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A multidirectional study on chemical fingerprints and biological activities of three *Cistus* extracts (*C. creticus, C. laurifolius,* and *C. salviifolius*) with ethnomedicinal uses

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Abstract: Humans have used medicinal plants to treat various diseases for thousands of years. *Cistus* species are also widely used in traditional medicine and have various medicinal applications; therefore, they deserve more in-depth research. The present study evaluated the chemical profile, antioxidant, enzyme inhibition, and cytotoxic properties of the twigs and leaves of *C. creticus* L., *C. laurifolius* L., and *C. salviifolius* L. grown in Türkiye. The methanolic extracts of the three species were rich in phenolics, mainly flavonoids. Exerted potent antioxidant activity with a methanolic extract from the leaves of *C. salviifolius* displayed the highest total phenolic (97.08-mg gallic acid equivalent/g) and flavonoid (49.60-mg rutin equivalent/g) contents, as well as antiradical (2,2-diphenyl-1-picrylhydrazyl) assay = 612.11 mg TE (trolox equivalent)/g; (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) = 804.66 mg TE/g), reducing ions (cupric reducing antioxidant capacity) = 690.54 mg TE/g; ferric reducing antioxidant power = 459.34 mg TE/g), and chelating (15.58 mg EDTAE/g) properties. It also revealed the best amylase and glucosidase inhibitory activity. Extracts from the twigs of the three *Cistus* species, except the leaves of *C. salviifolius* and *C. laurifolius*, displayed comparable acetylcholinesterase inhibitory activity (2.48–2.57 mg galanthamine equivalent (GALAE)/g). The twig of *C. laurifolius* also exerted the best antibutyrylcholinesterase (10.50 mg GALAE/g) and antityrosinase (73.15 mg kojic acid equivalent/g) activities. *C. creticus* leaves revealed toxicity toward the RAW cell line (cell viability reduced to 68.8%) and were not toxic to normal cells (S17). In conclusion, these three *Cistus* species were shown to be a rich source of bioactive compounds with the potential for future applications in the food, pharmaceutical, and cosmetic industries.

Key words: *Cistus* species, ethnomedicinal, antioxidant, enzyme inhibition, cytotoxicity

1. Introduction

Cistus (Cistaceae) is widely distributed in Europe, North Africa, the Middle East, and the Caucasus, mostly in maquis and garigue habitats. It is represented by 67 taxa (33 hybrids) worldwide and FIVE taxa (*C. creticus* L., *C. laurifolius* L., *C. monspeliensis* L., *C. parviflorus* Lam., and *C. salviifolius* L.) in Türkiye (Civeyrel et al., 2011; Güner et al., 2012; Szeremeta et al., 2018; Zalegh et al., 2021; Selvi et al., 2023;).

Cistus species are characterized by their woody stem, hard hairy leaves, white or pink/purple colored flowers, and trichomes that secrete a resin (ladano), which is responsible for their distinctive aromatic scent and appreciation in the perfume industry (Papaefthimiou et al., 2014).

Medicinal plants have been used for thousands of years to treat various diseases. *Cistus* species are also used in traditional medicine (Selvi et al., 2022; Selvi et al., 2023). The *Cistus* species considered in this study have ethnomedical uses among many rural populations. For example, *C. creticus* is used traditionally to treat sterility, ulcers, acne, and other skin disorders, as well as cuts, expectorant, constipation, and diabetes mellitus (Demirci Kayıran, 2023; Ozbekle, 2024). *C. laurifolius* is used to treat diabetes mellitus, rheumatism, and related inflammatory diseases (Yeşilada et al., 1997; Baytop, 1999), and *C. salviifolius* is traditionally used as an ointment or a cicatrizing or astringent agent (Baytop, 1999; Abdel-Massih and El Beyrouthy, 2022). Metabolomic analysis of *Cistus* species

