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THE EFFECT OF DIFFERENT SOLVENTS ON CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY, AND ANTIMICROBIAL POTENTIAL OF TURKISH *CISTUS CRETICUS* EXTRACTS

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ABSTRACT
The aims of this study were (1) to assess the extract yield, antioxidant activity, and antimicrobial activity of *Cistus creticus* extracts obtained from different locations in Türkiye (2) to evaluate the effect of water and methanol solvents on chemical composition, total polyphenol and flavonoid content, IC_{50}, and FRAP values of *C. creticus*. The extracts had a weak inhibitory effect against tested microorganisms. However, the results of antioxidant assays were very promising. The IC_{50} values of methanol and water extracts were 13.94 µg/mL and 34.41 µg/mL, respectively. Similarly, the FRAP value of methanolic extract (1.27 M/g) was higher than the water extract (0.72 M/g). The results of HPLC analysis demonstrated that rutin was found only in the methanol extract (13.252%). In addition, the methanol extract had a higher content of quercetin (7.909%), benzoic acid (4.226%), and chlorogenic acid (2.168%), whereas the water extract possessed a higher amount of gallic acid (13.705%).
Keywords: Plant extract, antioxidant activity, bioactive substances, flavonoids, phenolics

FARKLI ÇÖZÜCÜLERİN TÜRKİYE`DE YETİŞEN *CİSTUS CRETICUS* EKSTRAKLARININ KİMYASAL KOMPOZİSYON, ANTİOKSİDAN AKTİVİTE VE ANTİMİKROBİYAL POTANSİYELİNE ETKİSİ

ÖZ
Bu çalışmanın amaçları (1) Türkiye`nin farklı bölgelerinden toplanan *Cistus creticus* L. ekstrelerinin verim (%), antioksidan ve antimikrobiyal aktivitelerini değerlendirmek, (2) metanol ve su çözücülerinin *Cistus creticus* L ekstrelerinin kimyasal kompozisyon, toplam fenolik ve flavanoid miktarlarına, IC_{50} ve FRAP değerlerine etkisini araştırmaktır. Ekstreler zayıf bir antimikrobiyal etki gösterirken, antioksidan deneylerinin sonuçları çok umut vericidir. IC_{50} değerleri metanol ve su ekstreleri için sırasıyla 13.94 µg/mL ve 34.41 µg/mL'dir. Benzer olarak, metanol ekstresinin FRAP değeri (1.27 M/g) su ekstresininkinden (0.72 M/g) daha yüksek çıkmıştır. HPLC analizinin sonuçları

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rutinin sadece (%13.252) metanol ekstresinde olduğunu göstermektedir. Ayrıca, metanol ekstresi daha yüksek miktarda kuersetin (%7.909), benzoik asit (%4.226) ve klorojenik asit (%2.168) içerirken, buna karşın su ekstresi daha yüksek miktarda gallik asit değerine (%13.705) sahiptir.
Anahtar kelimeler: Bitki ekstresi, antioksidan aktivite, biyoaktif maddeler, flavanoidler, fenolikler

INTRODUCTION

Cistus species are evergreen shrubs with white, pink, or purple flowers. They are mostly found in the Mediterranean region. While there are 52 species of *Cistus* found in the world, 5 species are naturally present in Türkiye. These are *C. creticus* L., *C. laurifolius* L., *C. monspeliensis* L. *C. parviflorus* Lam., and *C. salviifolius* L. (Yeşilyurt, 2012). According to Şekeroğlu and Gezici (2021), *Cistus* species spread from the Mediterranean region to central Anatolia, Marmara, and the black sea region in Türkiye. Due to their aromatic and pharmacological properties, herbal brews, distillates, and resins of *Cistus* spp. have been used in folk medicine for ages to treat many ailments, such as diarrhea, peptic ulcers, skin rashes, urinary tract infections, and inflammation (Küpeli and Yesilada, 2007).

In recent years, scientific researches have been focused on the isolation and determination of chemical components in several *Cistus* species. According to the study by Papaefthimiou et al. (2014), 397 terpenes, 162 phenylpropanoids, 24 hydrocarbons, 35 fatty acids, 18 phytohormones, and vitamins were isolated from *Cistus* spp. In addition, 92 terpenes and 12 phenylpropanoids were reported in *C. creticus*. Terpenes are one of the principal constitutients of the plant essential oils providing smell and aroma specific to the plants. Terpenes can be found in the plant tissues generally as free, or bound to organic acid esters, glycosides, or proteins (Cox-Georgian et al., 2019). They demonstrate antimicrobial activity against bacteria, fungi and viruses (Paduch et al., 2007).

