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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PHENOLIC CONTENT AND ANTIBACTERIAL ACTIVITY OF *COTA PESTALOZZAE* BOISS. AERIAL PARTS

COTA PESTALOZZAE BOISS TOPRAK ÜSTÜ KISIMLARININ FENOLİK İÇERİĞİ VE ANTİBAKTERİYEL AKTİVİTESİ

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ABSTRACT

Objective: *The genus Cota was once considered a subgenus of Anthemis but is now recognized as an independent genus. The species belonging to Cota, including several endemic to Turkey, have been less extensively studied in the literature than other plants in the Asteraceae family. The current study aimed to examine the phenolic content and antibacterial activity of C. pestalozzae Boiss., an endemic species from Turkey.*
Material and Method: *The phenolic content of the methanol extract prepared from the aerial parts of C. pestalozzae Boiss. was analysed qualitatively and quantitatively using high-performance liquid chromatography (HPLC) analysis. This study calculated the limit of detection (LOD) and quantification (LOQ) for each detected compound. The antibacterial activity of the extract was also investigated against some Gram (+) and (-) bacteria strains.*
Result and Discussion: *In the current study, chlorogenic acid, 4,5-O-dicaffeoyl quinic acid, 3,5-O-dicaffeoyl quinic acid, rutin, hyperoside and isoquercetin were qualitatively and quantitatively detected in the methanolic extract of the aerial parts of C. pestalozzae Boiss. Extract of the aerial parts exhibited antibacterial activity only against Staphylococcus aureus ATCC 43300 (MRSA) with a MIC value of 10000 µg/ml. The research indicates that C. pestalozzae contains phenolic compounds and is effective against Gram (+) bacteria.*
Keywords: *Antibacterial activity, Asteraceae, Cota, flavonoids, HPLC, phenolic acids*

ÖZ

Amaç: *Cota cinsi, Anthemis cinsinin alt türleri arasında sınıflandırılırken günümüzde ayrı bir cins olarak kabul edilmektedir. Türkiye'de de endemik türleri bulunan Cota cinsine ait türler literatürde diğer Asteracea bitkilerine kıyasla daha az çalışılmıştır. Bu çalışmada, Türkiye'de endemik bir tür olan C. pestalozzae Boiis. türünün fenolik içeriği ve antibakteriyel aktivitesinin araştırılması amaçlanmıştır.*
Gereç ve Yöntem: *C. pestalozzae Boiss. türünün toprak üstü kısmından hazırlanan metanollü ekstrenin fenolik içeriği Yüksek Performanslı Sıvı Kromatografisi (HPLC) analizi ile kalitatif ve kantitatif olarak analiz edilmiştir. Bu çalışmada, tespit edilen bileşiklerin her biri için tespit limiti ve tayin limiti hesaplanmıştır. Ayrıca ekstrenin antibakteriyel aktivitesi bazı Gram (+) ve (-) bakteri türlerine karşı araştırılmıştır.*

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Sonuç ve Tartışma: *Yapılan çalışma, C. pestalozzae Boiss. bitkisinin toprak üstü kısmının metanollü ekstresinde klorojenik asit, 4,5-O-dikafeoil kinik asit, 3,5-O-dikafeoil kinik asit, rutin, hiperozit ve izokersetin kalitatif ve kantitatif olarak tespit edilmiştir. C. pestalozzae toprak üstü kısmı ekstresi sadece Staphylococcus aureus ATCC 43300'e (MRSA) karşı 10000 µg/ml MIC değeri ile antibakteriyel aktivite sergilemiştir. Çalışma sonuçları C. pestalozzae'nin fenolik bileşikler içerdiğini ve Gram (+) bakterilere karşı etkili olduğu göstermiştir.*
Anahtar Kelimeler: *Antibakteriyel aktivite, Asteraceae, Cota, fenolik asitler, flavonoitler, HPLC*