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revealed the presence of terpenoids, mainly labdane-type diterpenes and clerodanes, and phenylpropanoids, including flavonoids and ellagitannins (Papaefthimiou et al., 2014; Zalegh et al., 2021). The essential oil composition of *Cistus* species revealed the presence of sesquiterpenes, mono- and diterpenes with carvacrol, manoyl oxide, 13-epi-manoyl oxide, drimane-7,9(11)-diene, α-cadinene, δ-cadinene, α-cadinol, α-zingiberene, α-curcumene, (E)-β-caryophyllene, α-bisabolol, germacrene D, camphor and viridiflorol as major compounds in many oils (Zalegh et al., 2021). *These Cistus* species' high polyphenolic compound content enables them to withstand different biotic and abiotic stresses (Dixon and Paiva, 1995). Pharmacologically, they have been shown to possess antibacterial, antifungal, antiviral, and anticancer activities (Papaefthimiou et al., 2014; Zalegh et al., 2021).

The efficacy of antioxidant defense systems in living organisms is diminished due to several factors like aging and an unhealthy lifestyle, thereby hastening the onset of life-threatening diseases such as cardiovascular disease, diabetes, and cancer, among others (Yerlikaya et al., 2017; Mocan et al., 2018; Mohammed et al., 2020). Various natural products have been proven effective in alleviating many diseases (Roy et al., 2021; Wasihun et al., 2023).

Studies have demonstrated that *C. creticus* possesses antitumor (Ozbekle et al., 2024; Skorić et al., 2012), antiborrelia (Rauwald, et al., 2019), antiviral (Kuchta et al., 2020), antityrosinase (Gaweł-Bęben et al., 2020), antioxidant, antimicrobial, and antifungal (Lahcen et al., 2020) activities. C. *laurifolius* was found to possess antiinflammatory (Pekacar et al., 2024; Yeşilada et al., 1997), analgesic (Ark et al., 2004), antioxidant (Sadhu et al., 2006), antihepatotoxic (Küpeli et al., 2006), antinociceptive (Küpeli and Yesilada, 2007), and anticholinesterase (Akkol et al., 2012) activities. Previous studies have shown that *C. salviifolius* possesses antioxidant (Qa'dan et al., 2006), cytotoxic (El Euch et al., 2015), antiinflammatory, analgesic (Sayah et al., 2017a), tyrosinase, elastase, α-amylase, and α-glucosidase inhibitory (Chiocchio et al., 2018; Sayah et al., 2017b) activities.

A prior study demonstrated that the chemical composition and, hence, the biological properties of *Cistus* species were highly affected by many habitat factors and environmental conditions. Besides, no *Cistus* monograph was available despite the long traditional use of many *Cistus* species and their implication in diverse medicinal purposes (Lukas et al., 2021). Thus, more in-depth investigations on *Cistus* species grown in different geographical regions are needed to provide guidelines for their pharmacological and nutraceutical applications. This study evaluated the chemical profile, antioxidant, enzyme inhibition, and cytotoxic properties of *C. creticus*, *C. laurifolius*, and *C. salviifolius* grown in Türkiye.

2. Materials and methods

2.1. Plant collection

In 2021, plant materials were gathered from western Anatolia in Türkiye. Detailed information on this area is provided below. Dr. Selami Selvi performed the taxonomic identification, and a voucher specimen was stored in the herbarium of Balıkesir University. Leaves and twigs were carefully separated, dried in the shade at room temperature, ground, and stored in darkness.

1. *C. creticus*: Türkiye; B1 Balıkesir: Edremit, Doyran village road, maquis, 39°34'38.79"N, 26°42'53.75"E, 97 m, 12.05.2021, SV 3405

2. *C. salviifolius*: Türkiye; B1 Balıkesir: Ayvalık, Sarımsaklı-Ayvalık road, roadsides, 39°17'34.55"N, 26°40'11.34"E, 56 m, 12.05.2021, SV 3412

3. *C. laurifolius*: Türkiye; B3 Afyon: Sultandağı, Yakasinek village, roadsides, 38°32'30.66"N, 31°10'14.47"E, 1329 m, 24.06.2021, SV 3452

2.2. Plant extract preparation

Methanol was used to prepare the extracts. Approximately 10 g of the sample was soaked in 200 mL of methanol for 24 h at room temperature. The methanol evaporated under reduced pressure, and the extracts were kept at 4 °C until further analysis.

