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Original article

Flavonoids from the leaves of Iranian Linden; *Tilia rubra* subsp. *caucasica*

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Abstract

Background and objectives: Plants belonging to the genus *Tilia* L. (Tiliaceae) are often tall beautiful trees which are considered for various medicinal potentials of their flowers and leaves. The present study was an attempt to investigate the phytochemical constituents of *Tilia rubra* subsp. *caucasica* leaves from the hyrcanian forests of north of Iran. **Methods:** Chromatography on Silica gel (normal and reversed-phase) and Sephadex LH20 was applied for isolation and purification of the compounds from the hydroalcoholic extract of the plant leaves. The structures of isolated compounds were elucidated using UV, ^{1}H-NMR and ^{13}C-NMR spectral analyses. **Results:** Four flavonoid glycosides, quercetin-3-O-β-D-glucoside-7-O-α-L-rhamnoside (petiolaroside), quercetin-3-O-α-L-rhamnoside (quercitrin), apigenin-7-O-β-D-glucoside (cosmosiin) and luteolin-7-O-β-D-glucoside (cynaroside) were isolated from *T. rubra* subsp. *caucasica* leaves, which have been previously documented for their various biological activities. **Conclusion:** The results of this study introduc *T. rubra* subsp. *caucasica* as a source of bioactive flavonoid glycosides and highlight it as an appropriate option for further pharmacognostical studies.

Keywords: flavonoid, linden, namdar, Tiliaceae,*Tilia rubra* subsp. *caucasica*

Introduction

The genus *Tilia* L. (common names; linden and lime) from Tiliaceae family, consists of about 44 species and 2 hybrids, native to the temperate regions of Europe, Asia, and North America [1,2].

In Flora Iranica, Iranian *Tilia* trees have been listed as *Tilia platyphyllos* with two subspecies, *platyphyllos* and *caucasica* [3]. However, a comprehensive study by Zare *et al*. (2012) on taxonomical parameters of the various *Tilia* populations distributed throughout hyrcanian forests resulted in identification of four native taxa, *T. cordata*, *T. dasystyla*, *T. rubra* subsp. *caucasica* and *T. begoniifolia*, two new endemic taxa, *T. sabetii* and *T. stellato-pilosa*, as well as one hybrid, *Tilia* × *euchlora*, in north of Iran (Guilan, Mazandaran and Golestan provinces) [4].

Medicinal potentials of *Tilia* spp. make them valuable plants in pharmacognosy researches. In British Herbal Pharmacopeia, linden flower (*T. platyphyllos*, *T. cordata* and *T.* × *vulgaris*) has been mentioned useful for treatment of hypertension caused by arteriosclerosis and nervous tensions [5]. German commission E has also approved its use for treatment of colds and cold-related coughs [6]. Besides, linden leaf has been considered as diaphoretic which has not

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been supported by scientific evidences yet [6].
In Iran, *Tilia* trees are known as "namdâr" or "zirfoon" and the infusion of their flowers is used as sedative, diuretic, demulcent and for treatment of catarrh complaints [7,8].
Previous phytochemical investigations have reported several flavonoids such as kaempferol and quercetin, along with their various glycosides as main principles of the flowers and leaves of some *Tilia* spp. [9-13].
The present study was an attempt to investigate the phytochemical constituents of *Tilia rubra* subsp. *caucasica* leaves from the hyrcanian forest of north of Iran.

Experimental

Plant material

The leaves of *Tilia rubra* subsp. *caucasica* (Rupr.) V.Engl. (Syn. *T. caucasica* Rupr.) were collected in July 2012 from the Pareh-sar forest (Talesh, Guilan province, north of Iran) in partnership with the Natural Resources and Forestry Administration of Guilan, Iran.

Extraction

The shade-dried and powdered leaves (170 g) were macerated with 80% methanol in water (5 × 1 L) to obtain total hydroalcoholic extract. The total extract was concentrated by a rotary evaporator at 45 °C and then defatted using enough volumes of petroleum ether and chloroform, successively. The residue was dried and used for phytochemical investigations.

