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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION OF PHYTOCHEMICALS IN METHANOLIC HERBA EXTRACT OF *SILENE RUSCIFOLIA* BY LC-QTOF/MS AND GC/MS

SILENE RUSCIFOLIA METANOLİK HERBA EKSTRESİNDEKİ FİTOKİMYASALLARIN LC-QTOF/MS VE GC/MS İLE İNCELENMESİ

Kenan Can TOK[1], Muhammed Mesud HÜRKUL[2*], Nazmiye Neslihan BOZKURT[1], Ayhan İbrahim AYSAL[3], Şeyda YAYLA[2]

[1]Ankara University, Institute of Forensic Sciences, Department of Forensic Toxicology, Ankara, Türkiye

[2]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Türkiye

[3]Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Türkiye

ABSTRACT

Objective: *Silene L. (Caryophyllaceae) species are traditionally used for the treatment of inflammation, urinary inflammation, eye trouble, skin problem, stomach ache, dysentery, tooth decay, fever, headache, malaria, pimples and backache. Chemical ingredients of Silene species consist of flavonoids, anthocyanidins, terpenoids, triterpene saponins, phytoecdysteroids, benzenoids, vitamins, and show antioxidant, anti-inflammatory, antitumor and antiviral activities. Silene ruscifolia (Hub.-Mor. & Reese) Hub.-Mor. is called "gizli nakıl" in Türkiye.*

Material and Method: *The plant material was collected from Beynam Forest (Ankara/Türkiye). The aerial parts of the plant were extracted with methanol in an ultrasonic bath. HPLC system (Agilent 1260 Series) with an autosampler, binary pump, column oven, and a UV detector was coupled with an iFunnel Quadrupole Time-of-Flight LC-MS system (Agilent G6550A) with a Dual Spray Agilent Jet Stream electrospray ionization source. Agilent TC C-18 (4.6 mm x 150 mm x 5 µm) column was used for the separation of the compounds. The GC-MS analysis of extract was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973N quadrupole mass spectrometer (Agilent, USA). Mass Hunter software (Qualitative Analysis B.07.00) and the NIST Mass Spectral Library (2014) were used to determine and identify the compounds.*

Result and Discussion: *LC-MS Q-TOF analysis showed that S. ruscifolia contained rutin, narcissin, luteolin, isorhamnetin, rhamnetin and quercetin dimethyl ether. GC-MS analysis showed that the extract had*

* **Corresponding Author / Sorumlu Yazar:** Muhammed Mesud Hürkul
e-mail / e-posta: huerkulmm@gmail.com, **Phone / Tel.:** +903122033035

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the highest content of sugar (50.5%) and sugar alcohols (46.39%). It also contains carboxylic acid (0.47%), fatty acid (0.64%), sugar acid (0.42%), glycoside (0.48%), carotenoids (0.61%) and benzoic acid ester (0.49%). D-pinitol has the highest content in the extract with 41.14%.

Keywords: *GC/MS, Herba, LC-QTOF/MS, Silene ruscifolia*

ÖZ

Amaç: *Silene L. (Caryophyllaceae) türleri geleneksel olarak iltihap, idrar yolu iltihabı, göz rahatsızlığı, cilt sorunu, mide ağrısı, dizanteri, diş çürümesi, ateş, baş ağrısı, sıtma, sivilce ve sırt ağrısı tedavisinde kullanılmaktadır. Silene türlerinin kimyasal bileşenleri flavonoidler, antosiyanidinler, terpenoidler, triterpen saponinler, fitoekdisteroidler, benzenoidler, vitaminlerden oluşur ve antioksidan, antiinflamatuar, antitümör, antiviral aktivite gösterirler. Silene ruscifolia (Hub.-Mor. & Reese) Hub.-Mor. Türkiye'de "gizli nakıl" olarak adlandırılır.*