2.3. Assay for total phenolic and flavonoid contents

Following the procedures specified by Slinkard and Singleton (1977), total phenolics and flavonoids were measured. Gallic acid (GA) and rutin equivalents (RE) were used as references in the experiments, with the results presented as GA equivalents (GAE) and RE.

2.4. UHPLC-MS/MS analysis

Analysis of different extracts was carried out on liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS) using a system in which a UHPLC (Dionex Ultimate 3000RS, Thermo Fisher Scientific, Waltham, MA, USA) system was equipped with a Mass Spectrometer (Q-Exactive Orbitrap, Thermo Fisher Scientific, Waltham MA, USA). All analytical details are given in the supplemental materials section.

2.5. Assays for in vitro antioxidant capacity

Antioxidant tests were performed following the methods described by Grochowski et al. (2017). The findings of the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), radical scavenging, cupric reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP) tests were quantified in milligrams of Trolox equivalents (TE) per gram of extract. As indicated by the phosphomolybdenum assay, the antioxidant potential was quantified in millimoles of TE per gram of extract. The metal chelating activity was expressed as milligrams of disodium edetate equivalents (EDTAE) per gram of extract.

2.6. Inhibitory effects against key enzymes

According to established protocols (Grochowski et al., 2017), enzyme inhibition experiments were conducted on the samples. Amylase and glucosidase inhibition were quantified in acarbose equivalents (ACAE) per gram of extract. In contrast, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition were indicated in milligrams of galanthamine equivalents (GALAE) per gram of extract. Tyrosinase (Tyr) inhibition was assessed in milligrams of kojic acid equivalents (KAE) per gram of extract.

2.7. Antimicrobial activity

In vitro tests were conducted to evaluate the antimicrobial activity of *Cistus* extracts against a panel of four bacterial strains, both gram-negative and gram-positive: *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Bacillus subtilis* (PeruMyc 6), and *Salmonella typhy* (PeruMyc 7). Additionally, these extracts were tested for antifungal properties against several yeast and dermatophyte species, including *Candida tropicalis* (YEPGA 6184), *C. albicans* (YEPGA 6379), *C. parapsilopsis* (YEPGA 6551), *Trichophyton mentagrophytes* (CCF 4823), *Trichophyton tonsurans* (CCF 4834), *Arthroderma quadrifidum* (CCF 5792), *Trichophyton mentagrophytes* (CCF 5930), *Arthroderma insingulare* (CCF 5417), and *Auxarthron ostraviense* (DB7).

Candida parapsilosis (ATCC 22019) and *C. krusei* (ATCC 6258) were used as quality control strains in the antifungal tests, adhering to the protocols in CLSI documents M27-A3, M38-A2, M27-S4, and supplement M61. The PeruMycA culture collection at the University of Perugia, Italy, maintains these voucher cultures and provides them upon request. The minimal inhibitory concentration (MIC) of the *Cistus* extracts was assessed within the 1.562–200 μg mL−1 range. Controls included Ciprofloxacin (Sigma) at 1.56–200 μg mL−1, Fluconazole (Sigma) at 0.063–16 μg mL−1, and Griseofulvin (Sigma) at 0.03–8 μg mL−1 (Pagiotti et al., 2011).

The MIC endpoints for *Cistus* extracts were determined by the lowest concentration, which showed no visible growth. For Ciprofloxacin, Fluconazole, and Griseofulvin, these endpoints were the weakest concentrations that inhibited 80% of growth relative to the control (Angelini et al., 2021; CLSI, 2008a).

