alkaloids, flavonoids, steroids and coumarins are main metabolites of this genus [14]. Coumarins scopoletin and esculetin have been isolated *from Lycium chinense* [15].

Coumarins owe their class name to ‘Coumarou’, the vernacular name of the Tonka bean (Dipteryx odorata Willd. Fabaceae) from which coumarin itself was isolated in 1820 [16]. Coumarins are important natural products, and occupy a great place in synthetic organic chemistry [17]. Coumarins are found in the form of benzopyrene derivatives [18]. These compounds act as antioxidants, enzyme inhibitors, and precursors of toxic substances [19]. In addition to biological activities, they are used as additives in food, cosmetics and optical brightening agents [20].

To the best of our knowledge, any phytochemical study on *Lycium ruthenicum* has not been reported before. In the present research, we wish to report the isolation and identification of Scopoletin (Scheme 1), Esculetin (Scheme 2) and methyl 2-hydroxy-4-undecanoxy-trans-cinamate (Scheme 3) from the ethyl acetate extract of the aerial parts of this species.

Experimental

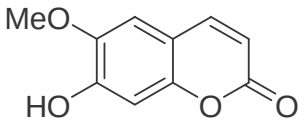
Materials

All reagents were purchased from Merck (Germany) and used without further purification. Silica gel (230–400 mesh, Merck, Germany) was employed for column chromatography.

Plant Material: The fresh aerial parts of the plant, *Lycium ruthenicum*, was collected from Mamaghan, East Azerbaijan province, Iran, in July 2011.

Characterization: Infrared spectra were recorded in KBr and were determined on a Perkin Elmer FT-IR spectrometer in the range of 4000–400 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance AC-400 MHz and 100 MHz respectively, using $CDCl_3$ and DMSO-d_6 as the deuterated solvents. Elemental analyses were carried out on a Perkin-Elmer 240C elemental analyzer and are reported in percent atomic abundance. Melting points are uncorrected and measured in open glass-capillaries using Stuart melting point apparatus. GC/MS analyses were carried out on a "Trace GC-Trace MS" instrument (England) with electron ionization source, quadruple analyzer, capillary DB-5MS column and Helium as carrier gas.

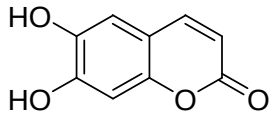
Isolation and purification: The extract was a column chromatographed on silica gel with a gradient elution of n-hexane/EtOAc (1:0→0:1) and afford to nine fractions: F1 (1.5 g), F2 (1.6 g), F3 (1.7 g), F4 (2.2 g), F5 (1.2 g), F6 (2.1 g), F7 (3.5 g), F8 (1.5 g) and F9 (1.4 g). F6 was subjected to column chromatography with n-hexane/ EtOAc (7:3→0:1) and finally purified by preparative thin layer chromatography with n-hexane/ EtOAc (2:8) to afford impure compound 1. Further purification of this impure crude by preparative thin layer chromatography with chloroform/methanol (9:1) obtained pure compound 1 (310 mg).



Scheme 1. Chemical structure of Scopoletin

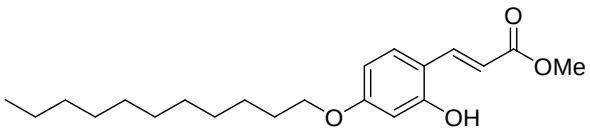
F8 was subjected to column chromatography with n-hexane/EtOAc (6:4→0:1) and then loaded on preparative thin layer chromatography with n-hexane/EtOAc (1.5:8.5) to afford impure further purification using pre-

parative TLC with chloroform/methanol (9:1) afford to pure compound 2 (220 mg).



Scheme 2. Chemical structure of Esculetin

F7 was subjected to column chromatography with n-hexane/EtOAc (6:4→0:1) and then purified using preparative thin layer chromatography with n-hexane/EtOAc (1:5) as eluent to afford pure compound 3 (520 mg).



