ORIGINAL ARTICLE / ÖZGÜN MAKALE



GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF CONSOLIDA THIRKEANA EXTRACT

CONSOLIDA THIRKEANA EKSTRESİNİN GAZ KROMATOGRAFİSİ-KÜTLE SPEKTROMETRESİ (GC-MS) ANALİZİ

Kenan Can TOK^{1*} (D, Şeyda YAYLA² (D)

¹Ankara University, Institute of Forensic Sciences, Department of Forensic Toxicology, Ankara,

Turkey

²Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey

ABSTRACT

Objective: Consolida thirkeana (Boiss.) Bornm. (Ranunculaceae) is considered endemic to Turkey and identified by pale lilac flowers, sessile follicles, and laciniae linear leaves. The genus Consolida can often be confused morphologically with Delphinium, in this respect, members of the genus Consolida need chemotaxonomic interest. In this study, the phytochemical content of C. thirkeana was clarified by GC/MS analysis.

Material and Method: The plant material was collected from Ayaş (Ankara/Turkey). A voucher specimen was deposited in the Ankara University Faculty of Pharmacy Herbarium. The aerial part of the plant was powdered in a grinder. The powdered plant parts were macerated with methanol. The GC-MS analysis of extracts 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 for determining and identifying compounds.

Result and Discussion: The analysis results of this study showed the presence of 15 compounds in C. thirkeana methanol extract. The extract contains 0.65% Glycoside, 0.68% Fatty acid, 0.91% Acylglycerol, 2.41% Carboxylic acid, 38.5% Sugar, 56.86% Sugar alcohol.

Keywords: Consolida thirkeana, endemic, GC/MS, herba, phytochemistry

ÖZ

Amaç: Consolida thirkeana (Boiss.) Bornm. (Ranunculaceae) Türkiye için endemik olarak kabul edilir ve soluk leylak çiçekleri, sapsız foliküller ve linear lasiniat yaprakları ile tanımlanır. Consolida cinsi genellikle morfolojik olarak Delphinium ile karıştırılabilir, bu bakımdan Consolida cinsinin üyelerinin kemotaksonomik

Accepted / Kabul: 21.04.2022

Corresponding Author / Sorumlu Yazar: Kenan Can Tok e-mail / e-posta: kctok@ankara.edu.tr, Phone / Tel.: +90 537 063 0808

olarak araştırılmasına ihtiyaç duyulur. Bu çalışmada, C. thirkeana'nın fitokimyasal içeriği GC/MS analizi ile aydınlatılmıştır.

Gereç ve Yöntem: Bitki materyali Ayaş'tan (Ankara/Türkiye) toplanmıştır. Bir herbaryum örneği Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu'na kaydedilmiştir. Bitkinin herbası bir öğütücüde toz haline getirildi. Toz haline getirilmiş bitki parçaları metanol kullanılarak maserasyon yöntemi ile ekstre edildi. Ekstrelerin GC-MS analizi, bir Agilent 5973N dört kutuplu kütle spektrometresi (Agilent, ABD) ile donatılmış bir Agilent 6890 gaz kromatografi 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: Bu çalışmanın analiz sonuçları, C. thirkeana metanol ekstresinde 15 bileşiğin varlığını göstermiştir. Ekstre %0.65 Glikozit, %0.68 Yağ asidi, %0.91 Açilgliserol, %2.41 Karboksilik asit, %38.5 Şeker, %56.86 Şeker alkolü içerir.

Anahtar Kelimeler: Consolida thirkeana, endemik, fitokimya, GC/MS, herba

INTRODUCTION

The Ranunculaceae family includes usually perennials herbs, the flowers of the family are bisexual and produce achenes, follicles, or baccate fruits [1-5]. The family members, which spread to all continents except Antarctica, are concentrated in the temperate and cold regions of the northern hemisphere [2,6]. Although many members of the family are poisonous, there are also various members that are used medicinally [2,7,8]. The most common chemicals in the Ranunculaceae family are alkaloids, other than that the family includes fatty acids, phytosterols, flavonoids, phenolic acids, and essential oils, in addition, different family members show antitumor, antimicrobial, insecticidal, antiparasitic, antioxidant and antiviral activities [7,9,10].

The genus *Consolida* Gray is called "mahmuzotu" in Turkey [11] and Anatolia is accepted as the center of diversity of the genus [3]. *Consolida thirkeana* (Boiss.) Bornm. is considered endemic to Turkey and is known as "Boz mahmuz" [11]. The plant is identified by pale lilac flowers, sessile follicles, and laciniae linear leaves [3,4]. The genus *Consolida* can often be confused morphologically with *Delphinium* [5,10], in this respect, members of the genus *Consolida* need chemotaxonomic interest. In this study, the phytochemical content of *C. thirkeana* was clarified by GC/MS analysis.