Isolation and purification of compounds

A portion of defatted hydroalcoholic extract (5 g) was moved on a C_{18} reversed-phase (mesh 230-400, fully end-capped, Sigma-Aldrich) column and eluted with a gradient mixture of MeOH-H_2O (9:1-10:0) to obtain eight fractions (A-H). Fraction C (280 mg) was chromatographed on a RP-18 column with H_2O-MeOH (8:2) to get four factions (C1-C4). Compound **1** (18 mg) was obtained followed by elution of fraction C2 (73 mg) over a Sephadex LH20 (Fluka) column with H_2O-MeOH (2:8) as the eluent. Reversed-phase column chromatography of fraction E (85 mg) with H_2O-CH_3CN (7:3) was resulted in isolation of compounds **2** and **3** as a mixture (4:6) (21 mg). Chromatography of fraction F (265 mg) on a RP-18 column with H_2O-MeOH (8:2-7:3) yielded five factions (F1-F5). Compound **4** (28 mg) was obtained from the fraction F3 (75 mg) on a RP-18 column (H_2O-CH_3CN, 6.5:3.5) and its impurities were removed by elution over a Sephadex LH20 column with H_2O-MeOH (2:8).
Thin layer chromatography was applied for monitoring all column chromatographies, and fractions showing similar spots under UV (254 and 366 nm) or after spraying natural product reagent (diphenylboric acid aminoethyl ester, 1% in methanol) (Sigma-Aldrich) were combined. ^{1}H-NMR and ^{13}C-NMR spectra of the isolated compounds were obtained on a Bruker Avance 400 DRX (400 MHz for ^{1}H and 100 MHz for ^{13}C) and UV spectra were recorded in methanol and after addition of diagnostic shift reagents on a Shimadzo A160 spectrophotometer [14].

Results and Discussion

Phytochemical investigation of the leaves of *T. rubra* subsp. *caucasica* resulted in the isolation and structure elucidation of four flavonoid glycosides. The isolated compounds were identified as quercetin-3-O-β-D-glucoside-7-O-α-L-rhamnoside (petiolaroside) (**1**), quercetin-3-O-α-L-rhamnoside (quercitrin) (**2**), apigenin-7-O-β-D-glucoside (cosmosiin) (**3**) and luteolin-7-O-β-D-glucoside (cynaroside) (**4**) (figure 1). Structure of these compounds were elucidated by NMR (^{1}H- and ^{13}C-NMR) and UV spectral analysis, as well as by comparing with related data published in the literature [15-18].
In 2014, Akyuz *et al*. analyzed phenolic compounds of the various blossom, leaf and trunk extracts of *T. rubra* subsp. *caucasica* growing in turkey by HPLC-UV and HPLC-UV-MS [19]. They reported high amounts of total phenolics
(17.548 ± 0.04 mg of gallic acid per g of dried sample) and total flavonoids (0.066 ± 0.19 mg of quercetin per g of dried sample) with presence of some phenolic compounds such as rutin,

