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Isolation and Identification of Main Compounds of *Lagochilus cabulicus*

S. SAEIDNIA[1], E. BARARI[2], A. SHAKERI[2] and A.R. GOHARI[1,*]

[1]Medicinal Plants Research Center, Tehran University of Medical Sciences, P. O. Box 14155-6451, Tehran, Iran
[2]Department of Chemistry, Faculty of Science, Golestan University, Gorgan, Iran

*Corresponding author: Fax/Tel: +98 21 64122330; E-mail: goharii_a@tums.ac.ir

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The genus, *Lagochilus* Bge., belongs to Lamiaceae family and consists of 44 species all over the world, 4 species of which are growing exclusively in Iran. Literature show that there is no report around the isolation and spectral elucidation of the main compounds of the Iranian species, *Lagochilus cabulicus* Benth. The aim of this study is to determine the main compounds of this species for the first time. Column and thin layer chromatographic methods were used for isolation and purification and spectroscopic data (^{1}H NMR and ^{13}C NMR) were employed for identification of the compounds isolated from ethyl acetate and methanol extracts. From the ethyl acetate and methanol extracts of *L. cabulicus*, four flavonoid, tricetin 3´-methyl ether (**1**), quercetin (**2**), quercetin 3-O-α-L-rhamnopyranosyl (1→6) β-D-glucopyranoside (**3**), quercetin 3-O-β-D-glucopyranoside (**4**), two steroids, sitosteryl acetate (**5**), stigmasteryl acetate (**6**) and one triterpene, lupeol (**7**), have been identified. The results show that the main compounds of *L. cabulicus* are biologically and pharmacologically active flavonol glucosides, sterol acetates and pentacyclic triterpene. There has not found any diterpene (lagochilin), as the main compound, in the aerial parts of *L. cabulicus*.

Key Words: *Lagochilus cabulicus*, Flavonoid, Lupeol, Sitosteryl acetate, Stigmasteryl acetate.

INTRODUCTION

The genus *Lagochilus* Bge, belongs to the plant family Lamiaceae and consists of 44 species all over the world, 33 of which grow in central Asia. Five species of this genus have been reported in Flora Iranica while, 4 species are exclusively growing in Iran and well-known as Lab-Khargushi in Persian language[1,2]. *Lagochilus cabulicus* Benth., which is called Lab-Khargushi-Kaboli, growing wildly in Iran, Afghanistan and Turkistan[2].

A literature review reveals that although, there are some reports around the chemical constituents of several species of this genus[3-6], there is no report on phytochemical investigation of *L. cabulicus*. Previous chemical studies on *Lagochilus* species have resulted in the isolation and identification of mostly diterpenes and diterpene lactone (*L. hirsutissimus* and *L. inebrians*)[3-5], flavonoids (*L. proskorjacovi* and *L. platycalyx*)[6,7] and polysaccharides (*L. usunachmaticus* and *L. zeravschanicus*)[8,9].

Anticoagulant activity was reported from the polysaccharides of *L. usunachmaticus*, which possessed a marked direct-action activity and exceeded heparin during affection[10]. Recently, the antimicrobial activity of *L. kotschyanus* oil was reported against four bacteria compared with standard antibiotics. Its flower oil was found to be active against *Streptococcus pyogenes*, *S. agalactia* and *Bacillus anthracis*. The leaves oil only showed inhibitory activity against *S. pyogenes*[11]. Based on unpublished data, these species were traditionally used for their sedative and intoxicating activities.

In this study, the separation and identification of some flavonoids, steroids and triterpene from the aerial parts of *L. cabulicus* is described.

EXPERIMENTAL

The aerial parts of *Lagochilus cabulicus* Benth., were collected during flowering stage in August (2009). Plant material was dried at ambient temperature and shade condition then cut into small pieces (1400 g) followed by extraction with ethyl acetate and methanol, respectively, using percolation method. Obtained extracts were concentrated by rotary-evaporator and dried by freeze drier to result in 11 g of ethyl acetate and 30 g of methanol extracts.