Gereç ve Yöntem: *Bitki materyali Beynam Ormanı'ndan (Ankara/Türkiye) toplandı. Bitkinin toprak üstü kısımları ultrasonik banyoda metanol ile ekstre edildi. Otomatik örnekleyici, ikili pompa, kolon fırını ve bir UV dedektörüne sahip HPLC sistemi (Agilent 1260 Serisi), Çift Sprey Agilent Jet Stream elektrosprey iyonizasyon kaynağına sahip bir iFunnel Quadrupole Time-of-Flight LC-MS system (Agilent G6550A) ile birleştirildi. Bileşiklerin ayrılması için Agilent TC C-18 (4.6 mm x 150 mm x 5 µm) kolonu kullanıldı. Ekstrenin GC-MS analizi, bir Agilent 5973N dört kutuplu kütle spektrometresi (Agilent, ABD) ile donatılmış bir Agilent 6890 gaz kromatografı kullanılarak yapıldı. Bileşiklerin belirlenmesi ve tanımlanması için Mass Hunter yazılımı (Qualitative Analysis B.07.00) ve NIST Mass Spectral Library (2014) kullanıldı.*

Sonuç ve Tartışma: *LC-MS Q-TOF analizi, S. ruscifolia'nın rutin, narsissin, luteolin, izoramnetin, ramnetin ve kersetin dimetil eter içerdiğini gösterdi. GC-MS analizi, ekstrenin şeker (%50.5) ve şeker alkolleri (%46.39) olarak en yüksek içeriğe sahip olduğunu göstermiştir. Ayrıca karboksilik asit (%0.47), yağ asidi (%0.64), şeker asidi (%0.42), glikozit (%0.48), karotenoidler (%0.61) ve benzoik asit esteri (%0.49) içerir. D-pinitol, %41.14 ile ekstredeki en yüksek içeriğe sahiptir.*

Anahtar Kelimeler: *GC/MS, Herba, LC-QTOF/MS, Silene ruscifolia*

INTRODUCTION

The Caryophyllaceae consists of herbaceous or mostly semi-shrub plants, usually with simple, entire and exstipulate leaves. Actinomorphic and solitary or in cymes flowers have anthophore or perigynous. Fruits are baccate or opening capsule [1-3]. The family contains 101 accepted genera worldwide [4].

Silene L. is a large and polymorphic genus. Subarctic-temperate zone to tropical mountains are the distribution areas of the genus in the world and includes 887 species [4]. Annual, biennial and perennial herbs (mostly semi-shrub) consist of this genus. The calyx is tube-shaped and vascularization is seen on it very clear. Fruit an opening capsule. Petals, stamens and ovary rise on anthophore [2,4]. *Silene ruscifolia* (Hub.-Mor. & Reese) Hub.-Mor. is perennial-herbaceous and has a strong woody stem at the base. Basal leaves are oblanceolate and cauline leaves are defined as ovate-cordate. Flowers in dichasia, and white petals rise on glabrous anthophore. Fruit is a capsule [2]. *S. ruscifolia* is called "*gizli nakıl*" in Türkiye [5]. Previous taxonomical studies have reported that *S. ruscifolia* is very similar to the Iranian *Silene commelinifolia* Boiss. [2]. According to current studies, *S. ruscifolia* is accepted as a synonym of *S. commelinifolia* [4].

Silene species are traditionally used against inflammation, urinary inflammation, eye trouble, skin problem, stomach ache, dysentery, tooth decay, fever, headache, malaria, pimples and backache [6-14]. Chemical ingredients of *Silene* species consist flavonoids, anthocyanidins, terpenoids, triterpene saponins, phytoecdysteroids, benzenoids, vitamins [15-19] and show antioxidant, anti-inflammatory, antitumor, antiviral activities [20-24].

MATERIAL AND METHOD

Plant materials

The plant material was collected from Beynam Forest (Ankara/Türkiye). A voucher specimen was deposited in the Herbarium of Ankara University Faculty of Pharmacy (AEF30851). Plant samples were dried in the shade and powdered with a grinder.