INTRODUCTION

The *Cota* J. Gay genus, also known as *Anthemis*, was previously classified as a subspecies of the *Anthemis* genus but is now recognised as a distinct genus within the Asteraceae family [1].The most important difference between the two morphologically similar genera is the shape of the achene, and *Cota* species are distinguished from *Anthemis* species by their dorsoventrally flattened, invertedly conical-shaped achenes with prominent ribs, either straight or with 3-10 ribs on each side [2]. *Cota* species are distributed in Europe (except for North European areas), North Africa, Central Asia, the Caucasus and Southwest Asia [3,4]. The genus *Cota* comprises 63 taxa worldwide, with 22 distinct taxa in Turkey. Notably, 9 of these are endemic, highlighting Turkey's significant role in preserving global biodiversity [2]. Studies on *Cota* species are limited, and as indicated in the literature, the flowers of *Cota* species are antiseptic and are used as healing plants [4]. In Europe, *Anthemis* species, the synonym of the genus *Cota*, are known to be used in tea, ointment, extract or tincture forms for sedative, antibacterial, anti-inflammatory and antispasmodic purposes [5]. The importance of *Cota* species in the pharmaceutical, food and cosmetic areas is increasing, with flavonoids and essential oils as major constituents [6]. In a study, the essential oil and fixed oil components of the endemic plant *C. hamzaoglui* Özbek & Vural were analysed by gas chromatography (GC). The essential oil was found to be composed of 59 components. The main structures of the compounds were fatty acids (34.7%), oxygenated sesquiterpenes (17.7%), alkanes (14%) and aliphatic aldehydes (8.3%). In a thorough analysis of lipid content, unsaturated fatty acids were identified as the most prevalent. The key fatty acids were found to be linoleic acid at 26.9%, linolenic acid at 13.2%, palmitic acid at 22.2%, and oleic acid at 20.9%. The study constructively pointed out the promising potential of both the plant's essential oil and fixed oil for practical applications in medical and cosmetic industries, paving the way for future exploration and development in these areas.[4]. A study investigated the antioxidant, antidiabetic, anti-inflammatory, and antimelanogenic properties, as well as the phytochemical composition, of the essential oil and methanolic extract derived from *the* aerial parts of *C. fulvida*. The essential oil's primary components were identified as follows: hexadecanoic acid is the predominant fatty acid; the most abundant sesquiterpenes include caryophyllene oxide, humulene epoxide, and spathulenol; and the major monoterpenes consist of camphor, 1,8-cineol, and α-pinene.). In the study, *C. fulvida* essential oil was found to have anti-inflammatory and antidiabetic effects. The essential oil also showed a weak antioxidant effect compared to the methanol extract. The extract has been shown to contain phenylpropenoid dimers, phenolic acids and flavonoids, and has been shown to have antioxidant, antidiabetic, anti-inflammatory and antimelanoma activity through tyrosinase inhibition [7]. In one study, *C. pestalozzae* showed antioxidant activity and the IC_{50} value in the DPPH (2,2-diphenyl-1-picrilhydrazyl) radical scavenging activity test was calculated as 18.66 mg/ml. The total phenol content of the plant, calculated in gallic acid equivalent, was found to be 42.59 milligrams of gallic acid equivalent per millilitre. The observed antioxidant activity was associated with the plant's phenol content [8]. Another study found *C. altissima* extracts to have antidiabetic, antioxidant, anti-inflammatory and antibacterial activity [9]. A study investigated the components of the essential oil obtained from *C. tinctoria* var. *tinctoria* and the antibacterial and lipase inhibitory activities of the essential oil and its extracts prepared with different solvents (*n*-hexane, acetonitrile, water and methanol). The study identified the essential oil's primary components as terpenes, with the predominant terpenes being borneol, camphor, and *β*-pinene. The study demonstrated that *C. tinctoria* var. *tinctoria* exhibits antibacterial and lipase enzyme inhibitory effects [10]. The study of the biological activities of *C. tinctoria* by methanol and aqueous infusion indicated that the plant exhibited cholinesterase inhibitory,

tyrosinase inhibitory and antidiabetic activities in addition to its potent antioxidant activity [11]. In a further study, the hydroalcoholic extract of *C. tinctoria* demonstrated cytotoxic activity against gastric and liver cancer cell lines and the cytotoxic activity depended on the dose. The mechanism underlying this cytotoxic effect was associated with oxidative stress, cancer cell cycle and apoptosis. Furthermore, it was emphasised that the phenolic compounds present in the plant may play a role in the observed cytotoxic effect [6]. The *C. palaestina* subsp. *syriaca* is an endemic species of *Cota* genus in the Eastern Mediterranean region. The isolation and purification of a compound from the plant, identified as 1-β,10-epoxy-6-hydroxy-1,10H-inunolide, a sesquiterpene lactone, has demonstrated anti-inflammatory, cytostatic and antimetastatic effects when tested on breast cancer cell lines [12]. This study sought to explore the antibacterial properties and the phytochemical composition of a methanol extract derived from the aerial parts of the endemic plant *C. pestalozzae* Boiss. This research aimed to uncover the potential medicinal benefits of this species, examining its ability to inhibit bacterial growth and the specific chemical compounds present in the extract.