In addition, *Cistus* spp. is also known to have a variety of polyphenols and flavonoids. Plants synthesize these secondary metabolites via phenylpropanoid pathways. These compounds are produced by plants to adapt to stress conditions (Skorić et al., 2022). Various phenylpropanoids have been identified in different *Cistus* spp. For instance, Gürbüz et al. (2018) reported the presence of trans-tiliroside, a mono-coumaroyl kaempferol glucoside, hyperin, and myricetin 3-O-ss-galactopyranoside in *C. salviifolius*, *C. creticus*, and *C. laurifolius* methanolic extracts from Turkey. In another study, quercetin and myricetin derivatives were found in abundance in the aqueous extracts of *C. albilus*, *C. clusii*, and *C. salviifolius*, which were collected from dry and arid areas in Spain (Tomás-Menor et al., 2013). Moreover, Akkol et al. (2012) found quercetin and kaempferol derivatives in the ethanol extract of *C. laurifolius* leaves from Bolu, Turkey. Many factors, such as species, location, and climatic conditions, can affect the chemical constituents of the plant. Regarding this, Barrajón-Catalán et al. (2011) evaluated the influence of subgenus, soil, and climate on the chemical composition of *Cistus*. They found that higher amounts of flavonoids and very little or no ellagitannins were present in the *Cistus* subgenus (*C. clusii*, *C. laurifolius,* and *C. monspeliensis*), while the opposite was observed for the *Leucocistus* and *Halimioides* subgenus (*C. ladanifer*, *C. salviifolius*, *C. populifolius*, and *C. libanotis*). They also discovered that the presence of phenolic compounds was significantly influenced by the *Cistus* subgenus more than the soil and the climate. Besides, it has been found that many of these polyphenolic compounds carry a high level of bioactivity. Güvenç et al. (2005) reported that a butanol extract of *C. creticus* leaves demonstrated antimicrobial activity against gram-positive *Staphylococcus aureus*. In a recent study, Carev et al. (2020) found that *C. creticus* and *C. salviifolius* had high antioxidant activity with 43 different phytochemicals.

Despite the growing interest in determining the chemical composition and bioactivity of different plant materials, the *C. creticus* plant has not been fully studied yet for its biological activity. Therefore, the objectives of this study were first to scan different places in Türkiye for *C. creticus* extract yield, total phenolic and flavonoid content, DPPH free radical scavenging activity,

and antimicrobial activity; second, to determine the effect of methanol and water extraction techniques on *C. creticus* extracts' chemical composition, total phenolic and flavonoid content, and antioxidant properties.

MATERIALS AND METHODS

Materials

C. creticus L. plant materials were gathered from nature reserves and parks in different regions of Türkiye and they were identified by Prof. Dr. Menşure Özgüven. Then, they were kept at room temperature separately based on their location until reached to constant weight. All parts of the plant material, including wood or stalks, bark, and leaves, were powdered to a particle size smaller than 5 mm using a mechanical mill. After that, the ground *C. creticus* powders were kept in containers, labeled, and stored until further analysis.

Methanol extraction procedure

The ground *C. creticus* (100 g) was placed in a bottle with 1 L methanol and shaken for 24 h (Toros, Turkey; 400 rpm). Later, the mixture was strained using a vacuum pump and Whatman No.1 filter paper. Then, the supernatant was concentrated using a rotary evaporator (Rotavapor® R-300, Buchi, Italy) at 50 ºC and the obtained samples were freeze-dried using a lyophilizer (Christ Alpha 1-4 LD Plus, Germany).

Conventional water extraction procedures

The sample-to-solvent ratio was also kept at 1/10 (w/v) in this procedure. The mixture of ground plant material and water was boiled at 100 ºC for 5 hours with a continuous stirrer (Wisd MSH-20A, South Korea). Then the mixture was filtered with Whatman No.1 filter paper. The supernatant was taken to a new beaker and evaporated to dryness without boiling at 65-75 ºC. Afterwards, the dried extracts were freeze-dried.