Extraction process

The aerial parts of the plant were extracted with methanol in ultrasonic bath for 30 min. The extract was filtered from 0.45 μm filters before injection to the LC-QTOF/MS and GC/MS systems [25].

LC-QTOF/MS analysis

HPLC system (Agilent 1260 Series) with an autosampler, binary pump, column oven, and a UV detector coupled with an iFunnel Quadrupole Time-of-Flight LC-MS system (Agilent G6550A) with a Dual Spray Agilent Jet Stream electrospray ionization source (Agilent Technologies, Inc., CA, USA) was used for analysis of the extracts of the sample. The instrument was operated with negative and positive electrospray ionization in a 2 GHz extended dynamic range mode. During the run of the samples, all ions mode with collision energies 0 eV, 10 eV, and 40 eV were used with the acquisition rate was 1 spectra/second. Agilent TC C-18 (4.6 mm x 150 mm x 5 μm) column was used for the separation of the compounds. Agilent MassHunter software B 06.00 was used for the analysis of the data. Metlin Metabolite database, Massbank.eu, and Pubchem databases were used for the identification of compounds [26-27]. The detailed instrumental conditions are tabulated in Table 1.

GC/MS analysis

The two-step derivatization procedure was performed before the GC/MS analysis. During the procedure, methoxymation followed by silylation was applied before the analysis [28-29]. Methoxymation was used to prevent split or duplicated signals of sugars onto the chromatogram.

Methoxyamine HCl (Merck, Darmstadt, Germany) solution was prepared daily as 25 mg/ml pyridine. After the sample (100 μl) was evaporated the nitrogen flowed gently, and 50 μl methoxyamine HCl solution was added to the dried sample. After waiting for 1.5 hours at about 30°C, 50 μl of

Bis(trimethylsilyl)trifluoroacetamide + 1% Trimethylchlorosilane (BSTFA + 1% TMCS) (Sigma-Aldrich, Germany) was added, and kept for another 45 min. at 70°C for a complete silylation. GC/MS system was used for the rest of the analysis (Agilent 6890/5973N model), (Santa Clara, USA). Crossbonded diphenyl dimethyl polysiloxane phase-coated Restek Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) (Bellefonte, USA) was preferred for the analysis with the carrier gas (99.999%) helium at a 1.5 ml/min flow rate. 1 µl of the derivatized sample solution was injected in the splitless mode. The transfer line, as well as the injection port, were set at 280°C. Quadrupole was arranged at 150°C while the ion source was maintained at 230°C. The temperature program initially started at 50ºC and was held for 2 min, then increased to 280°C at the rate of 3°C/min. In this stage, it was held for 12 min. to complete the analysis (90 min.). Mass Hunter software (Qualitative Analysis B 07.00) and the NIST Mass Spectral Library (2014) were used for the determination and identification of the compounds.

Table 1. Instrumental parameters for LC-QTOF/MS system

Column	TC C-18 (4.6 mm x 150 mm x 5 µm)
Column Temperature (°C)	30
Injection volume (µl)	10
Run Time (min.)	82
Mobile Phase A	0.1% acetic acid in water
Mobile Phase B	0.1% acetic acid in acetonitrile
Flow rate (ml/min)	0.65
Gradient (time-B%)	0 min-5 % B; 4 min-5 % B; 12 min-10 % B; 15 min-10 % B; 28 min-20 % B; 48 min-40 % B; 60 min.-60 % B; 65 min-70 % B; 66 min-90 % B; 72 min-90 % B; 72.1 min-5 % B
UV (nm)	280
Ionization mode	Negative and positive electrospray ionization mode with jet stream technology
Drying gas temp (°C)	200
Drying gas flow (l/min)	14 l/min
Nebulizer pressure (psi)	40
Sheath gas temperature (°C)	350
Sheath gas flow (l/min)	11
Capillary Voltage (V)	1500
Nozzle Voltage (V)	1000
Mass range (amu)	30-1700
Reference ions	980.0147, 1033.9881 for negative run, 922.0098 for positive run