MATERIAL AND METHOD

Plant Material and Extraction

C. pestalozzae Boiss. was collected from Konya Ermenek Road on 19/05/2023, and Prof. Dr. A. Mine Gençler-Özkan identified the plant from the Pharmaceutical Botany Department of the Faculty of Pharmacy. The herbarium specimen is stored in AUEF Herbarium (AEF31016).

To perform HPLC analysis, 1 gram of the aerial plant material was extracted with 25 ml of methanol for 30 minutes in an ultrasonic water bath. To assess the antibacterial activity, a methanolic extract was prepared from the aerial parts of the plant (32 g). The plant material was extracted with 300 ml methanol three times using an ultrasonic water bath for 30 min. The obtained methanol extract was evaporated to dryness under a vacuum to obtain 1.95 g of methanol extract (yield was calculated as 6.09%).

HPLC Chromatography Conditions for Qualitative and Quantitative Analysis

In our study extract and standard compounds were analysed by HPLC (Agilent LC 1260 Cromatograph) with gradient elution. The analysis conditions are given in Table 1. The diode array detector (DAD) was configured to a wavelength of 210 nm, after which peak areas were automatically integrated by the computer using Agilent Software. Different concentrations were prepared of each standard compound to obtain calibration curves (1; 0.5; 0.4; 0.2; 0.1; 0.05 mg/ml). To make the calibration curve tested, compounds and 20 mg/ml concentration of extract were injected into HPLC three times. Peak areas were used against the concentrations; calibration curves and equations were obtained. Absorbances were measured at 210 nm for extract and standard compounds. The limit of detection (LOD) and the limit of quantitation (LOQ) values were calculated for each compound by employing the "signal/noise x 3" and the "signal/noise x 10" equations, respectively. The compounds were then injected into the HPLC 6 times.

Table 1. HPLC analysis conditions

Column	ACE 5 C18, 250mm; 4.6mm; 5µm		
Detector	Diode Array Detector		
Column temperature	40°C		
Flow rate	1 ml/min		
Injection volume	10µl		
Analyse time	40 min. with 5 min. post-time		
Mobil phase	Time (min)	Water with %0.2 phosphoric acid (%)	Acetonitrile (%)
	0	90	10
	36	70	30
	36.01	10	90
	40	10	90

Antibacterial Activity Assay

In the antibacterial activity test, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 (methicillin-susceptible, MSSA) and *S. aureus* ATCC 43300 (methicillin-resistant, MRSA) were used as test bacteria. The *C. pestalozzae* extract (40 mg) was dissolved in dimethyl sulfoxide (10% DMSO, 1 ml). The extract's minimum inhibitory concentration (MIC) values were determined by broth microdilution [13]. Serial two-fold dilutions of the extract ranging from 10000 to 78.125 µg/ml were prepared in Mueller Hinton Broth (Merck, Germany). The inoculums were prepared from subcultures maintained for 24 hours. Subsequently, the final test concentration of the test bacteria was adjusted to $5x10^5$ cfu/ml, after which the microplate was incubated at 35°C for 18-24 hours. The last well that demonstrated complete inhibition of visual microbial growth was identified as the MIC value (µg/ml). To establish a baseline for comparison, the solvent (10% DMSO) was used as the negative control, and ciprofloxacin and gentamicin were employed as standard antibiotics.

RESULT AND DISCUSSION

Results of HPLC Analysis

In the present study, HPLC analysis results have revealed that the methanol extract contains chlorogenic acid, caffeoyl quinic acid, rutin, hyperoside, and isoquercitrin. Figure 1 gives chromatograms and retention times (Rt) of the standard compounds.

The primary objective of this study was a thorough qualitative and quantitative analysis of these compounds. Figure 2 clearly displays the HPLC chromatogram of the extract, highlighting the identified peaks that correspond to the standard compounds. The quantities of the detected compounds were accurately determined by injecting solutions of standard compounds at various concentrations. Several standards were prepared for this analysis, each at a different concentration to create a comprehensive dataset. Following this preparation, these standard solutions were injected into the analytical system, and a calibration curve was constructed based on the measured peak areas corresponding to each concentration. These calibration curves are used to calculate the amount of each compound as µg/ 100 mg plant material. The average content of each standard compound and standard deviations are presented in Table 2 together with the equations of the calibration curves of the standards. Table 2 also displays LOD and LOQ values indicating the validation of the analysis.