The spectra for ^{1}H- and ^{13}C NMR were measured on a Brucker Advance 500 DRX spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given in d parameter. Silica gel 60 F_{254} pre-coated plates (Merck) were used for TLC and the detection of compounds was accomplished by exposure to UV light at 254 and 366 nm followed by spraying with anisaldehyde-H_2SO_4 reagent and then heating at 120 ºC for 5 min.

Isolation: The methanol extract (30 g) was subjected to silica gel column chromatography (CC) with chloroform, chloroform: ethyl acetate (1:1), ethyl acetate, ethyl acetate: methanol (1:9, 1:1) and methanol as eluent, to give six fractions (A-F). The fraction A (3300 mg) was further fractionated by a silica gel CC with chloroform: methanol (19:1, 9:1 and 1:1) to obtain five fractions (A_1-A_5). The fraction A_4 was submitted to silica gel CC with chloroform: methanol (7:3, 1:1) to gain two fractions (A_{41}, A_{42}). Compound **1** (4 mg) was obtained from A_{42} (130 mg) using sephadex LH_{20} CC with methanol: chloroform (8:2). The fraction B (3480 mg) was subjected to silica gel CC with chloroform: methanol (19:1, 9:1 and 1:1) and methanol as eluent to result in five fractions (B_1-B_5). Compound **2** (3.7 mg) was obtained from B_2 (430 mg) using sephadex LH_{20} CC followed by twice elution with methanol: chloroform (8:2). The fraction E (4500 mg) was submitted to sephadex LH_{20} CC with methanol to give five fractions (E_1-E_5). The fraction E_4 was subjected to reverse phase silica gel CC (RP, C_{18}) with methanol: water (6:4, 7:3, 8:2 and 9:1) and methanol to give five fractions (E_{41}-E_{45}). The compound **3** (4 mg) and **4** (3 mg) were obtained from E_{42} (37 mg) using sephadex LH_{20} CC with methanol.

The ethyl acetate extract (11 g) was subjected to silica gel CC with hexane:chloroform (1:0, 7:3, 3:7 and 0:1), chloroform: ethyl acetate (1:1 and 0:1) and finally ethyl acetate: methanol (1:1) to obtain seven fractions (Et_1-Et_7). The fraction Et_5 (1100 mg) was submitted to silica gel CC with hexane: chloroform (3:2, 2:3, 1:4 and 0:1) to gain five fractions (Et_{51}-Et_{55}). The fraction Et_{52} (35 mg) was subjected to silica gel CC with hexane: ethyl acetate (19:1 and 9:1) to give compounds **5**, **6** (9 mg) and compound **7** (17 mg).

The ^{1}H and ^{13}C NMR data (δ, ppm) of the isolated compounds **1-7** from the methanol and ethyl acetate extracts of *L. cabulicus* are given below:

Tricetin 3′-methyl ether (1): Yellow crystal (R_f = 0.56 in C_9M_1). ^{1}H NMR (500 MHz, methanol-d_4), δH: 7.11 (1H, d, *J* = 2 Hz, H-2'), 7.09 (1H, d, *J* = 2 Hz, H-6'), 6.57 (1H, s, H-3), 6.44 (1H, d, *J* = 2.1 Hz, H-8), 6.20 (1H, d, *J* = 2.1 Hz, H- 6), 3.93 (3H, s, OMe). ^{13}C NMR (125 MHz, DMSO-d_6), δC: 163.80 (C-2), 103.23 (C-3), 181.64 (C-4), 161.40 (C-5), 98.78 (C-6), 164.17 (C-7), 93.85 (C-8), 157.26 (C-9), 103.64 (C-10), 120.36 (C-1′), 102.40 (C-2′), 148.59 (C-3′), 138.67 (C-4′), 145.92 (C-5′), 107.49 (C-6′), 56.22 (C-OMe).