RESULT AND DISCUSSION

LC-MS Q-TOF systems were used for the identification of compounds using mass spectra of both precursor and product ions within a mass error of 5 ppm. Using different collision energies (0-10-40 eV) precursor ions were fragmented and product ions gave information about the compounds' functional groups and allowed to structure elucidation. Sample extract was analyzed using in gradient elution LC

program to get enough resolution to identify each compound. The UV chromatogram at 280 nm showed main composition of the extract and the most abundant 7 compounds were identified using reference standards and database search via Metlin and Pubchem. MassHunter generated molecular formula and product ions were used for identification. In case of compound 3 in Table 2, m/z 665 produced 315 ion (Isorhamnetin), so the compound can be classified as a glycoside of Isorhamnetin. The same product ion pattern was achieved for Narcissin as well. Isorhamnetin/Rhamnetin seperation on column (retention time 51.5 min. and 52.8 min. respectively) and product ion spectrum of compounds were used identification of compounds. Rhamnetin spectra showed m/z 165 product ion as a base peak while Isorhamnetin had m/z 151. All identified compounds were confirmed both by generated precursor ions and their fragments with mass error less than 5 ppm. The main components of the sample were eluted between 40-60 min. interval. Total ion chromatogram (A) and UV chromatogram (B) at 280 nm are shown below.