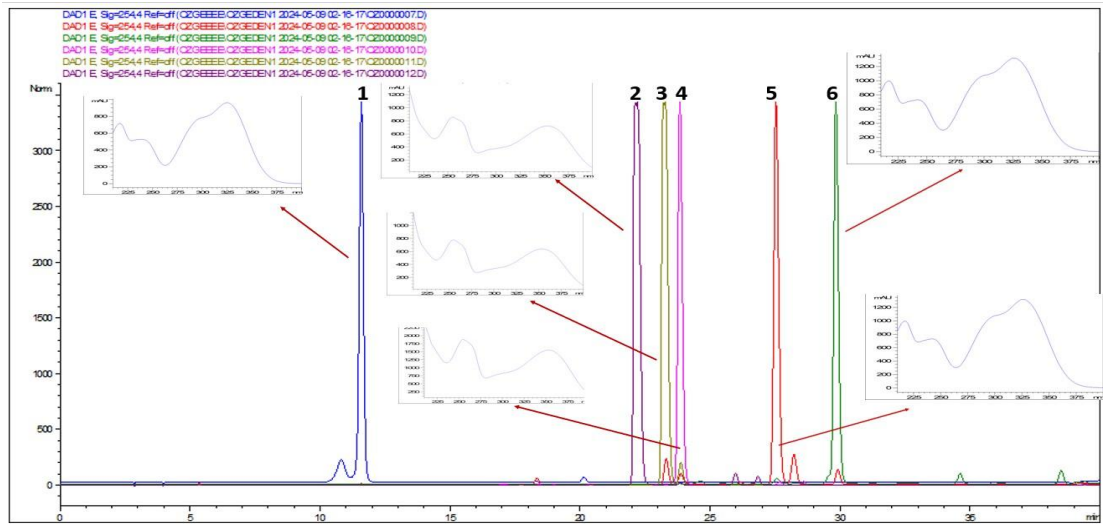

Figure 1. Chromatograms, UV spectrums and Rt (min.) of the detected compounds. Chlorogenic acid (1) Rt: 11.591, rutin (2) Rt: 22.236, hyperoside (3) Rt: 23.284, isoquercetin (4) Rt: 23.840, 3,5-*O*-dicaffeoyl quinic acid (5) Rt: 27.539, 4,5-*O*-dicaffeoyl quinic acid (6) Rt: 29.842

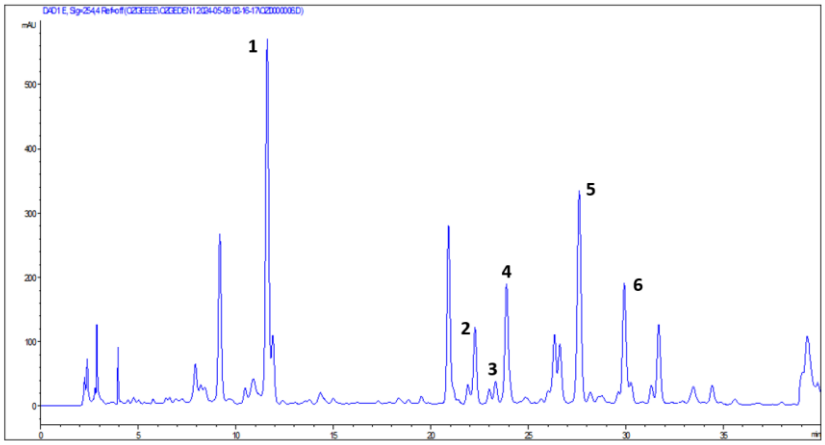

Figure 2. HPLC chromatogram of the extract and the peaks identified as belonging to the standard compounds

In a previous study, the phytochemical content of a methanol extract prepared from the aerial part of *C. fulvida* was analysed by LC-MS/MS. The results demonstrated the presence of several compounds, including 3,5-*O*-dicaffeoylquinic acid, syringic acid, 4,5-*O*-dicaffeoylquinic acid, caffeic acid derivatives, chicoric acid, quercetin glycosides and luteolin derivatives [7]. Another study revealed that methanol extract prepared from *C. tinctoria* comprises various phenolic acids and flavonoids. These include *p*-hydroxybenzoic acid, protocatechuic acid, benzoic acid, chlorogenic acid, caffeic acid, and p-coumaric acid. Additionally, flavonoids such as rutin, quercetin, kaempferol, hesperidin, and apigenin are present, along with catechin derivatives, including (+)-catechin and epicatechin. The observed potent antioxidant activity in the study was associated with the phenolic content of the plant [11]. The analysis of the phytochemical content of another *Cota* species, *C. altissima*, utilizing LC-MS/MS revealed that the main components were 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid, quercetin, and isorhamnetin glycoside [9]. The present analysis indicates the phytochemical structure of the methanol extract from *C. pestalozzae* utilizing high-performance liquid chromatography (HPLC). Drawing inspiration from previous studies on *Cota* species, this research has revealed intriguing compounds such as caffeoylquinic acid derivatives, caffeic acid derivatives, and distinct flavonoid derivatives.