Quercetin (2): Yellow crystal (R_f = 0. 6 in C_8M_2): ^{1}H NMR (500 MHz, methanol-δ$_4$), δH: 7.73 (1H, d, *J* = 2.1 Hz, H-2'), 7.63 (1H, dd, *J* = 8.4, 2.1 Hz, H-6'), 6.87 (1H, d, *J* = 8.5 Hz, H-5'), 6.38 (1H, d, *J* = 2 Hz, H-8), 6.17 (1H, d, *J* = 2 Hz, H- 6). ^{13}C NMR (125 MHz, DMSO-d_6), δC: 146.83 (C-2), 135.68 (C-3), 175.81 (C-4), 160.68 (C-5), 98.13 (C-6), 163.91 (C-7), 93.31 (C-8), 156.12 (C-9), 102.98 (C-10), 121.93 (C-1′), 115.02 (C-2′), 145.06 (C-3′), 147.68 (C-4′), 115.56 (C-5′), 119.92 (C-6′).

Quercetin 3-O-α-L-rhamnopyranosil (1→6) β-D-glucopyranoside (3): Yellow crystal (R_f = 0. 48 in ethyl acetate:water:acetic acid:formic acid, 9:2.6:1.1:1.1).

^{1}H NMR (500 MHz, methanol-d_4), δH: 6.20 (1H, d, *J* = 2 Hz, H-6), 6.40 (1H, d, *J* = 2.1 Hz, H-8), 7.67 (1H, d, *J* = 2.1 Hz, H-2′), δH: 6.86 (1H, d, *J* = 8.5 Hz, H-5′), 7.62 (1H, dd, *J* = 8.6, 2.2 Hz, H-6′), 5.10 (1H, d, *J* = 7.3 Hz, H-1′′), 3.25-3.47 (4H, m, H-2′′, H-3′′, H-4′′, H-5′′), 3.38 (2H, m, H6′′), 4.51 (1H, d, *J* = 1.2 Hz, H-1′′′), 3.63 (1H, dd, *J* = 3.5/1.5 Hz, H-2′′′), 3.53 (1H, dd, *J* = 9.5/3.5 Hz, H-3′′′), 3.28 (1H, m, H-4′′′), 3.44 (1H, m, H-5′′′), 1.28 (3H, d, *J* = 6.0 Hz, CH_3-6′′′). ^{13}C NMR (125 MHz, methanol-d_4): δC: 158.56 (C-2), 135.66 (C-3), 179.45 (C-4), 163.01 (C-5), 99.98 (C-6), 166.05 (C-7), 94.90 (C-8), 159.42 (C-9), 105.70 (C-10), 123.17 (C-1′), 117.72 (C-2′), 145.85 (C-3′), 149.84 (C-4′), 116.08 (C-5′), 123.58 (C-6′), 104.72 (C-1′′), 75.71 (C-2′′), 77.27 (C-3′′), 71.45 (C-4′′), 78.22 (C-5′′), 68.60 (C-6′′), 102.41 (C-1′′′), 72.12 (C-2′′′), 72.29 (C-3′′′), 73.94 (C-4′′′), 69.73 (C-5′′′), 17.87 (C-6′′′).

Quercetin 3-O-β-D-glucopyranoside (Isoquercitrin) (4): Yellow crystal (R_f = 0.5 in ethyl acetate:water:acetic acid: formic acid, 9:2.6:1.1:1.1).