Extraction yield

The crude extract yield (%) was determined for *C. creticus* samples collected from different locations according to the methanol extraction procedure. The following formula was used to calculate the extract yield (%).

$$\text{Extract yield (\%)} = (\text{Extract mass}/\text{Sample mass}) \times 100 \quad (1)$$

Antimicrobial susceptibility testing

The inhibitory effect of methanolic extracts against some foodborne pathogenic bacteria, yeast, and mold was determined based on a disc diffusion method (Hudzicki, 2009). Mueller Hinton agar (MHA, Merck) was used to test bacteria and mold without supplementation, while 2% glucose and 0.5 mg/mL methylene blue were used to supplement MHA agar for yeast testing. Overnight cell cultures were prepared using a tryptic soy broth (Merck). After centrifugation, the cell pellets were washed twice and suspended in a 0.85% NaCl solution. Inoculation sizes were adjusted to 10^8 CFU/mL for bacterial cell cultures and 10^6 CFU/mL for yeast and mold cells. Then the agar surface was inoculated with 100 µL of cell cultures. Following this, a sterile 6 mm empty disc impregnated with a 20 µL of *Cistus* extract was placed onto the inoculated agar surface and incubated at 35 ± 2 ºC for up to 24 hours. After the incubation, the diameter of the inhibition zones was measured, and the results were provided in mm.

HPLC analysis

Details of the standard preparation, HPLC condition, and apparatus used for this study are provided in Table 1. An external standard curve of the phenolic standards was used to calculate the percentage of phenolic compounds present in the *Cistus* extracts.

Total phenolic content

Total phenolic content was determined according to Singleton and Rossi (1965). Simply, the sample extract and Folin-Ciocalteu's reagent were mixed at a 1:4 (v/v) ratio. After 5 min of incubation, 7.5% Na_2CO_3 solution was added to the mixture at a 1:1 (v/v) ratio. The incubation was continued for 90 min in the dark, and the absorbance of mixtures was measured at 760 nm. A six-point gallic acid standard curve was prepared to calculate the TPC of *Cistus* extracts. The TPC was expressed as gallic acid equivalents (mg GA/g) for dry extract.

Table 1. HPLC conditions used for the determination of chemical composition of *Cistus creticus*

Instrument	Waters model W2690/5 autosampler equipped with Waters 2695 pumps
Detector	Waters 2489 UV detector
Type of column	ACE C_{18} (5 μm – 4.6 × 250 mm) column (Advanced Chromatography Technologies Ltd., Aberdeen, Scotland)
Column temperature	25 °C,
Injection volume	20 μL
Flow rate	1.2 mL/min
Mobile phases	A: 2% acetic acid B: equal volumes of acetonitrile and 0.5% acetic acid solution C: acetonitrile
Gradient program	A: 95%, B: 5% 5 min A: 80%, B: 20% 8 min A: 78%, B: 22% 10 min A: 75%, B: 25% 17 min A: 73%, B: 27% 19 min A: 60%, B: 40% 30 min A: 55%, B: 45% 35 min A: 35%, B: 65% 40 min B: 10%, C: 90% 45 min C:100% 50 min A: 95%, B: 5% 55 min

Total flavonoid content

The plant extract and 2% $AlCl_3$ methanolic solution were mixed at a 1:1 (v/v) ratio. The mixture was kept at room temperature for 15 min and the absorbance values were determined at 430 nm. The quercetin standard curve was used to evaluate the TFC of *Cistus* extracts. The TFC was stated as quercetin equivalents (mg QUE/g) for dry extract (Djeridane et al., 2006).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The modified method of Cuendet et al. (2001) was used to assess the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity of the *Cistus* extracts. First, dried samples were dissolved in methanol at different concentrations. Next, the resulting samples and a 0.004% (w/v) DPPH methanolic solution were mixed (1/100, v:v). The mixture was kept at dark for 30 min, then absorbance was read at 517 nm against a blank. The inhibitions of the DPPH radical (%) were calculated according to the following formula, where A_{blank} denotes the absorbance of the control (DPPH radical) and A_{sample} implies the absorbance value of the extract (sample and DPPH radical).

$$I\ (\%) = (A_{blank} - A_{sample}\ /A_{blank}) \times 100 \qquad (2)$$

After obtaining I (%) values of the *Cistus* extracts at different concentrations, the extract concentrations were plotted against the inhibition values (%). Finally, the sample concentration corresponding to 50% inhibition (IC_{50}) of the DPPH radical was determined from the plot.

The ferric reducing antioxidant power (FRAP) assay

Required acetate buffer (300 mM, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine solution (10 mM TPTZ in 40 mM HCl), and $FeCl_3.6H_2O$ (20 mM). These were mixed (10:1:1, v:v:v) to prepare the FRAP reagent. Then the assay was carried out by mixing the FRAP reagent with distilled water and plant extract (30:10:1, v:v:v) and incubating the mixture at 37 °C for 15 min. After that, the absorbance of the *Cistus* extracts and the blank (FRAP reagent) was measured at 595 nm. The $FeSO_4.7H_2O$ standard curve was prepared at different concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM)

to calculate the FRAP values of the *Cistus* extracts, and the results were expressed as M of Fe^{+2}/g of dry extract (Riahi et al., 2013).