MATERIAL AND METHOD

Plant materials

The plant material was collected from Ayaş (Ankara/Turkey). A voucher specimen was deposited in the Ankara University Faculty of Pharmacy Herbarium in Turkey.

Extraction process

The collected plant materials were dried in the shade. The aerial part of the plant was powdered in a grinder. The powdered plant parts were macerated with methanol for 8 hours x 3 days. After the

extraction process, the fractions were combined and the extract was dried under reduced pressure at 40°C [12].

GC/MS analysis

For the analysis of the extracts, two-step derivatization was performed before the analysis. For derivatization, methoxymation combined with silulation was applied before analysis. We used methoxymation to prevent multiple chromatographic peaks of sugars on the chromatogram [12,13]. Before the methoxymation, we prepared the methoxyamine hydrochloride (MOX) (Sigma-Aldrich, Germany) solution daily in pyridine (25 mg/ml). After the 100 μ l sample was evaporated under a gentle nitrogen flow, 50 μ l MOX solution was added to the dried sample and waited for 90 minutes at 30°C degrees. Afterward, 50 μ l of BSTFA + 1% TMCS (Sigma-Aldrich, Germany) was added, and it was kept for another 45 minutes at 70°C for silvlation.

Agilent 6890/5973N model GC/MS (Santa Clara, USA) was used for the analysis. Restek RTX-5MS ($30 \text{ m} \times 0.25 \text{ mm}$ i.d. $\times 0.25 \mu\text{m}$) gas chromatography capillary column was used as an analytical column (Bellefonte, USA). Gas chromatography grade (99.999%) helium at a 1.5 ml/min flow rate was used as the carrier gas. One μ l of the derivatized sample solution was injected in the splitless mode. The injection port and transfer line (AUX) temperatures were set at 280°C. Ion source and quadrupole temperatures were maintained at 230°C, and 150°C, respectively. The GC oven temperature started at 50°C and was held for 2 min, then increased to 280°C at 3°C/min and held for 12 min. The total analysis time was 90 min. The mass spectrum was obtained by electron ionization at 70 eV. 50-500 atomic mass units were scanned by the detector. Mass Hunter software (Qualitative Analysis B.07.00) and the NIST Mass Spectral Library (2014) are used for determining and identifying compounds.

RESULT AND DISCUSSION

Figure 1 shows the major compounds identified in *C. thirkeana* extract by GC-MS. The analysis shows the presence of 15 compounds (Table 1) in methanol extract. Methanol extract of *C. thirkeana* contains 0.65% Glycoside, 0.68% Fatty acid, 0.91% Acylglycerol, 2.41% Carboxylic acid, 38.5% Sugar, 56.86% Sugar alcohol.

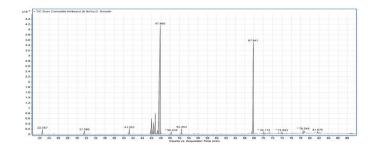


Figure 1. Compounds identified by GC-MS in C. thirkeana extract.

#	RT (min)	Identified Compounds	Area Sum %	Classification
1	22.567	Glycerol	1.09	Sugar alcohol
2	31.58	Malic acid	0.94	Carboxylic acid
3	41.251	Aconitic acid	1.47	Carboxylic acid
4	46.005	D-fructose	4.38	Sugar
5	46.368	D-(-)-fructose	3.22	Sugar
6	46.811	D-Glucose	5.81	Sugar
7	47.312	D-Allose	1.1	Sugar
8	47.865	D-Glucitol (Sorbitol)	54.57	Sugar alcohol
9	50.234	Palmitic acid	0.68	Fatty acid
10	52.453	Myo-Inositol	1.2	Sugar alcohol
11	67.841	Sucrose	22.79	Sugar
12	70.112	Maltose	0.58	Sugar
13	73.943	D-Lactose	0.62	Sugar
14	78.548	β-D-Galactopyranaside	0.65	Glycoside
15	81.675	Glycerol monostearate	0.91	Acylglycerol

Table 2. Compounds identified by GC-MS in *C. thirkeana* extract.

The analysis results of this study showed that sugar alcohols were the highest content in the extract at 56.86%. The sugar alcohols in the extract were determined as D-Glucitol (Sorbitol) 54.57%, Myo-Inositol 1.2%, and Glycerol 1.09%. D-Glucitol (Sorbitol) (54.57%) constitutes the highest content of the extract. Sorbitol, a sugar alcohol with a sweet taste, is used as a sugar substitute and metabolized slowly in the human body [14]. Sugar alcohols can be one of the ingredients of pharmaceutical products such as throat lozenges [15] and they do not cause dental caries and also are important for diabetics because of their low calorie and glycemic index [16]. Moreover, sugar alcohols act on intestinal health with their prebiotic properties [17].