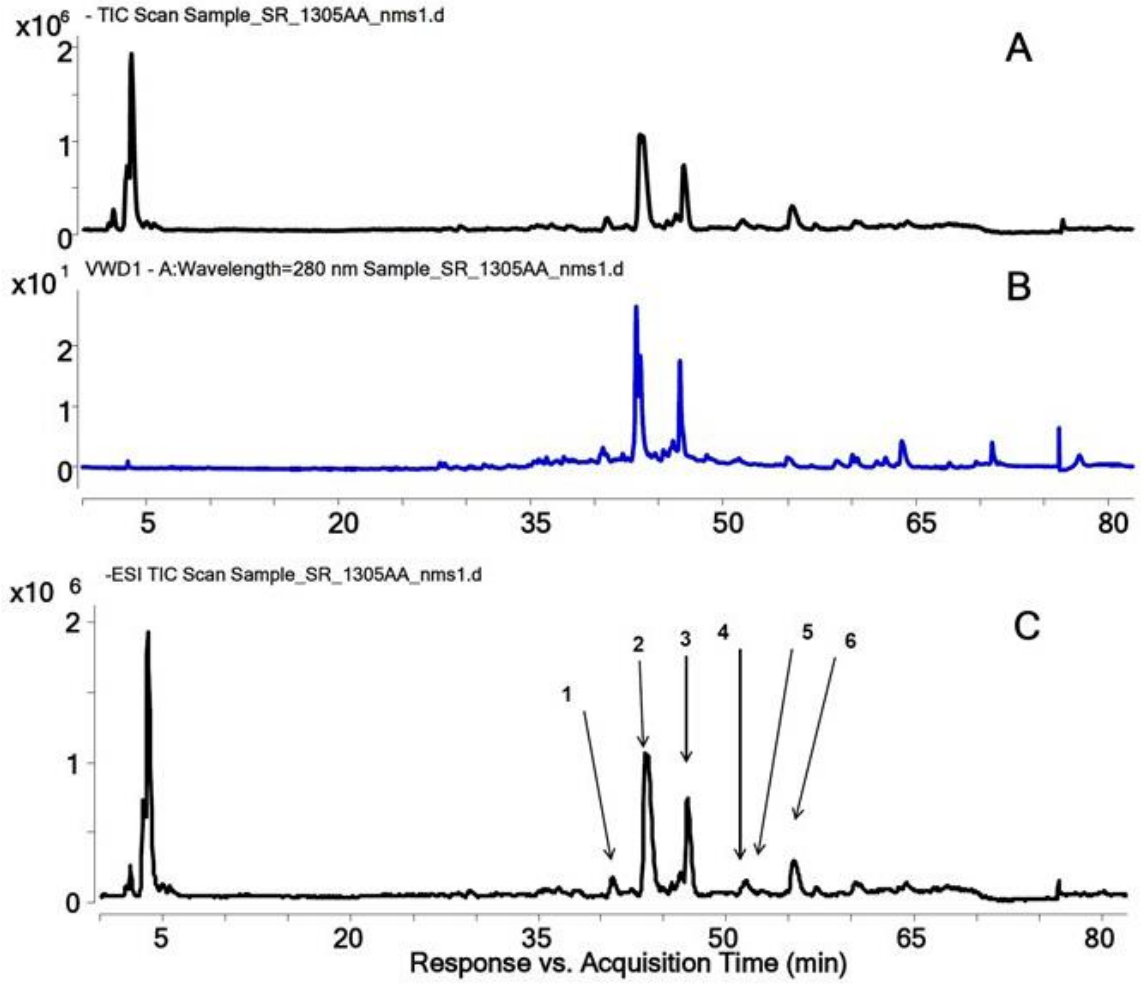

Figure 1. Total ion chromatogram of sample, negative mode MS (A) and 280 nm UV chromatogram (B) and the identified compounds (C)

LC-MS Q-TOF analysis showed that *S. ruscifolia* contained rutin, narcissin, luteolin, isorhamnetin, rhamnetin, and quercetin dimethyl ether. Phenolic compounds are produced as secondary metabolites in plants and are common [30-32]. They show sedative, hepatoprotective, antibacterial, antifungal, anticonvulsant, wound healing and antiviral activities [33-44]. Moreover, they also have antidepressant, diuretic, antiulcer, antidiabetic, antioxidant, anti-hypercholesterolemic, anticancer, and cardioprotective effects [45-60].

Table 2. Details of identified compounds in the extract

No.	Name	Formula	Calculated ion	Found ion	Error,ppm	rt, min
1	Rutin	$C_{27}H_{30}O_{16}$	609.1461	609.1441	3.28	41.2
2	Narcissin	$C_{28}H_{32}O_{16}$	623.1618	623.1615	0.48	43.4
3	Isorhamnetin glycoside compound	$C_{30}H_{34}O_{17}$	665.1723	665.1706	2.56	46.9
4	Luteolin	$C_{15}H_{10}O_6$	285.0405	285.0396	3.16	51.2
5	Isorhamnetin	$C_{16}H_{12}O^7$	315.0510	315.0512	-0.63	51.5
6	Rhamnetin	$C_{16}H_{12}O_7$	315.0510	315.0513	-0.95	52.8
7	Quercetin dimethylether	$C_{17}H_{14}O_7$	329.0667	329.0673	-1.82	55.4