2.8. Antibacterial/antifungal susceptibility testing

Antibacterial susceptibility testing was conducted to determine the MIC of *Cistus* extracts using a microdilution method following the Clinical and Laboratory Standards Institute M07-A9 protocol (CLSI, 2012a). Antifungal susceptibility testing for yeasts and filamentous fungi was conducted according to the guidelines specified in CLSI M27-A3 and M38-A2 protocols (CLSI, 2008a; CLSI, 2012b; CLSI, 2008b; CLSI, 2012a).

2.9. Cell culture

The HepG2, RAW 264.7, and S17 cell lines, representing human hepatocarcinoma, murine macrophages, and mouse bone marrow stromal cells, respectively, were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 2 mM L-glutamine (1%), and penicillin (50 U/mL)/ streptomycin (50 μg/mL) (1%), kept at 37 °C with 5% CO_2 in a humidified atmosphere.

2.10. Determination of cellular viability

Cells were seeded in 96-well plates at a density of 5×10^3 cells/well for HepG2 and S17 and 1×10^4 cells/well for RAW 264.7. After incubating overnight, the cells were treated with 100 μg/mL extracts for 72 h. Cells treated with 0.5% dimethylsulfoxide (DMSO) served as the control. Cellular viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, as previously described by Rodrigues et al. (2016). The percentage of cellular viability was calculated relative to the DMSO (0.5%) control.

3. Statistical analysis

The results were given as mean ± SD of the three parallel experiments. Differences in extract levels were assessed using ANOVA with Tukey's test ($p < 0.05$). GraphPad 9.0 was used for all analyses.

4. Results and discussion

4.1. Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid (TFC) content in the methanolic extracts of the leaves and twigs of *C. creticus, C. laurifolius,* and C. *salviifolius* were determined, and the results are depicted in Table 1. The TPC was between 36.09 and 97.08 mg GAE/g, with the highest significant ($p < 0.05$) value recorded from the leaves of C. *salviifolius.* The twigs of the three species and the leaves of *C. creticus* displayed comparable values (90.35–93.15 mg GAE/g; $p \geq 0.05$). The TFC was in the range of 8.10, and 49.60 mg RE/g, with the leaves of C. *salviifolius* showing the highest significant ($p < 0.05$) content, followed by the leaves of C. *laurifolius* and *C. creticus,* respectively. The leaves of the three species accumulated TFC more than twice that obtained from their respective twigs. These results indicate that the three species were rich in phenolic compounds, which aligns with previous studies (Orhan, 2013; Sayah et al., 2017).

4.2. Chemical characterization

The chemical profile of the three *Cistus* species was determined, and the results are presented in Tables 2–4. Seventy-eight compounds were detected in *C. creticus* and C. *laurifolius,* and 100 in C. *salviifolius.* Extracts of the three species revealed the presence of variable classes of

Table 1. Extraction yields (%) and total phenolic and flavonoid contents in methanolic extracts of leaves and twigs from three *Cistus* species.

Species	Parts	Extraction yields (%)	TPC (mg GAE/g)	TFC (mg RE/g)
Cistus creticus	Leaves	17.26	90.53 ± 0.12[b]	36.21 ± 0.16[c]
	Twigs	7.81	92.68 ± 1.87[b]	15.92 ± 0.36[d]
Cistus laurifolius	Leaves	17.27	36.09 ± 0.54[c]	37.66 ± 0.31[b]
	Twigs	11.93	90.88 ± 0.55[b]	14.07 ± 0.18[e]
Cistus salviifolius	Leaves	18.45	97.08 ± 1.08[a]	49.60 ± 0.38[a]
	Twigs	9.66	93.15 ± 0.87[b]	8.10 ± 0.25[f]

*Values are reported as mean ± SD of three parallel measurements. GAE: Gallic acid equivalents; RE: Rutin equivalents. Different superscripts indicate significant differences between the tested extracts ($p < 0.05$).