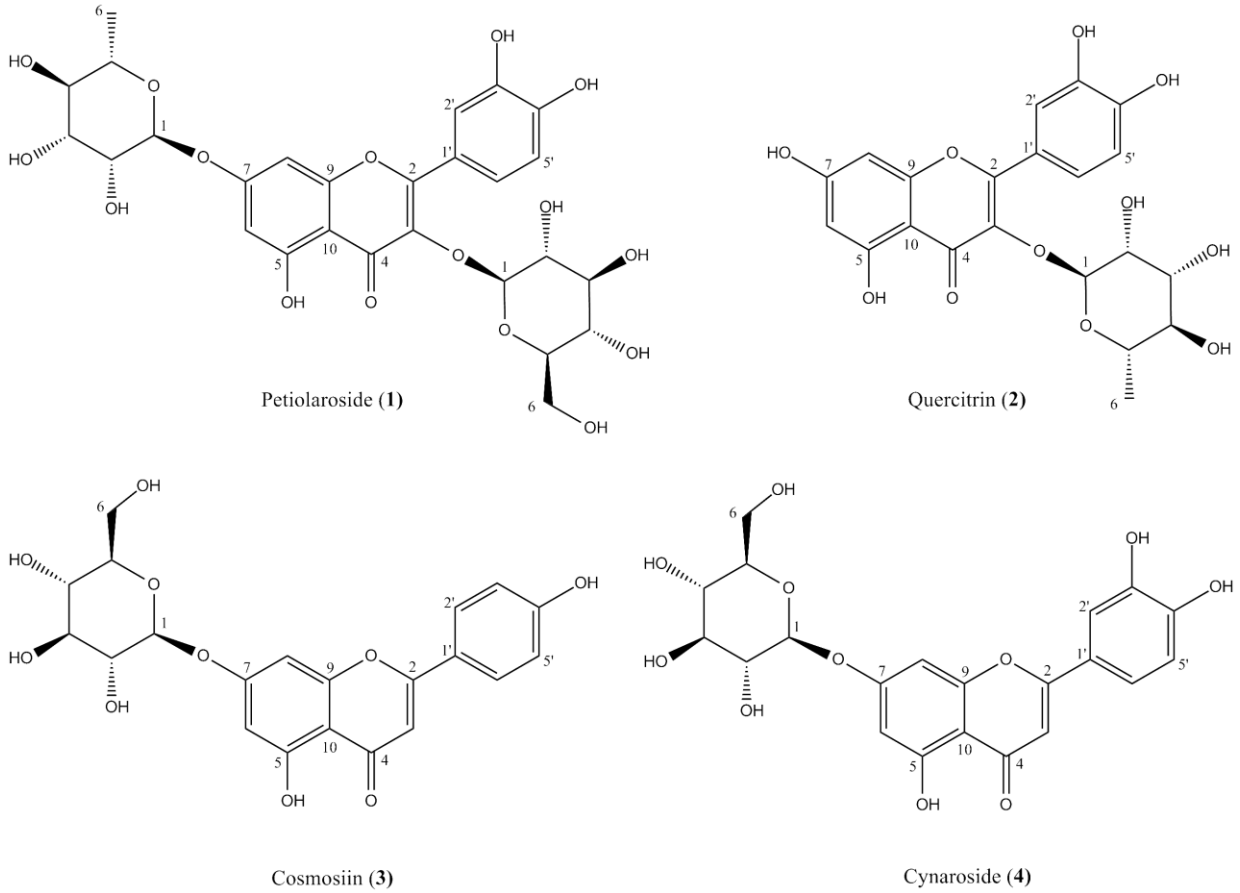

Figure 1. Structures of the isolated compounds (**1**-**4**) from *T. rubra* subsp. *caucasica* leaves

quercetin, catechin, caffeic acid, ferulic acid, chlorogenic acid, *p*-hydroxy benzoic acid, gallic acid and protocathechuic acid in *T. rubra* subsp. *caucasica* leaf [19].Our study, however, reported the isolation of petiolaroside (**1**), quercitrin (**2**), cosmosiin (**3**) and cynaroside (**4**) from *T. rubra* subsp. *caucasica* leaves for the first time. Furthermore, this is the first report on isolation of flavone glycosides, **3** and **4**, from *Tilia* genus. Petiolaroside (**1**) and quercitrin (**2**) have been previously reported from *T. platyohyllos*, *T. cordata* and *T. americana* var. *mexicana* [9-12]. Quercitrin (**2**) has also been detected by HPLC in *T. rubra* and *T. argentea* leaves [13].