^{1}H NMR (500 MHz, methanol-d_4): δH: 7.70 (1H, d, *J* = 2.7 Hz H-2′), 7.58 (1H, dd, *J* = 8.4,2.6 Hz, H-6′), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 6.40 (1H, d, *J* = 2.5 Hz, H-8), 6.20 (1H, d, *J* = 2.5 Hz, H-6) and 5.24 (1H, d, *J* = 7.6, H-1′′). ^{13}C NMR (125 MHz, DMSO-d_6), δC: 156.05 (C-2), 133.53 (C-3), 177.41 (C-4), 161.08 (C-5), 98.63 (C-6), 164.09 (C-7), 93.51 (C-8), 156.15 (C-9), 103.98 (C-10), 121.09 (C-1′), 115.26 (C-2′), 144.80 (C-3′), 148.36 (C-4′), 116.31 (C-5′), 121.50 (C-6′), 100.92 (C-1′′), 74.11 (C-2′′), 76.49 (C-3′′), 69.95 (C-4′′), 77.49 (C-5′′) and 60.90 (C-6′′)[18,19].

β-Sitosteryl acetate (5): White crystal (R_f = 0.7 in chloroform): ^{1}H NMR (500 MHz, $CDCl_3$), δH: 5.38 (1H, m, H-6), 4.60 (1H, m, H-3), 2.05 (3H, s, Me of acetate), 1.02 (3H, s, Me-19), 0.94 (3H, d, *J* = 6.4 Hz, Me-21), 0.84 (6H, m, Me-26 and Me-29), 0.82 (3H, d, *J* = 6.9 Hz, Me-27), 0.68 (3H, s, Me-18). ^{13}C NMR (125 MHz, $CDCl_3$), δ C: 37.02 (C-1), 27.80 (C-2), 74.01 (C-3), 38.14 (C-4), 139.68 (C-5), 122.65 (C-6), 31.91 (C-7), 31.91 (C-8), 50.07 (C-9), 36.62 (C-10), 21.05 (C-11), 39.75 (C-12), 42.34 (C-13), 56.71 (C-14), 24.31 (C-15), 28.25 (C-16), 56.07 (C-17), 11.87 (C-18), 19.32 (C-19), 36.18 (C-20), 18.80 (C-21), 34.00 (C-22), 26.13 (C-23), 45.88 (C-24), 29.20 (C-25), 19.05 (C-26), 19.76 (C-27), 23.01 (C-28), 12.25 (C-29), 170.62 (C=O), 21.56 (Me of Acetate)[20].

Stigmasteryl acetate (6): White amorphous crystal (R_f = 0.7 in chloroform).

^{1}H NMR (500 MHz, $CDCl_3$), δH: 5.15 (1H, m, H-22), 5.03 (1H, m, H-23), 4.60 (1H, m, H-3), 2.05 (3H, s, Me of acetate), 1.02 (3H, s, Me-19), 0.99 (3H, d, *J* = 6.4 Hz, Me-21), 0.85 (3H, d, *J* = 6.9 Hz, Me-27), 0.81 (3H, t, *J* = 7.8 Hz, Me-29), 0.79 (3H, d, *J* = 6.8 Hz, Me-26), 0.68 (3H, s, Me-18). ^{13}C NMR (125 MHz, $CDCl_3$), δC: 37.02 (C-1), 27.80 (C-2), 74.01 (C-3), 38.14 (C-4), 139.68 (C-5), 122.65 (C-6), 31.91 (C-7), 31.91 (C-8), 50.07 (C-9), 36.62 (C-10), 21.05 (C-11), 39.75 (C-12), 42.34 (C-13), 56.71 (C-14), 24.31 (C-15), 29.20 (C-16), 56.07 (C-17), 12.00 (C-18), 19.32 (C-19), 40.50 (C-20), 21.33 (C-21), 138.32 (C-22), 129.32 (C-23), 51.26 (C-24), 31.92 (C-25), 19.05 (C-26), 21.05 (C-27), 25.66 (C-28), 12.25 (C-29), 170.50 (C=O), 21.42 (Me of acetate)[20].

Lupeol (7): White needle crystal (R_f = 0.8 in hexane-ethyl acetate (9:1).