metabolites like flavonoids, tannins, coumarins, phenolic acids, fatty acids, and their glycosides and derivatives (Tables S1–S6). A higher concentration of flavonoids was observed after scrutinizing the biochemical levels across all extracts from the three species. Both *C. creticus* and *C. laurifolius* extract accumulated the highest number of flavonoid compounds (60%), followed by the leaf extracts of *C. salviifolius* (54%). The latter had a relatively high number of compounds belonging to tannins (26%), while the two other species showed a lower presence of tannin compounds (9%). Furthermore, punicalagin and isomers were detected in *C. salviifolius* and *C. criticus* but not in *C. laurifolius*. Phenolic acids and coumarins represented, respectively, 6%–9% and 3%–10% of the phytoconstituents in the three species. Lukas et al. (2021) proposed a classification of selected *Cistus* species, including *C. creticus* and *C. salviifolius*, into two main chemovariants: flavonol-rich, purple-flowered clade (*C. creticus*) and the more ellagitannin-rich, white- or whitish-pink-flowered clade (*C. salviifolius*). However, in the present study, *C. laurifolius* (white flower) is instead associated with the flavonol-rich chemovariant. Lukas et al.'s (2021) classification is based on the chemical profile of leaves' aqueous extracts, while methanol was used to prepare the extracts in the present study, and, therefore, the extraction solvent might have affected compound recovery (Hemmer et al., 2024). In addition, Lukas et al. (2021) reported that the separation of the purple-flowered and the white- and whitish-pink-flowered clade was not entirely perfect, and more investigations are needed, as many Italian, Croatian, and Cypriot accessions of *C. creticus* exhibited comparatively high percentages of punicalagin derivatives (tannins). Considering the distribution of the other metabolites, four diterpenes, namely isoabienol and manool or 13-epimanool, sclareol, and labda-7,14-dien-13-ol, in addition to abscisic acid (a sesquiterpenoid phytohormone), were only identified in the *C. creticus* extracts. The latter compound is characteristic of drought-resistant species, including *C. creticus* (Munné-Bosch et al., 2009). Furthermore, many labdane-type diterpenes were identified in *Cistus* species, and manoyl oxide and 13-epimanoyl oxide were observed in Cretan *C. creticus* subsp. *eriocephalus* leaves' extracts (Demetzos et al., 2002). Although these labdane-types diterpenes were not detected in the extracts of the other two species, the two compounds mentioned above, in addition to other diterpenes, were previously identified in *C. salviifolius* (Demetzos et al., 2002; Loizzo et al., 2013) and *C. laurifolius* (Teresa et al., 1986). This variation in results could be attributed to many factors, including diurnal, seasonal, ecological, drought, temperature, plant age, organ type, and the type of trichomes the organs contain (Papaefthimiou et al., 2014).

4.3. Antioxidant activity

Six complementary assays, including DPPH, ABTS, CUPRAC, FRAP, chelating, and total antioxidant activity, via phosphomolybdenum assay, were performed to evaluate the antioxidant properties of the three *Cistus* species. The results are depicted in Table 5. The antioxidant activity of the three species varied according to species, plant part, and antioxidant assays. The leaves of *C. salviifolius* exerted ($p < 0.05$) the highest radical scavenging (DPPH assay = 612.11 mg TE/g; ABTS = 804.66 mg TE/g), reducing ions (CUPRAC = 690.54 mg TE/g; FRAP = 459.34 mg TE/g), and chelating (15.58 mg EDTAE/g) activities. The twigs of the three species also displayed the highest total antioxidant activity via the phosphomolybdenum assay (3.30–3.48 mmol TE/g; $p \geq 0.05$). The twigs of *C. creticus* and *C. salviifolius* exhibited the second-best values ($p \geq 0.05$) in the DPPH and CUPRAC assays, while the twig of the former showed the second-best values in the ABTS and FRAP assays. However, the leaves of *C. creticus* and *C. laurifolius* recorded ($p \geq 0.05$) the second-best chelating capacity. Overall, the extracts of the three *Cistus* species displayed remarkable antioxidant activity. These results are

Table 2. Chemical composition in the extracts of *C. creticus*.