The literature search showed that this phytochemical study on *C. thirkeana*, a medicinally important plant belonging to the Ranunculaceae family, is the first report except for some studies [4,18]. For genera, such as *Delphinium* and *Consolida*, that are close in morphological characteristics, the use of different classification tools such as chemical characters is required for an exact diagnosis. This study may provide chemotaxonomic benefits for *C. thirkeana*.

ACKNOWLEDGMENTS

The authors thank Dr. M. Mesud HÜRKUL for the collection of plant materials.

AUTHOR CONTRIBUTIONS

Conception: *K.C.T.*, *Ş.Y.*; Design: *K.C.T.*, *Ş.Y.*; Supervision: *K.C.T.*; Resources: *K.C.T.*, *Ş.Y.*; Materials: *K.C.T.*, *Ş.Y.*; Data collection and/or processing: *K.C.T.*; Analysis and/or interpretation: *K.C.T.*, *Ş.Y.*; Literature search: *K.C.T.*, *Ş.Y.*; Writing manuscript: *K.C.T.*; Critical review: *K.C.T.*, *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.

REFERENCES

- 1. Brummitt, R.K. (1992). Vascular plant families and genera. Richmond, UK: Royal Botanic Gardens, Kew.
- 2. Heywood, V.H., Brummitt, R.K., Culham, A., Seberg, O. (2007). Flowering plant families of the world. Ontario: Firefly Books.
- 3. Davis, P.H. (1965). Flora of Turkey and the East Aegean Islands, (Vol. 1, pp.119-134). Edinburgh, UK: Edinburgh University Press.
- 4. Hürkul, M.M. (2021). Leaf, stem and root anatomy of *Consolida thirkeana* (Boiss.) Bornm. (Ranunculaceae). *Journal of Research in Pharmacy*, 25(4), 415-419. [CrossRef]
- 5. Gümüşok, S., Hürkul, M.M. (2021). Anatomy of *Consolida orientalis* (Gay) Schröd. (Ranunculaceae): Root, stem and leaf. *İstanbul Journal of Pharmacy*, *51*(3), 3-4. [CrossRef]
- 6. POWO. (2022). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org Accessed: 10.04.2022.
- 7. Evans, W. (2002). Trease and Evans pharmacognosy (15th ed.). WB Saunders.
- 8. Metcalfe, C.R., Chalk, L. (1965). Anatomy of dicotyledones. (Vol. 2). Oxford, UK: Clarendon Press.
- 9. Hao, D.C. (2018). Ranunculales medicinal plants: Biodiversity, chemodiversity and pharmacotherapy. London, UK: Academic Press.

- Yin, T., Cai, L., Ding, Z. (2020). A systematic review on the chemical constituents of the genus *Consolida* (Ranunculaceae) and their biological activities. *RSC Advances*, 10(58), 35072-35089. [CrossRef]
- 11. Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M. T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyigit Botanik Bahçesi Yayınları, Flora Dizisi I.
- 12. Kıymacı, M.E., Tok, K.C., Hürkul, M.M. (2022). A study on phytochemical analysis and antibacterial activity of *Quercus macranthera* subsp. *syspirensis* (K. Koch) Menitsky branch and leaf extracts. *Journal of Faculty of Pharmacy of Ankara University*, 46(1), 160-169. [CrossRef]
- 13. Blau, K., Halket, J.M. (1993). Handbook of derivatives for chromatography. Wiley.
- 14. Awuchi, C.G., Echeta, K.C. (2019). Current developments in sugar alcohols: Chemistry, nutrition, and health concerns of sorbitol, xylitol, glycerol, arabitol, inositol, maltitol, and lactitol. *International Journal of Advanced Academic Research*, 5, 1-33.
- 15. Saraiva, A., Carrascosa, C., Raheem, D., Ramos, F., Raposo, A. (2020). Natural sweeteners: The relevance of food naturalness for consumers, food security aspects, sustainability and health impacts. *International Journal of Environmental Research and Public Health*, *17*(17), 6285. [CrossRef]
- Kroger, M., Meister, K., Kava, R. (2006). Low-calorie sweeteners and other sugar substitutes: a review of the safety issues. *Comprehensive Reviews in Food Science and Food Safety*, 5(2), 35-47. [CrossRef]
- Ruiz-Ojeda, F.J., Plaza-Díaz, J., Sáez-Lara, M.J., Gil, A. (2019). Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. *Advances in Nutrition*, 10, 31-48. [CrossRef]
- Mericli, A.H., Yazici, S., Eroglu-Ozkan, E., Sen, B., Kurtoglu, S., Ozcelik, H., Mericli, F. (2012). Norditerpenoid alkaloids from *Consolida thirkeana* and *Consolida sulphurea*. *Chemistry of Natural Compounds*, 48(3), 525-526.