Statistical analysis

All analyses were conducted in three replications, and the data was stated as the mean ± standard deviation. The results of HPLC analysis, the TPC and TFC assays, the DPPH radical scavenging activity, and the FRAP assay of methanol and water extracts of *C. creticus* were evaluated by a two-sample t-test. The significance between the treatments was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Pre-assessment screening of *Cistus* extracts from different locations

The extract yield of *Cistus* samples taken from four regions (the Mediterranean, Aegean, Marmara, and Middle Black Sea regions) is displayed in Table 2. Almost all the samples were *C. creticus*, except C24 from Karşıyaka, İzmir, which was *Cistus parviflorus*. The highest extract yield was obtained from C27 (Amasya) with 16.8%, followed by C1 (Gülnar, Mersin) with 16%, C30 (Muğla) with 15.3%, and C31 (Amasya) with 15.2%. In addition, when the extracts were compared according to their antioxidant activity, the IC_{50} value of *Cistus* extract was lowest for sample C29 (16.77 μg/mL) from Dim, Alanya, and followed by C21 (16.89 μg/mL) from Hisarönü, Muğla, C4 (17.13 μg/mL) from Gülnar, Mersin, and C31 (17.65 μg/mL) from Amasya (Table 2). The lower IC_{50} indicates a higher antioxidant activity. Moreover, the results of TPC and TFC (Table 2) correspond with the results of the half-maximal inhibitory concentration. In other words, the samples with a high DPPH antioxidant activity (lower IC_{50} values) also had high total phenolic and flavonoid contents. For instance, the C4 sample, which was collected from Gülnar, Mersin, had the highest TPC (132.99 mg/mL) and TFC (10.93 mg/mL), which coincided with a low IC_{50} value of 17.13 μg/mL. Additionally, the C4 samples from Gülnar, Mersin were chosen to carry the second part of the assays due to their high phenolic and flavonoid contents.

It has been shown that plant flavonoids with high antioxidant capacities are also associated with antimicrobial properties (Górniak et al., 2019). Therefore, the assessment of antimicrobial activity was performed with nine *Cistus* samples carrying higher antioxidant activity. The results showed that methanolic extracts of *C. creticus* possessed none or very weak antimicrobial activity against tested bacteria, yeast, and mold (Table 3). Only noticeable inhibitory effect was found for the C19 samples from Biga, Çanakkale, providing 7- and 6-mm weak inhibition zones against *Listeria monocytogenes* and *Candida albicans*, respectively. Since the results were insignificant, antimicrobial testing was not pursued for the next stage of the study.

As previously mentioned the phenolic compounds with high antioxidant capacity are high likely to hold antimicrobial properties (Górniak et al., 2019). However, the *Cistus* extracts obtained in this study did not demonstrate similar results. In a previous study, Alcaraz et al. (2000) explored the relationship between structures of the polyphenol compounds with their antimicrobial activity. Phenolic compounds with open heterocyclic rings tend to have high antimicrobial properties. Nevertheless, binding of methoxy or acetyl groups to heterocyclic rings at different positions can diminish their antimicrobial activities (Alcaraz et al, 2000; Avila et al., 2008). In addition, it has been also found that the hydroxyl groups on these heterocyclic rings possess strong antioxidant activity, and replacing hydroxyl groups with lipophilic compounds increases the antimicrobial activity of phenolic compounds (Liu et al., 2008). In contrast to our results, lipophilic extracts of *Cistus* spp. demonstrated inhibitory effect against different microorganisms in previous studies (Paduch et al., 2007; Papaefthimiou et al., 2014). These findings suggest that molecules attach to active binding cites of phenolic compounds in the *Cistus* extracts carry high antioxidant activity with low antimicrobial properties.

Table 2. The extract yield, antioxidant activity, total flavonoid and phenolic content of *Cistus creticus* samples obtained from different locations in Türkiye