Table 2. Amounts of the standard compounds in the extract, LOD and LOQ values of the compounds

Standard Compounds	**Calibration Equations and R^2 Values**	***C. pestalozzae* Aerial Part (µg/100 mg plant material) Mean±SD**	**LOD (µg/ml)**	**LOQ (µg/ml)**
Isoquercetin	y = 38.873x + 78.959 R^2 = 0.9998	544.98± 5.03	0.9	3
Chlorogenic acid	y = 16.86x + 226.21 R^2 = 0.9981	4084.967±165	0.2	0.6
4,5-*O*-dicaffeoyl quinic acid	y = 13.613x – 9.7593 R^2 = 1	1696.12±74.77	0.6	2
3,5-*O*-dicaffeoyl quinic acid	y = 11.271x – 28.347 R^2 = 0.9999	3570.00±89.95	1.2	4
Rutin	y = 25.152x – 45.754 R^2 = 0.9999	56.34±9.11	0.3	1
Hyperoside	y = 34.803x – 550.74 R^2 = 0.9988	125.52±11.28	1.2	4

Results of Antibacterial Activity Assay

The results of the current study demonstrate the *C. pestalozzae* aerial part extract exhibited antibacterial activity against *S. aureus* ATCC 43300 (MRSA) with a MIC value of 10000 µg/ml. However, no antimicrobial activity was observed against the other tested bacteria within the 10000-78.125 µg/ml concentration range (Table 3). A study investigating the antimicrobial activity of essential oil and extracts obtained from *C. tinctoria* var. *tinctoria* revealed that the essential oil and extracts were effective against gram (+) bacteria. The study indicated that the extract prepared with acetonitrile demonstrated antibacterial activity against the *Mycobacterium smegmatis* (MIC value 548 µg/ml), *S. aureus* (MIC value 274 µg/ml) and *Bacillus cereus* (MIC value 274 µg/ml) strains. In addition, it was observed that the extracts did not show any effect against *E. coli*, *P. aeruginosa*, and *Enterococcus faecalis* [10]. In another study, it was observed that n-hexane extract prepared from the *C. altissima* plant showed an antibacterial effect against the *S. aureus* strain and the MIC value was calculated as 312.5 µg/ml [9]. The findings corroborate the literature data and demonstrate that the methanol extract of *C. pestalozzae* exhibits no antibacterial activity against the test strains of Gram-(-) bacteria.

Table 3. MIC values (µg/ml) of the *C. pestalozzae* extract against test bacteria

	Gram-positive Test Bacteria		**Gram-negative Test Bacteria**		
Sample	***S. aureus* ATCC 25923 (MSSA)**	***S. aureus* ATCC 43300 (MRSA)**	***E. coli* ATCC 25922**	***K. pneumoniae* ATCC 13383**	***P. aeruginosa* ATCC 27853**
***C. pestalozzae* extract (40 mg/ml)**	-	10000	-	-	-
Ciprofloxacine	<0.00025	0.0005	<0.00025	<0.00025	0.0625
Gentamicin	0.0005	<0.00025	0.0005	<0.00025	NT

NT: not tested, (-): no activity

The present study provides evidence of the limited antibacterial activity of *C. pestalozzae* and identifies the presence of phenolic acids and flavonoids in the methanolic extract derived from the plant's aerial parts, both qualitatively and quantitatively. The results indicate that *C. pestalozzae* may serve as a promising source of naturally occurring secondary metabolites. Further research is warranted to isolate and identify these compounds in order to elucidate the phytochemical structure of the plant.

AUTHOR CONTRIBUTIONS

Concept: Ö.B.A.; Design: Ö.B.A.; Control: Ö.B.A.; Sources: Ö.Y., Ö.B.A.; Materials: Ö.Y., S.S.R., Ö.B.A.; Data Collection and/or Processing: Ö.Y.; Analysis and/or Interpretation: Ö.Y., S.S.R., Ö.B.A.; Literature Review: Ö.Y., Ö.B.A.; Manuscript Writing: Ö.Y., S.S.R., Ö.B.A.; Critical Review: Ö.Y., S.S.R., Ö.B.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

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

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