A review of the literature revealed that the isolated flavonoid glycosides have been reported for their various biological and pharmacological activities [20-34]. Loscalzo *et al.* exhibited the neurosupressive effects of quercetin-3-O-glucoside-7-O-rhamnoside (petiolaroside) (**1**), isolated from *T. petiolaris* inflorescences, in the hole board, locomotor activity and thiopental-induced loss of righting reflex tests in mice [20]. Quercitrin (**2**) has been also reported as a compound with antioxidant [21], intestinal anti-inflammatory [22], antinociceptive [23], antidiabetic [24], antileishmanial [25] and antidiarrhoeal [26] activity. Moreover, antidiabetic [27] and aldose reductase inhibitory activity [28] of cosmosiin (**3**), as well as antioxidant [29], anti-inflammatory [30], α-glucosidase and amylase inhibitory [31], antiasthmatic [32], antimicrobial [33] and anticarcinogenic [34] potentials of cynaroside (**4**)

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of the isolated compounds (**1-4**) in DMSO-d_6

	^{1}H chemical shifts (δ) in ppm				^{13}C chemical shifts (δ) in ppm			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
2	-	-	-	-	156.6	148.9	164.8	165.2
3	-	-	6.60 *s*	7.27 *s*	133.4	134.7	103.7	103.8
4	-	-	-	-	177.3	163.8	182.6	182.7
5	-	-	-	-	160.7	159.1	162.2	161.8
6	6.23 *br s*	6.47 *br s*	6.47 *br s*	7.02 *d* (1.7 Hz)	99.3	99.7	100.1	100.2
7	-	-	-	-	161.4	163.1	163.5	163.5
8	6.41 *br s*	6.66 *br s*	6.67 *br s*	7.36 *d* (1.7 Hz)	94.2	92.4	95.4	95.5
9	-	-	-	-	155.8	155.1	157.5	157.6
10	-	-	-	-	105.5	103.1	105.9	106.1
1'	-	-	-	-	121.7	121.2	121.7	122.0
2'	7.80 *d* (2.0 Hz)	7.86 *d* (2.0 Hz)	7.92 *d* (8.8 Hz)	8.01 *br s*	116.3	114.7	129.2	114.3
3'	-	-	7.09 *d* (8.8 Hz)	-	144.8	144.7	116.6	146.5
4'	-	-	-	-	148.5	146.6	161.6	150.7
5'	6.90 *dd* (8.4, 2.0 Hz)	6.99 *dd* (8.4, 2.0 Hz)	7.09 *d* (8.8 Hz)	7.51 *br d* (8.4Hz)	115.1	115.5	116.6	116.8
6'	7.52 *d* (8.4 Hz)	7.80 *d* (8.4 Hz)	7.92 *d* (8.8 Hz)	8.00 *d* (8.4 Hz)	121.5	119.6	129.2	119.9
Glu-1	5.15 *d* (6.2 Hz)	-	4.96 *d* (6.2 Hz)	5.63 *d* (6.2 Hz)	100.5	-	100.5	100.3
Glu-2	3.1-4.2*	-	3.1-4.2*	3.2-4.5*	74.1	-	73.7	73.8
Glu-3	3.1-4.2*	-	3.1-4.2*	3.2-4.5*	76.5	-	77.8	77.1
Glu-4	3.1-4.2*	-	3.1-4.2*	3.2-4.5*	69.9	-	70.1	70.2
Glu-5	3.1-4.2*	-	3.1-4.2*	3.2-4.5*	77.6	-	77.2	77.8
Glu-6	3.1-4.2*	-	3.1-4.2*	3.2-4.5*	60.9	-	61.2	61.4
Rha-1	4.70 *d* (2.0 Hz)	4.71 *d* (2.0 Hz)	-	-	98.4	100.6	-	-
Rha-2	3.1-4.2*	3.1-4.2*	-	-	69.8	68.1	-	-
Rha-3	3.1-4.2*	3.1-4.2*	-	-	70.1	70.2	-	-
Rha-4	3.1-4.2*	3.1-4.2*	-	-	71.6	70.5	-	-
Rha-5	3.1-4.2*	3.1-4.2*	-	-	70.2	75.1	-	-
Rha-6	0.86 *d* (5.7 Hz)	1.2 *d* (6.0 Hz)	-	-	17.9	17.5	-	-

* Overlapped peaks.

have been shown during previous *in vivo* and *in vitro* biological investigations. The results of this study on occurrences of these biologically active principles in *T. rubra* subsp. *caucasica* leaves suggest the species as a valuable medicinal plant for further pharmacological and toxicological studies.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Radoglou K, Dobrowolska D, Spyroglou G, Nicolescu V. A review on the ecology and silviculture of limes (*Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa* Moench.) in Europe. *Die Bodenkultur*. 2009; 9(60): 9-19.