Sample number	Location	Yield (%)	DPPH (IC_{50} value, µg/mL)	TFC (mg QUE/g)	TPC (mg GAE/g)
C1	Gülnar/Mersin	16.0	27.69	4.22	57.22
C2	Gülnar/Mersin	14.0	27.18	3.78	43.81
C3	Gülnar/Mersin	10.1	26.59	3.56	47.39
C4	Gülnar/Mersin	13.5	17.13	10.93	132.99
C5	Gazipaşa/Antalya	13.6	20.82	7.72	92.16
C6	Gazipaşa/Antalya	13.8	30.74	4.67	58.13
C7	Antalya	11.6	33.21	3.75	78.06
C8	Köyceğiz/Muğla	13.2	27.35	4.82	73.50
C9	Bucak Dağı	11.3	27.67	4.80	70.75
C10	Antalya/Serik	10.5	28.02	4.17	80.64
C11	Dim Orman İşl.	11.4	29.63	4.65	68.90
C12	İzmir Orman İşl.	12.0	24.51	5.18	87.54
C13	Antalya	14.0	21.77	4.38	99.79
C14	Alanya	9.3	24.01	4.14	91.25
C15	Antalya	11.3	20.14	4.83	88.05
C16	Lapseki/Çanakkale	12.6	34.76	3.98	59.44
C17	Biga/Çanakkale	12.3	21.93	5.92	108.45
C18	Çanakkale	12.4	30.72	4.43	52.59
C19	Biga/Çanakkale	15.0	19.77	2.66	76.65
C20	Amasya	12.8	27.7	2.35	67.12
C21	Hisarönü/Muğla	17.2	16.89	2.43	72.81
C22	Muğla	11.5	26.76	3.03	64.94
C23	Manavgat/Antalya	13.5	21.12	2.54	72.80
C24*	Karşıyaka/İzmir	11.4	27.87	2.07	68.28
C25	Alanya	10.1	19.62	2.73	85.98
C26	Datça/Muğla	12.5	23.62	2.90	73.14
C27	Amasya	16.8	24.37	2.45	76.19
C28	Alanya	14.9	20.05	2.69	86.55
C29	Dim/Alanya	12.6	16.77	2.80	84.80
C30	Muğla	15.3	22.32	2.46	60.32
C31	Amasya	15.2	17.65	2.96	99.01
C32	Marmaris/Muğla	14.8	23.94	3.11	77.40
C33	Marmaris/Muğla	13.5	25.04	2.31	91.04
C34	Muğla	12.8	21.56	2.93	82.48
C35	Feke	9.6	19.11	2.81	85.98

*Only this sample was identified as *Cistus parviflorus*.

DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: 50% maximum inhibitory concentration; TFC: Total flavonoid content; TPC: Total phenolic content.

Table 3. The results of disc diffusion assay (mm)

	Methanolic extracts of *C. creticus*								
Type of microorganism	C4	C15	C19	C21	C24	C25	C29	C31	C35
Bacillus cereus	5.0	5.0	5.0	5.0	5.0	5.0	4.0	4.0	5.0
Enterococcus faecalis	4.0	-	4.0	4.0	2.0	3.0	2.0	3.0	2.0
Listeria monocytogenes	2.0	3.0	7.0	5.0	1.0	0.5	0.5	1.5	1.5
Staphylococcus aureus	4.0	2.0	2.0	3.0	-	2.0	2.0	2.0	6.0
Escherichia coli	1.0	2.0	2.0	1.0	5.0	3.0	1.0	1.0	2.0
E. coli O157:H7	4.0	5.0	2.0	6.0	5.0	5.0	-	6.0	7.0
Salmonella Typhimurium	1.0	4.0	3.0	4.0	2.0	1.0	1.0	1.0	5.0
Candida albicans	4.0	4.0	6.0	5.0	3.0	4.0	5.0	5.0	6.0
Saccharomyces cerevisiae	5.0	4.0	3.0	3.0	3.0	3.0	3.0	5.0	5.0
Aspergillus brasiliensis	2.0	2.0	1.0	-	2.0	2.0	2.0	2.0	2.0

Phenolic composition of *Cistus* extracts

The polyphenolic compounds of the methanolic and water extracts of plants were analyzed qualitatively and quantitatively by HPLC. Table 4 summarizes the HPLC results on the phenolic content of *C. creticus* L. According to all available data, rutin was the principal substance in terms of quantity (Figure 1). It was not found in water extract; however, it was particularly plentiful in methanolic extract (13.252%) (Table 4). The other ten standards except rutin were detected in all extracts. Quercetin was the main compound in the methanolic and water extracts, with 7.909% and 5.415%, respectively. Gallic acid was plentiful in the water extract (13.705%) and less in the methanolic extract (1.501%). Benzoic and chlorogenic acids were found in different amounts among the extracts. They were found to be higher in the methanolic extract than the water extract (4.226% and 2.168%, respectively). No difference was observed between the extracts of other phenolic substances (Figure 1).