Compounds	leaves	twigs
Quinic acid	+	+
2,3-Hexahydroxydiphenoylglucose	+	+
Citric acid	+	+
Gallic acid (3,4,5-Trihydroxybenzoic acid)	+	+
Punicalin	+	-
5-O-Galloylquinic acid	+	+
Gallocatechin	+	+
Prodelphinidin B isomer 1	+	+
Galloylshikimic acid isomer 1	+	+
Galloylshikimic acid isomer 2	+	+
Procyanidin B	+	+
Punicalagin	+	-
Prodelphinidin B isomer 2	+	+
Uralenneoside	+	+
Catechin	+	+
Scopoletin-7-O-glucoside (Scopolin)	+	+
Caffeic acid	+	+
Fraxin (Fraxetin-8-O-glucoside)	+	+
Dihydrokaempferol-O-hexoside	+	+
Fraxetin (7,8-Dihydroxy-6-methoxycoumarin)	+	+
p-Coumaric acid	+	+
Scopoletin (7-Hydroxy-6-methoxycoumarin)	+	+
Taxifolin (Dihydroquercetin)	+	+
Ellagic acid-O-hexoside	+	+
Quercetin-O-dirhamnosylhexoside	+	+
Myricetin-3-O-glucoside (Isomyricitrin)	+	+
Myricetin-3-O-rutinoside	+	+
Myricetin-O-pentoside	+	+
Dihydrokaempferol (3,4',5,7-Tetrahydroxyflavanone)	+	+
Myricitrin (Myricetin-3-O-rhamnoside)	+	+
Hyperoside (Quercetin-3-O-galactoside)	+	+
Ellagic acid-O-pentoside	+	+
Rutin (Quercetin-3-O-rutinoside)	+	+
Ellagic acid	+	+
Myricetin (3,3',4',5,5',7-Hexahydroxyflavone)	+	+
Quercitrin (Quercetin-3-O-rhamnoside)	+	+
Eriodictyol (3′,4′,5,7-Tetrahydroxyflavanone)	+	+
Kaempferol-3-O-rutinoside (Nicotiflorin)	+	+
Isorhamnetin-3-O-glucoside	+	+
Abscisic acid	+	+
Isorhamnetin-3-O-rutinoside (Narcissin)	+	+
Afzelin (Kaempferol-3-O-rhamnoside)	+	+
Helichrysoside (Quercetin-3-O-[p-coumaroyl-(→6)glucoside])	+	+
Naringenin (4',5,7-Trihydroxyflavanone)	+	+
Quercetin (3,3',4',5,7-Pentahydroxyflavone)	+	+
Luteolin (3',4',5,7-Tetrahydroxyflavone)	+	+
Tiliroside (6"-O-trans-p-Coumaroylastragalin)	+	+

Table 2. (Continued.)