Scheme 3. Chemical structure of methyl-2-hydroxy-4-undecanoxy-trans-cinamate

Structure elucidation of isolated compounds Scopoletin (1)

White needles. MP: decomposes at 204-205ºC. Ref: 0.55 ($CHCl_3$-MeOH, 9:1). IR (KBr): 3540.8, 1716.4, 1609.2, 1211.9 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.88 (3H, s, Me), 6.28 (1H, d, *J* = 9.24 Hz), 7.11 (1H, s), 7.70 (1H, d, *J* = 9.24 Hz), 6.72 (1H, s), 6.12 (1H, s); ^{13}C NMR (100 MHz DMSO-d_6): δ 58.65, 102.32, 107.67, 110.43, 113.43, 142.35, 143.11, 149.88, 150.56, 161.22. MS (EI, 70 eV): *m/z* (%) = 192 [M^+] (65). Anal. Calced for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.52; H, 4.22.

Escalation (2)

White powder. MP: decomposes at 271-273ºC. Ref: 0.48 ($CHCl_3$-MeOH, 9:1). IR (KBr): 3285.5, 2922.2, 1590.9, 1453.4. 1212.6 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 6.15 (1H, d, *J* = 9.50), 6.74 (1H, s, PhH), 6.93 (1H, s, PhH), 7.75 (1H, d, *J* = 9.50), 6.12 (1H, s), 5.98 (1H, s); ^{13}C NMR (100 MHz DMSO-d_6): δ 109.04(CH), 112.60(CH), 115.18(CH), 117.33(C), 124.02(CH), 145.76(C), 145.10(C), 149.62(C), 161.12(CO). MS (EI, 70 eV): *m/z* (%) = 179 [M^+] (93). Anal. Calced for $C_9H_6O_4$: C, 60 .68; H, 3.39. Found: C, 60.67; H, 3.42.

Methyl-2-hydroxy-4-undecanoxy-trans-cinamate (3)

White powder. MP: decomposes at 68-69ºC. R_f : 0.52 (EtOAc- *n*-Hexane, 1:5). IR (KBr): 3411 (broad, OH), 1707 (CO), 1636, 1597, 1123 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.63 (1H, d, *J* = 16.6), 7.10 (1H, d, *J* = 8.6), 7.08 (1H, s), 7.94 (1H, d, *J* = 8.6), 6.32 (1H, d, *J* = 16.6), 5.89 (1H, broad, OH), 4.21 (2H,

t, *J* = 6.80), 3.95 (3H, s, OMe), 1.51-1.23 (18H, broad), 0.90 (3H, t, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.42, 147.89, 146.75, 144.65, 127.07, 123.07, 115.69, 114.70, 109.26, 64.15, 55.94, 31.95, 29.73, 29.69, 29.63, 29.58, 29.39, 29.34, 28.79, 26.03, 22.72, 14.16. MS (EI, 70 eV): *m/z* (%) = 348 [M^+] (17), 317 (34), 207 (100). Anal. Calced for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.41; H, 9.23.

Results and discussion

The dried aerial parts (2 kg) of the plant were ground to very small size powder and extracted by maceration with solvent ethyl acetate (3 × 10 L) for 3 weeks at room temperature. Concentration of solution of ethyl acetate under reduced pressure by rotary evaporator gave 39 g of the related solvent extract. The extract was subjected to silica gel column chromatography. Two coumarin and one cinamate derivatives were obtained from this phytochemical study. Structure elucidation of isolated compounds were carried out using several techniques such as ^{1}H-NMR, ^{13}C-NMR and FT- IR spectroscopy, MS spectrometry and elemental analysis. Results showed the presence of biologically important secondary metabolites in the extract of this medicinal species for the first time in Iran. These findings could support some of the traditional uses of this herb. We hope this report could be a start point for medicinal uses of this widely distributed but useless plant in Iran.

Conclusion

The phytochemical study of the ethyl acetate extract obtained from the air-dried aerial part of *Lycium ruthenicum* afforded to two known coumarins and methyl 2-hydroxy-4-undecanoxy-trans-cinamate. These compounds were purified and their structures were elucidated by a detailed spectroscopic analysis, elemental analysis and comparison of their spectroscopic and physical data with those reported in the literature.

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