Table 4. Composition of phenolic compounds of *Cistus creticus* L. obtained by methanol and water extraction

Phenolic Compound (%)	Approximate Rt (min)	*Cistus creticus* L.	
		Methanol	Water
Gallic acid	4.942	1.50a ± 0.19	13.71b ± 0.18
4-Hyrdoxybenzoic acid	13.515	1.32a ± 0.11	1.35a ± 0.01
Chlorogenic acid	14.033	2.17a ± 0.07	0.58b ± 0.03
Vanillic acid	15.402	0.27a ± 0.05	0.55b ± 0.02
Syringic acid	17.835	0.11a ± 0.01	0.27b ± 0.01
Cumaric acid	24.217	0.12a ± 0.02	0.12a ± 0.08
Rutin	29.577	13.25 ± 2.19	-
Benzoic acid	30.565	4.23a ± 0.52	0.90b ± 0.02
Cinnamic acid	35.873	0.93a ± 0.19	0.07b ± 0.01
Rosmarinic acid	38.037	0.72a ± 0.01	0.11b ± 0.04
Quercetin	44.296	7.91a ± 0.98	5.42b ± 0.12

Data is expressed as mean ± standard deviation (n = 3).

[a,b] Different letters in the same row denote a significant difference, two sample t test ($P \leq 0.05$).

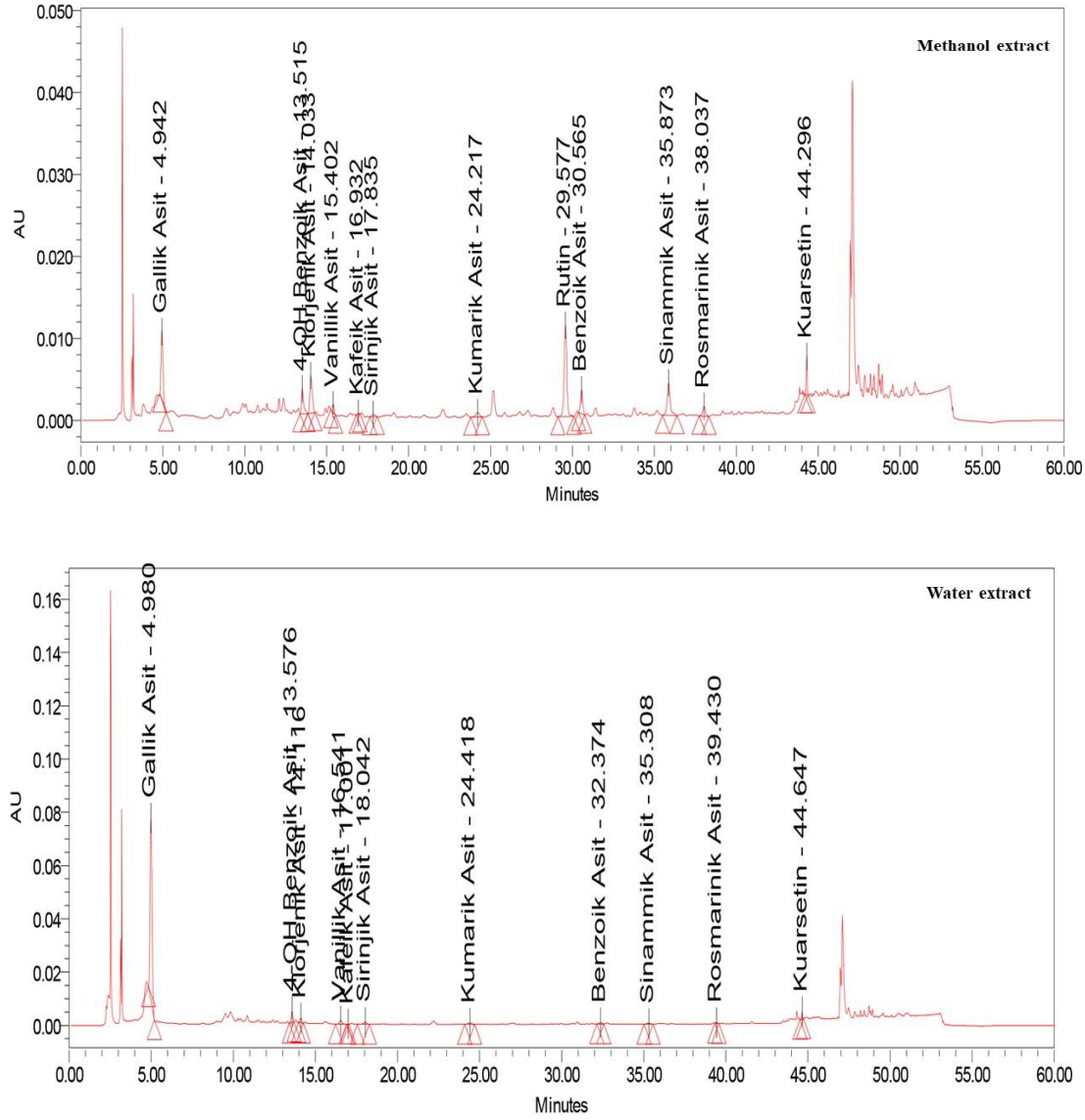

Figure 1. HPLC chromatograms of *Cistus creticus* L. extracts.

