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Original Article

Flavonoid Glycosides from *Tribulus terrestris* L. *orientalis*

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Abstract

Tribulus terrestris L. var. *orientalis* (Kerner) G. Beck is widely used in the traditional medicine of many countries. Several flavonoides are identified from the plant material. Aerial parts which collected from northeast of Iran are used in this study. Three flavonoid glycosides were isolated and characterized. The flovonoids were found to be based on quercetin and kaempferol. Flavonoid glycosides which separated were consisted of quercetin 3-O-glycoside, quercetin 3-O-rutinoside and kaempferol 3-O-glycoside. The latest one was a new for the plant.

Keywords: Flavonoid glycosides; Kaempferol; Quercetin; *Tribulus terrestris*.
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1. Introduction

The genus *Tribulus* L. (Zygophyllaceae) has 25 species in the world [1]. In the Flora Iranica four species have been reported for this genus which include *T. terrestris* L., *T. pentandrus* Forssk., *T. ochroleucus* Maire and *T. longipetalus* subsp. *macropeterus*. The former one is characterized by having four spines in mericarps of fruits in which two of them are shorter than two other ones. This species includes with three varieties: *T. terrestris* var. *orientalis* (Kerner) G. Beck, *T. terrestris* var. *robustus* Boiss., and *T. terrestris* var. *bicornis* (C. A. Mey) Hadidi [2].

T. terrestris L. var. *orientalis* is a widespread and summer- tolerant plant which distributed in arid and semi-arid parts of the world include Africa, Middle East, East Asia, East, Center and South of Europe and Australia, Mediterranean region and SW Asia. The plant is distributed in most parts of Iran [2].

T. terrestris L. is used in the traditional medicine of many countries for treatment of cardiac diseases, edema, eye trouble, skin disorders, urinary troubles and stones in the bladder and as a diuretic, aphrodisiac [3]. It has been shown to increase the free serum testosterone [4] and to be effective in the treatment of sexual and erectile dysfunction by conversion of its phytochemical derivative, protodioscine to dehydro-epi androsterone (DHEA) [5]. It has protective effect on genetic damage [6] and stimulates melanocyte proliferation in the treatment of vitiligo [7]. Moreover, a nematocidal activity has been reported [8]. Its action on motor activity, muscle tone and restorative tonic for vigor, and mainly used to improve performance in sports [9]. The plant is reported to contain steroidal

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Table 1. UV data of flavonoid glycosides from *T. terrestris* var. *orientalis*.

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$-HCl	NaOAc	NaOAc-H_3BO_3
Quercetin 3-O-glycoside	257	276	275	271	268	262
Quercetin 3-O-rutinoside	257	278	273	268	270	262
Kaempferol 3-O-glycoside	266	275	276	276	276	256

saponins, alkaloides, and flavonoides [10-16]. *T. terrestris* is also known for having antimicrobial, antiacetylcholine and haemolytic activity [17-19]. For treatment of kidney troubles, it is either used alone or in combination with *Zea mays* [20]. The quantities and presence of important metabolites depend on the various parts of the plant used [16].

There are no investigations on the flavonoides of it in Iran. In this article, we report the isolation and the structural characterization of two quercetin glycosides and one kaempferol glycoside.

2. Materials and methods

2.1. Plant material

Aerial parts of the plant were collected which grows as weed in farming lands as dominated plant after harvesting crops. A voucher specimen (Golestan Province, 9 km E Kalaleh, near to Ajansangarly village, *ca*. 200 m, 11.8.2006, Ajani 10050) is deposited in the Central Herbarium of Medicinal Plants (ACECR), Karaj, and Tehran Province, Iran.

2.2. General experimental procedures

Melting points were taken on a Reichert-Jung apparatus (Vienna, Austria). Ultraviolet spectra were recorded on a Shimadzu 160A spectrometer (Kyoto, Japan). Electron Ionization Mass Spectra (EIMS) were determined on a Finnigan MAT SQ 70 (California, USA) at 70eV. H-NMR and C-NMR spectra were measured in $CDC1_3$ with tetramethylsilane (TMS) as an internal standard using a varian 400 Unity plus spectrometer. FTIR spectra were recorded on a Nicolet 550 spectrometer (Madison, WI, USA). Column chromatography (CC) was conducted with silica gel (kieselgel 60, 60-100 mesh ASTM; Merck, Darmstalt, Germany) and thin-layer chromatography (TLC) with Merck silica gel 60 F254 on glass plates.