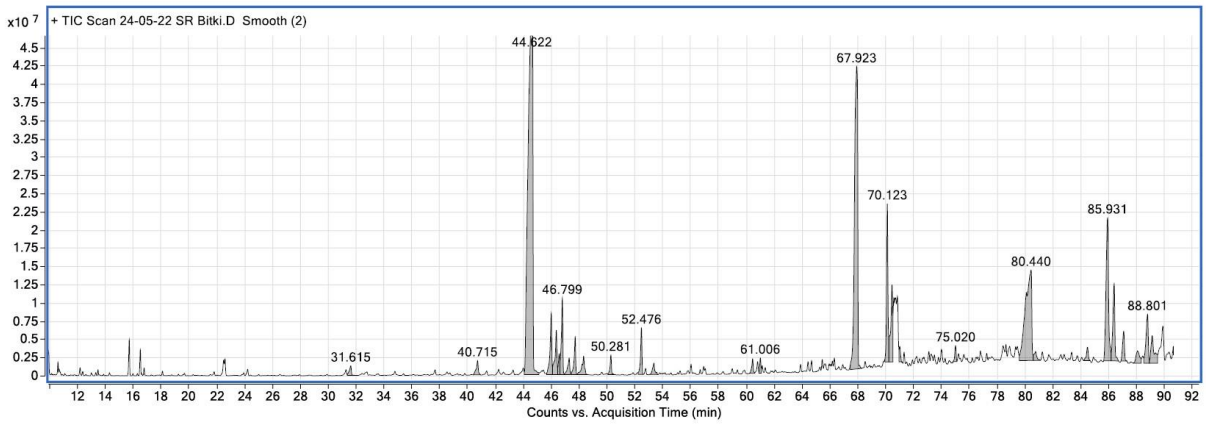

Figure 2. GC-MS Chromatogram for the extract

As a result of GC/MS analysis, 38 compounds were detected and 23 of them were identified (Table 3). These 23 compounds constitute 70.19% of the total peak area. GC-MS analysis showed that the extract had the highest content of sugar (50.5%) and sugar alcohols (46.39%). It also contains carboxylic acid (0.47%), fatty acid (0.64%), sugar acid (0.42%), glycoside (0.48%), carotenoids (0.61%) and benzoic acid ester (0.49%). D-pinitol has the highest content in the extract with 41.14%. D-pinitol, first isolated from *Pinus monticola*, is the most common inositol ether in plants, and also has anti-inflammatory, antidiabetic, antitumoral, antioxidant and cancer chemopreventive effects [61].

Table 3. Identified compounds in the extract by GC/MS.

#	RT (min)	Identified Compounds	Area Sum % of Identified Compounds	Area Sum % of Detected Compounds	Classification
1	31.615	Malic acid	0.47	0.33	Carboxylic acid
2	40.715	Xylitol	0.69	0.49	Sugar alcohol
3	44.622	D-Pinitol	41.14	29.3	Sugar alcohol
4	46.002	D-Fructose	2.52	1.8	Sugar
5	46.369	D-(-)-Fructose	2.01	1.43	Sugar
6	46.572	D-Allose	0.69	0.49	Sugar
7	46.799	D-(+)-Talose	2.71	0.97	Sugar
8	47.289	D-Allose	0.81	0.58	Sugar
9	47.719	D-Sorbitol	1.37	0.97	Sugar alcohol
10	48.331	D-Pinitol	1.13	0.81	Sugar alcohol
11	50.281	Palmitic Acid	0.64	0.46	Fatty acid
12	52.476	Myo-Inositol	1.55	1.1	Sugar alcohol
13	53.367	D-Allose (isomer 1)	0.6	0.42	Sugar
14	60.453	Dulcitol	0.51	0.37	Sugar alcohol
15	61.006	D-(+)-Galacturonic acid	0.42	0.3	Sugar acid
16	67.923	Sucrose	22.79	16.23	Sugar
17	70.123	D-(+)-Trehalose	5.04	3.53	Sugar
18	71.014	Aucubin	0.48	0.34	Glycoside
19	75.02	Methyl 2,4-dihydroxybenzoate	0.49	0.35	Benzoic Acid Ester
20	80.761	Lycopene	0.61	0.42	Carotenoids
21	85.931	Lactose	8.52	6.07	Sugar
22	88.801	D-(-)-Fructofuranose	2.62	1.87	Sugar
23	89.133	Maltose (isomer 1)	2.19	1.56	Sugar

AUTHOR CONTRIBUTIONS

Conception: *K.C.T., M.M.H.*; Design: *K.C.T., M.M.H.*; Supervision: *M.M.H.*; Resources: *K.C.T., M.M.H., N.N.B., A.İ.A., Ş.Y.*; Materials: *M.M.H., Ş.Y.*; Data collection and/or processing: *K.C.T., M.M.H., N.N.B., A.İ.A., Ş.Y.*; Analysis and/or interpretation: *K.C.T., M.M.H., N.N.B., A.İ.A., Ş.Y.*; Literature search: *K.C.T., M.M.H., N.N.B., A.İ.A., Ş.Y.*; Writing manuscript: *M.M.H.*; Critical review: *K.C.T., M.M.H., N.N.B., A.İ.A., Ş.Y.*; Other: -

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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