[2] Kew: The Plant List database. Version 1.1; Available from: http://www.theplantlist.org/.

[3] Browičz K. *Tiliaceae* in KH. Rechinger (ed.) *Flora Iranica* no: 148. Graz: Akademische Druck-u Verlagsanstalt, 1981.
[4] Zare H, Amini T, Assadi M. A review of the genus *Tilia* L. (Tiliaceae) in Iran, new records and new species. *Iran J Bot.* 2012; 18(2): 175-190.
[5] Association BHM. *British herbal pharmacopoeia*. Bournemouth: British Herbal Medicine Association, 1996.
[6] Blumenthal M, Busse W, Goldberg A, Gruenwaid J, Hall T, Klein S, Riggins C, Rister R. *The complete German commission E monographs: therapeutic guide to herbal medicines*. Austin: American Botanical Council, 1998.
[7] Amin GR. *Popular medicinal plants of Iran*. Tehran: Iranian Research Institute of Medicinal Plants, 1991.
[8] Parsa A. Medicinal plants and drugs of plant origin in Iran. IV. *Plant Food Hum Nutr*. 1960; 7(1): 65-136.
[9] Pietta P, Mauri P, Bruno A, Zini L. High-performance liquid chromatography and micellar electrokinetic chromatography of flavonol glycosides from *Tilia*. *J Chromatogr A*. 1993; 638(2): 357-361.
[10] Negri G, Santi D, Tabach R. Flavonol glycosides found in hydroethanolic extracts from *Tilia cordata*, a species utilized as anxiolytics. *Rev Bras Plantas Med*. 2013; 15(2): 217-224.
[11] Aguirre-Hernández E, González-Trujano ME, Martínez AL, Moreno J, Kite G, Terrazas T, Soto-Hernández M. HPLC/MS analysis and anxiolytic-like effect of quercetin and kaempferol flavonoids from *Tilia americana* var. *mexicana*. *J Ethnopharmacol*. 2010; 127(1): 91-97.
[12] Martínez AL, González-Trujano ME, Aguirre-Hernández E, Moreno J, Soto-Hernández M, López-Muñoz FJ. Antinociceptive activity of *Tilia americana* var. *mexicana* inflorescences and quercetin in the formalin test and in an arthritic pain model in rats. *Neuropharmacology*. 2009; 56(2): 564-571.
[13] Toker G, Aslan M, Yeşilada E, Memişoğlu M, Ito S. Comparative evaluation of the flavonoid content in officinal *Tiliae* flos and Turkish lime species for quality assessment. *J Pharmaceut Biomed*. 2001; 26(1): 111-121.
[14] Mabry TJ, Markham KR, Thomas MB. *The systematic identification of flavonoids*. New York: Springer-Verlag, 1970.
[15] Kim AR, Ko HJ, Chowdhury MA, Chang YS, Woo ER. Chemical constituents on the aerial parts of *Artemisia selengensis* and their IL-6 inhibitory activity. *Arch Pharm Res*. in press.
[16] Su YQ, Shen YH, Tang J, Zhang WD. Chemical constituents of *Incarvillea mairei* var. *grandiflora*. *Chem Nat Compd*. 2010; 46(1): 109-111.
[17] Lee JH, Park KH, Lee MH, Kim HT, Seo WD, Kim JY, Baek IY, Jang DS, Ha TJ. Identification, characterization, and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean *Perilla* (*Perilla frutescens*) cultivars. *Food Chem*. 2013; 136(2): 843-852.
[18] Yassa N, Saeidnia S, Pirouzi R, Akbaripour M, Shafiee A. Three phenolic glycosides and immunological properties of *Achillea millefolium* from Iran, population of Golestan. *Daru J Pharm Sci*. 2007; 15(1): 49-52.
[19] Akyuz E, Şahin H, Islamoglu F, Kolayli S, Sandra P. Evaluation of phenolic compounds in *Tilia rubra* subsp. *caucasica* by HPLC-UV and HPLC-UV-MS/MS. *Int J Food Prop*. 2014; 17(2): 331-343.
[20] Loscalzo LM, Wasowski C, Marder M. Neuroactive flavonoid glycosides from Tilia *petiolaris* DC extracts. *Phytother Res*. 2009; 23(10): 1453-1457.
[21] Wagner C, Fachinetto R, Dalla Corte CL, Brito VB, Severo D, Dias G, Morel AF, Nogueira CW, Rocha JB. Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation *in vitro*. *Brain Res*. 2006; 1107(1): 192-198.