2.3. Extraction procedures

The shade dried and powdered aerial parts (50 g) of the plant were extracted with 80% MeOH (2 l) by perculation for 72 h. The solvent was evaporated by vacuum distillation at 45 ºC to produce a gummy residue and 50% of methanolic extract was re-dissolved in MeOH and the 50% MeOH soluble part was partitioned by ethyl acetate. The 50% MeOH phase was subjected to column chromatography over silica gel (60-120 mesh) and eluted with $CHCl_3$: MeOH mixtures with increasing concentrations methanol to give 4 fractions. Fraction 2 was re-chromatographed on a sephadex LH- 20 column using MeOH as eluent, to yield 1 and 2 (150, 100 mg). Fraction 4 was chromatographed on a silica gel CC and eluted with EtOAC: MeOH mixtures with increasing concentrations of methanol to give 3 fractions. Fraction 3 was rechromatographed on a sephadex LH- 20 column using MeOH as eluent to yield 3 (12 mg).

2.4. Identification

The isolated flavonoides were identified by UV, ^1H and ^{13}C NMR and mild acid hydrolysis. UV, ^1H and ^{13}C NMR data of the isolated flavanones are shown in Tables 1 and 2.

Acid hydrolysis: Compounds 1, 2 and 3 each in a mixture of HCl 8% and MeOH (2 ml) were separately refluxed for 2 h. The reaction mixtures were reduced in vacuum to dryness, dissolved in H_2O (2 ml) and neutralized with Na_2CO_3. The neutralized products were subjected to TLC analysis (eluent: EtOAc- MeOH- H_2O- HOAC, 6:2:1:1 and pc reluent: N-BuOH-HOAC-H_2O (4:5:7) and C_6H_6-N- BuOH-H_2O-Pyridin (1:5:3:3).

Table 2. NMR data of flavonoid glycosides from *T. terrestris* var. *orientalis*.

	Compound 1		Compound 2		Compound 3	
	^{13}C	^{1}H delta, m, *J*(Hz)	^{13}C	^{1}H delta, m,*J*(Hz)	^{13}C	^{1}H delta, m,*J*(Hz)
2	159.8		156.4		159.3	
3	136.5		133.3		135.8	
4	179.8		177.4		179.8	
5	163.0		161.2		163.4	
6	99.9	6.20,d(2.1)	98.7	6.18,d(2.1)	100.8	6.17,d(1.9)
7	167.4		164.2		167.9	
8	95.6	6.39,d(2.1)	93.6	6.38,d(2.1)	95.5	6.36,d(1.9)
9	158.1		156.6		159.0	
10	107.6		103.9		105.7	
1'	122.4		121.2		123.2	
2'	132.0	7.69,d(2.1)	115.2	7.63,d(2.1)	132.7	8.05,dd(2.1, 9.15)
3'	116.6		144.8		116.5	6.88,dd(1.8, 9.15)
4'	161.8		148.4		162.o	
5'	116.5	6.87,d(8.3)	116.3	6.87,d(8.3)	116.5	6.88,dd(1.8, 9.15)
6'	132.0	7.58,dd(2.1 , 8.3)	121.6	7.62,d(8.3)	132.7	8.05,dd(2.1, 9.15)
1"	103.9	5.24,d(7.6)	101.2	5.28,d(7.2)	104.7	5.22,d(7.6)
2"	75.8	3.48,t(8.4)	74.1	3.63,t(8.0)	76.1	3.44,t(9.2)
3"	78.4	3.43,t(8.9)	76.4	3.49,t(8.8)	78.5	3.41,t(9.2)
4"	71.9	3.35,t(9.2)	70.6	3.38,t(9.6)	71.8	3.31,t(9.8)
5"	79.2	3.22m	75.9	3.45m	78.8	3.19m
6"	62.9	3.70,d(11.8)	67.0	3.58m	63.0	3.53,dd(5.5, 11.8)
		3.57,dd(5.5 ,11.9)		4.04,dd(1.1, 11.6)		
1"'			100.8	4.63,d(2.1)		
2"'			70.4	4.94,dd(2.1, 4.3)		
3"'			70.0	3.78,dd(4.3, 9.8)		
4"'			71.8	3.29,t(9.8)		
5"'			68.3	3.57m		
6"'			17.8	1.12,d(6.2)		