Although the same flavonoids discussed in this article have not been addressed in any literature-based papers, there are a few studies that explain the development of a comparable gradient method for the analysis of various polyphenols from *Cistus* species. Generally, studies have been examined in terms of the quercetin, myricetin, and rutin contents of the Cistus plant. No studies were found to evaluate vanillic, syringic, cumaric, benzoic, cinnamic, rosmarinic, and gallic acids. In a study examining the polyphenol contents of 5 different *Cistus* species endemic to Turkey (Onal et al., 2023), quercetin-3-rutinoside (50.61 ppm) was the highest content in the *C. creticus* species. In another study investigating *Cistus* species endemic to Turkey (Gürbüz et al., 2018), the outcomes showed that quercetin in *C. salviifolius* and kaempferol in all examined samples remained below the limit of detection. Despite this, mono-coumaroyl kaempferol glucoside trans-tiliroside was found to be the most prevalent flavonoid in *C. salviifolius* and *C. creticus* (0.276 g and 0.253 g in 100 mL extract, respectively), whereas hyperin and myricetin 3-O-galactopyranoside were found

to be the most potent flavonoids in *C. laurifolius* samples. On the contrary, rutin and quercetin were prominent in this study. It can be interpreted that the plants may have changed depending on the year they were collected, the soil, the climate, and the extraction techniques.

Mastino et al. (2018) examined the phenolic contents of three different *Cistus* species. In that study, they used 51 phenolic standards and detected 37 different compounds in the *C. creticus* species. The phenolic acids represented the main fraction in all three samples. The methyl derivatives of rosmarinic acid observed in all three *Cistus* species were 3-O-methylrosmarinic acid, dihydroxy-dihydroferuloyl methyl rosmarinic acid, and feruloyl dimethyl rosmarinic acid. The study results of Mastino et al. (2018) differ from our study because of the regional difference. They used *C. creticus* from Sardinia, Italy. Some of our phenolics, such as rutin and quercetin, were not detected in *Sardinia creticus* species. So, we can say that the growing place of the plant affects the composition.

In our study, phenolic makeup was also evaluated in terms of extraction procedure. The various extraction techniques employed in the investigations, as well as the plant's origin and edaphic variables, result in varying outcomes. The health benefits of *C. creticus* L. are associated with its high content of rutin and qurcetin (Hitl et al., 2022). Its high antioxidant capacity can be associated with these molecules. Good for heart and brain health, lowering blood pressure, and reducing oxidative stress are the proven effects of rutin and quercetin molecules (Ciumărnean et al., 2020).

Total phenolic and flavonoid content and antioxidant activity of *Cistus* extracts

The results of the total phenolic content of the methanol and water extracts of *C. creticus* are presented in Table 5. The methanol extract (135.24 mg GAE/g) had a statistically significantly ($P \leq 0.05$) higher total phenolic content than the water extract (114.35 mg GAE/g). According to the HPLC results, gallic acid was in higher concentration in the water extract, while benzoic acid was found in higher proportions in the methanolic extract. As is known, the total phenolic content analysis covers the presence of various phenolic compounds, including phenolic acids and flavonoids. Because of the presence of phenolic acids and the high content of flavonoids such as rutin and quercetin, the TPC value of the *C. creticus* methanolic extract was higher than the water extract (Tables 4 & 5). In a recent study by İnan et al. (2021), an aqueous extract of *C. creticus*, which was obtained from Kayışdağı, İstanbul, had a much higher TPC (275. 47 mg GAE/g) in comparison to this study. On the contrary, a lower TPC (69.34 mg GAE/g) was reported for a methanolic extract of the Syrian *C. creticus* (Waed et al., 2016). When the TPC results of *C. creticus* extracts were compared with other *Cistus* species, our extracts had a higher TPC than the *C. albidus* (L.) (112.48 mg GAE/g) and *C. monspeliensis* (L.) (79.19 mg GAE/g) (Bouyahya et al., 2016), and a lower TPC than the *C. salviifolius* (560.3 mg GAE/g) (Rebaya et al., 2016).