[22] Camuesco D, Comalada M, Rodríguez-Cabezas ME, Nieto A, Lorente MD, Concha A, Zarzuelo A, Gálvez J. The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. *Brit J Pharmacol*. 2004; 143(7): 908-918.

[23] Gadotti VM, Santos AR, Meyre-Silva C, Schmeling LO, Machado C, Liz FH. Antinociceptive action of the extract and the flavonoid quercitrin isolated from *Bauhinia microstachya* leaves. *J Pharm Pharmacol*. 2005; 57(10): 1345-1351.

[24] Babujanarthanam R, Kavitha P, Pandian MR. Quercitrin, a bioflavonoid improves glucose homeostasis in streptozotocin-induced diabetic tissues by altering glycolytic and gluconeogenic enzymes. *Fund Clin pharmacol*. 2010; 24(3): 357-364.

[25] Muzitano MF, Cruz EA, Almeida AP, Da Silva SA, Kaiser CR, Guette C, Rossi-Bergmann B, Costa SS. Quercitrin: an antileishmanial flavonoid glycoside from *Kalanchoe pinnata*. *Planta Med*. 2006; 72(1): 81-83.

[26] Galvez J, Crespo M, Jimenez J, Suarez A, Zarzuelo A. Antidiarrhoeic activity of quercitrin in mice and rats. *J Pharm Pharmacol*. 1993; 45(2): 157-159.

[27] Rao YK, Lee MJ, Chen K, Lee YC, Wu WS, Tzeng YM. Insulin-mimetic action of rhoifolin and cosmosiin isolated from *Citrus grandis* (L.) Osbeck leaves: enhanced adiponectin secretion and insulin receptor phosphorylation in 3T3-L1 cells. *Evid-Based Compl Alt*. 2011; 1-9.

[28] Shimizu M, Ito T, Terashima S, Hayashi T, Arisawa M, Morita N, Kurokawa S, Ito K, Hashimoto Y. Inhibition of lens aldose reductase by flavonoids. *Phytochemistry*. 1984; 23(9): 1885-1888.

[29] Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. *J Agr Food Chem*. 2001; 49(6): 2774-2779.

[30] Park CM, Song YS. Luteolin and luteolin-7-*O*-glucoside inhibit lipopolysaccharide-induced inflammatory responses through modulation of NF-κB/AP-1/PI3K-Akt signaling cascades in RAW 2647 cells. *Nutr Res Pract*. 2013; 7(6): 423-429.

[31] Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci Biotech Bioch*. 2000; 64(11): 2458-2461.

[32] Jin M, Yang JH, Lee E, Lu Y, Kwon S, Son KH, Son JK, Chang HW. Antiasthmatic activity of luteolin-7-*O*-glucoside from *Ailanthus altissima* through the down-regulation of T helper 2 cytokine expression and inhibition of prostaglandin E2 production in an ovalbumin-induced asthma model. *Biol Pharm Bull*. 2009; 32(9):1500-1503.

[33] Akroum S, Bendjeddou D, Satta D, Lalaoui K. Antibacterial activity and acute toxicity effect of flavonoids extracted from *Mentha longifolia*. *Am-Euras J Sci Res*. 2009; 4(2): 93-96.

[34] Baskar AA, Ignacimuthu S, Michael GP, Al Numair KS. Cancer chemopreventive potential of luteolin-7-*O*-glucoside isolated from *Ophiorrhiza mungos* Linn. *Nutr Cancer*. 2011; 63(1): 130-138.