Quercetin-3-O-methyl ether	+	+
Methoxy-trihydroxy(iso)flavanone	+	+
Kaempferol (3,4',5,7-Tetrahydroxyflavone)	+	+
Isorhamnetin (3'-Methoxy-3,4',5,7-tetrahydroxyflavone)	+	+
Apigenin (4',5,7-Trihydroxyflavone)	+	+
Chrysoeriol (3'-Methoxy-4',5,7-trihydroxyflavone)	+	+
Methoxy-trihydroxy(iso)flavone isomer 1	+	+
Dimethoxy-trihydroxy(iso)flavone	+	+
Dihydroxy-methoxy(iso)flavanone	+	+
Rhamnetin (7-Methoxy-3,3',4',5-tetrahydroxyflavone)	+	+
Traumatic acid (2-Dodecenedioic acid)	+	+
Trihydroxy-trimethoxy(iso)flavone isomer 1	+	+
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	+	+
Methoxy-trihydroxy(iso)flavone isomer 2	+	+
Trihydroxy-trimethoxy(iso)flavone isomer 2	+	+
Dihydroxy-trimethoxy(iso)flavone isomer 1	+	+
Methoxy-trihydroxy(iso)flavone isomer 3	+	+
Methoxy-trihydroxy(iso)flavone isomer 4	+	+
Bisapigenin	+	+
Dihydroxy-methoxy(iso)flavone	+	+
Dihydroxy-trimethoxy(iso)flavone isomer 2	+	+
Dihydroxy-tetramethoxy(iso)flavone	+	+
Hydroxy-tetramethoxy(iso)flavone	+	+
Dimethoxy-hydroxy(iso)flavone	+	+
Hydroxy-trimethoxy(iso)flavone	+	+
Isoabienol	+	+
Manool or 13-Epimanool	+	+
α-Linolenic acid	+	+
Sclareol	+	+
Linoleic acid	+	+
Labda-7,14-dien-13-ol	+	+

+: present; -: absent.

Table 3. Chemical composition in the extracts of *C. salviifolius*.

Compounds	Leaves	twigs
Quinic acid	+	+
Hexahydroxydiphenoylglucose	+	+
Citric acid	+	+
Galloylglucose isomer 1	+	-
Galloylglucose isomer 2	+	-
Gallic acid (3,4,5-Trihydroxybenzoic acid)	+	+
Prodelphinidin B isomer 1	+	+
Punicalin	+	-
Galloylglucose isomer 3	+	-
Protocatechuic acid (3,4-Dihydroxybenzoic acid)	+	+
Prodelphinidin B isomer 2	+	+
Gallocatechin	+	+
1-O-(3-Hydroxy-5-methoxyphenyl)glucose	+	+

Table 3. (Continued.)

Prodelphinidin B isomer 3	+	+
Punicalagin isomer	+	-
Pedunculagin	+	-
Corilagin or isomer	+	-
Unidentified tannin isomer 1	+	-
Procyanidin B isomer 1	+	-
Flavogallonic acid dilactone or isomer	+	-
Punicalagin	+	-
Prodelphinidin B isomer 4	+	+
Uralenneoside	+	-
Procyanidin B isomer 2	+	+
Unidentified tannin isomer 2	+	-
Catechin	+	-
Epigallocatechin	+	-
Unidentified tannin isomer 3	+	+
Caffeic acid-O-hexoside	+	+
Procyanidin B isomer 3	+	+
Prodelphinidin B isomer 5	+	+
Caffeic acid	+	+
Dihydroxy-methoxycoumarin	+	-
3,4-Dihydroxyphenylacetone-3-O-glucoside	+	+
Epigallocatechin-3-O-gallate (Teatannin II)	+	+
Procyanidin B isomer 4	+	+
Dihydrokaempferol-4'-O-glucoside	+	+
Epicatechin	+	+
1-O-(3-Hydroxy-5-methoxyphenyl)-6-O-galloylglucose	+	+
Fraxetin (7,8-Dihydroxy-6-methoxycoumarin)	+	+
Gallocatechin-3-O-gallate	+	+
p-Coumaric acid	+	+
Vicenin-2 (Apigenin-6,8-di-C-glucoside)	+	-
Scopoletin (7-Hydroxy-6-methoxycoumarin)	+	+
Catechin-3-O-gallate	+	+
Taxifolin (Dihydroquercetin)	+	+
Myricetin-O-galloylhexoside	+	+
Ellagic acid-4-O-glucoside	+	+
Epicatechin-3-O-gallate	+	+
Myricetin-3-O-glucoside (Isomyricitrin)	+	+
Myricetin-3-O-rutinoside	+	+
Myricetin-3-O-xyloside	+	+
Quercetin-O-galloylhexoside	+	+
Myricetin-3-O-arabinofuranoside	+	+
Dihydrokaempferol (3,4',5,7-Tetrahydroxyflavanone)	+	+
Myricitrin (Myricetin-3-O-rhamnoside)	+	+
Quercetin-O-pentosylhexoside	+	+
Myricetin-3-O-arabinopyranoside	+	+
Myricetin-O-malonylhexoside	+	+
Hyperoside (Quercetin-3-O-galactoside)	+	+
Ellagic acid-O-pentoside	+	+