^{1}H NMR (500 MHz, $CDCl_3$), δH: 4.70 (1H, s, H-29b), 4.58 (1H, s, H-29a), 3.20 (1H, dd, *J* = 10.2, 4.4 Hz, H-3), 1.70

(3H, s, Me-30), 0.98 (3H, s, Me-24), 0.96 (3H, s, Me-27), 0.84 (3H, s, Me-25), 0.80 (3H, s, Me-28), 0.77 (3H, s, Me-23). ^{13}C NMR (125 MHz, $CDCl_3$), δC: 38.7 (C-1), 27.44 (C-2), 79.0 (C-3), 38.86 (C-4), 55.30 (C-5), 18.32 (C-6), 34.27 (C-7), 40.83 (C-8), 50.44 (C-9), 37.17 (C-10), 20.93 (C-11), 25.14 (C-12), 38.05 (C-13), 42.83 (C-14), 27.44 (C-15), 35.58 (C-16), 43.0 (C-17), 48.30 (C-18), 48.0 (C-19), 150.98 (C-20), 29.84 (C-21), 40.0 (C-22), 27.98 (C-23), 15.36 (C-24), 16.12 (C-25), 15.97 (C-26), 14.54 (C-27), 18.0 (C-28), 109.32 (C-29), 19.30 (C-30)[21].

RESULTS AND DISCUSSION

From the aerial parts of *L. cabulicus*, four flavonoids, two steroids and one triterpene were isolated and identified as tricetin 3´-methyl ether (**1**)[12], quercetin (**2**)[13,14], quercetin 3-O-ramnoglucoside (**3**)[15,16], quercetin 3-O-glucoside (**4**)[16], β-sitosteryl acetate (**5**), stigmasteryl acetate (**6**)[17] and lupeol (**7**)[18] based on the spectroscopic spectra (^{1}H NMR, ^{13}C NMR) compared to the known standard compounds which reported in the literature. To our best of knowledge, this is the first report on the isolation and structural elucidation of these compounds from the species, *L. cabulicus*.

Among the isolated flavonoids, the compound **3** is a well-known flavonol glycoside named rutin and previously isolated from *L. Platycalyx*[19]. Other derivatives of quercetin and tricetin (compounds, **1**, **2** and **4**) are reported for the first time from the genus, *Lagochilus*. The isolated steroids are the acetate form of β-sitosterol and stigmasterol which separated as a mixture and have not been previously reported from *Lagochilus*. There is only one report about the isolation of β-sitosterol from *L. pubescens*[5]. Lupeol (**7**) is also isolated for the first time from the genus, *Lagochilus*.

As shown in the literature, the anti-proliferative effect of quercetin 3-O-rhamnoglucoside (**3**) and quercetin 3-O-glucoside (**4**) has been reported on six various cancer cell lines including colon, breast, hepatocellular and lung cancer[20]. Their results indicated that quercetin 3-O-glucoside showed the most potent growth inhibition, whereas rutin has the least potency. Also, it is reported that the flavonol quercetin 3-O-glucoside can inhibit *in vitro* absorption of cyaniding 3-glucoside[21]. A literature review shows that stigmasterol, stigmasterol acetate and β-sitosterol were evaluated for their anti-nociceptive activity. Their results revealed that stigmasterol and stigmasterol acetate (50-200 mg/kg) can exhibit significant analgesic activity against both acetic acid and formalin-induced nociception in mice[22]. Lagochilin is a bitter alcoholic diterpene which is found in various species of the genus *Lagochilus*, most notably *Lagochilus inebrians* and reported to be responsible for the sedative and hypotensive activities of this plant[23]. But in the present study, there was not found as one of the main components. It seems that lagochilin may be in a trace amount in the aerial parts of *L. cabulicus*. Therefore, the phytochemical investigation of the rhizome and root extract of *L. cabulicus* is suggested.

In conclusion, the results of this paper show that the main compounds of *L. cabulicus* are biologically and pharmacologically active flavonol glucosides, sterol acetates and pentacyclic triterpene, lupeol. The results indicated that there has not found any diterpene (especially lagochilin), as the main compound, in the aerial parts of *L. cabulicus*.

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