Table 5. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of *Cistus creticus* L. attained by methanol and water extraction

Parameters	*Cistus creticus* L.	
	Methanol	Water
TPC (mg GAE/g DW)	135.24a ± 12.83	114.35a ± 10.89
TFC (mg QUE/g DW)	34.34a ± 6.33	19.78b ± 1.66
DPPH (IC_{50}, µg/ml)	13.94a ± 0.96	34.41b ± 2.28
FRAP (M Fe^{+2}/g)	1.27a ± 123.79	0.72b ± 33.42

DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power.
Data is expressed as mean ± standard deviation (n = 3).
[a,b] Different letters in the same row denote a significant difference, two sample t test ($P \leq 0.05$).

The total flavonoid content of the *C. creticus* extracts was significantly ($P \leq 0.05$) affected by the extraction methodology. Similar to the TPC results, the TFC of methanolic extract was higher than water extract (Table 5). The presence of rutin and quercetin was associated with a higher TFC value for the methanolic extract than the water extract with just quercetin present. It should also be noted that since the chromatography analysis was limited to the quantification of the available standards, unidentified flavonoids may also have an influence on the TFC values. Contrarily, in a previous study, a higher ($P \leq 0.05$) TFC value was found for the water extract of *C. creticus* than the methanolic extract (Waed et al., 2016). In another study, Skorić et al. (2012) investigated the effect of shoots and roots on the TPC and TFC values of a *C. creticus* ethanolic extract and found that extracts obtained from shoots had significantly ($P \leq 0.05$) higher TPC and TFC values than the roots. Besides, İnan et al. (2021) reported 24.15 mg QUE/g for the water extract of *C. creticus*, which was higher than our results for the water extract (19.78 mg QUE/g).

It has long been known that phenolic acids and flavonoids are major antioxidant compounds. They can exhibit their antioxidant activity by the mechanism of donating electrons or protons to scavenge free radicals, to chelate metal ions, or to inhibit enzymes responsible for radical generation (Irigoiti et al., 2021). In previous studies, IC_{50} values of some of the pure antioxidant compounds have been reported as follows: gallic acid (3.53 μg/mL), quercetin (6.55 μg/mL), rutin (9.44 μg/mL) (Singh et al., 2018), and ascorbic acid (5.59 μg/mL) (Gedikoğlu et al., 2022). In this study, *C. creticus* extracts demonstrated strong radical scavenging activity for the DPPH radical. Despite the water extract possessing a higher content of gallic acid, possibly due to the presence of higher proportions of quercetin, benzoic acid, and rutin that are only found in the methanolic extract, the *C. creticus* methanolic extract (13.94 μg/mL) had a significantly ($P \leq 0.05$) higher antioxidant activity than the water extract (34.41 μg/mL) (Table 5). When the studied extracts compared to mentioned pure antioxidants, *Cistus* extracts had a relatively lower antioxidant capacity. On the other hand, İnan et al. (2021) found compatible results with these antioxidant compounds (5.63 μg/mL). As is known, the radical scavenging activity of extracts can differ according to methodology, type of solvent used in the assays, material location, vegetative stages of the plant, part of the plant used for the extraction, and the presence and quantity of polyphenols (Papaefthimiou et al., 2014; Matlok et al., 2020). Therefore, the differences in the IC_{50} values could be associated with some of the aforementioned factors. When the antioxidant activity was evaluated by the FRAP assay, the results were similar to the DPPH radical scavenging activity (Table 5). The methanolic extract of *C. creticus* exhibited strong ferric reducing antioxidant activity and provided statistically significantly ($P \leq 0.05$) higher antioxidant activity than the water extract. There are no reports of FRAP values found for *Cistus* spp. in the literature, as far as in our knowledge. Besides, when the *Cistus* extracts were compared to natural extracts (Gedikoğlu, 2022) and essential oils (Gedikoğlu et al., 2019), both the methanolic and water extracts of *Cistus* had significantly better results.

CONCLUSION

In this study, the phenolic composition and antioxidant activity of *Cistus creticus* L. was evaluated according to extraction methods. The presence and concentration of polyphenolic compounds in the extracts and their antioxidant activities were significantly ($P \leq 0.05$) affected by the extraction techniques. The methanolic extracts exhibited the highest antioxidant activities in both the DPPH and FRAP assays. In addition, rutin was only present in the methanolic extracts. Furthermore, quercetin and benzoic acid were found in significantly ($P \leq 0.05$) higher amounts in the methanolic extracts. On the other hand, gallic acid content was higher ($P \leq 0.05$) in the aqueous extracts.

AUTHOR CONTRIBUTIONS

Ayça Gedikoğlu: Data collection; formal analysis; project administration; writing, reviewing, and editing.

Hale İnci Öztürk: Data collection, writing, reviewing, and editing.

Ezgi Aytaç: Processing plant material, data collection, and writing.

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CONFLICT OF INTEREST
The authors have no competing interests to declare.

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