The chromatograms were sprayed with aniline hydrogen phthalate followed by heating. The sugars were identified after comparison with authentic samples.

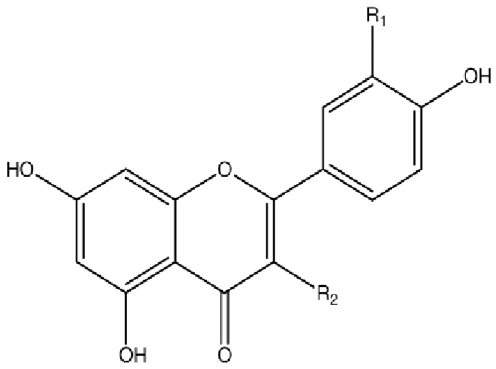
3. Results and discussion

From the aerial parts of *T. terrestris* var. *orientalis*, three flavonoid glycosides were isolated and characterized. The glycosides were found to be based on quercetin and kaempfrol. Compounds 1, 2 showed UV absorption mixture of 257 and 352, 252 which were characteristic for quercetin derivatives.

The compound 1 was identified by mass spectrometry, which gave molecular ion peak at m/z 464, corresponding to the molecular formula $C_{21}H_{20}O_{12}$. Singlets at 6.39 ppm and 6.20 ppm in the ^{1}HNMR spectrum of compound 1 revealed the peaks of 5,7-dihydroxy flavonol. A doublet-doublet signal at 7.58 ppm (*J*=2.1, 8.3 Hz) and two doublet at 7.69 ppm (*J*=2.1) and 6.87 ppm (*J*= 8.3 Hz) showed 3',4'-dihydroxy functional structure corresponding to aromatic β-ring of quercetin. The glycosidic signal shown by ^{1}H and ^{13}C NMR spectra (Table 2) was concluded as β-D-glycoside. Therefore, compound 1 was determined to be quercetin-3-O-β-D-glucopyranoside (Scheme 1).

Compound 2 showed in their ^{13}C spectrum besides quercetin signals, 12 additional peaks attributable for rutinosides. Mass spectrum of 2 gave molecular ion peak at 664 m/z, corresponding to the quercetin-3-O-rutinoside.

Compound 3 was characterized by mass spectrometry, which gave molecular ion peak at m/z 448, corresponding to the molecular formula $C_{21}H_{20}O_{11}$. Two doublet-doublets at 6.17 ppm and 6.36 ppm (*J*= 1.9 Hz) in the ^{1}H NMR spectrum of 3 (Table 1) showed 2H Ax system in the aromatic A-ring of 5.7-dihydroxy flavonol. Two doublet-doublets at 8.05 ppm (*J*= 2.1, 9.15) and 6.88 ppm (*J*= 1.8,



Scheme 1. Structure of flavonoid glycosides isolated from T. terrestris.

1- Quercetin-3-O-Glycoside	R_1: OH	R_2: Glucose
2- Rutin	R_1: OH	R_2: Rutinose
3- Kaempferol-3-O-Glycoside	R_1: H	R_2: Glucose

9.15 Hz) and the ^{1}H NMR spectrum of 2 also revealed a AB system in the aromatic ring β because the integration showed four protons in this AB system. Those data indicated that 3 had kaempferol as on aglycone. ^{1}H and ^{13}C NMR spectra of 3 shown glycosidic signal that was concluded as β-D-glucoside.

In conclusion, three flavonol glycosides have been isolated from *T. terrestris*. One of them (**3**) was reported for the first time from *T. terrestris.*

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