Table 3. (Continued.)

Rutin (Quercetin-3-O-rutinoside)	+	+
Eschweilenol C (Ellagic acid-4-O-rhamnoside)	+	-
Avicularin (Quercetin-3-O-arabinofuranoside)	+	+
Ellagic acid	+	+
Kaempferol-7-O-glucoside	+	+
Quercetin-O-malonylhexoside	+	+
Guaijaverin (Quercetin-3-O-arabinopyranoside)	+	+
Myricetin (Cannabiscetin, Myricetol, 3,3',4',5,5',7-Hexahydroxyflavone)	+	+
Quercitrin (Quercetin-3-O-rhamnoside)	+	+
Kaempferol-3-O-rutinoside (Nicotiflorin)	+	+
Isorhamnetin-3-O-glucoside	+	+
Ducheside A (3-O-Methylellagic acid-4'-O-xyloside)	+	+
Kaempferol-O-malonylhexoside	+	+
Quercetin-O-(p-coumaroyl)hexoside	+	+
Naringenin (4',5,7-Trihydroxyflavanone)	+	+
Quercetin (3,3',4',5,7-Pentahydroxyflavone)	+	+
Tiliroside (6"-O-trans-p-Coumaroylastragalin)	+	+
Quercetin-3-O-methyl ether	+	+
3"-O-trans-p-Coumaroylastragalin	+	+
Methoxy-trihydroxy(iso)flavanone	+	+
Kaempferol (3,4',5,7-Tetrahydroxyflavone)	+	+
Isorhamnetin (3'-Methoxy-3,4',5,7-tetrahydroxyflavone)	+	+
Apigenin (4',5,7-Trihydroxyflavone)	+	+
Isokaempferide (3-Methoxy-4',5,7-trihydroxyflavone)	+	+
Dimethoxy-trihydroxy(iso)flavone	+	+
Dihydroxy-methoxy(iso)flavanone	+	+
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	+	+
Pinocembrin (5,7-Dihydroxyflavanone)	+	+
Dihydroxy-trimethoxy(iso)flavone isomer 1	+	+
Pinellic acid (9,12,13-Trihydroxy-10E-octadecenoic acid)	+	+
Acacetin (5,7-Dihydroxy-4'-methoxyflavone)	+	+
Amentoflavone (3",8-Bisapigenin)	+	+
Genkwanin (4',5-Dihydroxy-7-methoxyflavone)	+	+
Ermanin (5,7-Dihydroxy-3,4'-dimethoxyflavone)	+	+
Dihydroxy-trimethoxy(iso)flavone isomer 2	+	+
5,7-Dihydroxy-3,3',4',8-tetramethoxyflavone (Gossypetin-3,3',4',8-tetramethyl ether)	+	+
Flindulatin (5-Hydroxy-3,4',7,8-tetramethoxyflavone)	+	+
Apigenin-4',7-dimethyl ether (4',7-Dimethoxy-5-hydroxyflavone)	+	+
Kaempferol-3,4',7-trimethyl ether (5-Hydroxy-3,4',7-trimethoxyflavone)	+	+

+: present; -: absent.

Table 4. Chemical composition in the extracts of *C. laurifolius*.

Compounds	Leaves	Twigs
Quinic acid	+	+
Gallic acid (3,4,5-Trihydroxybenzoic acid)	+	+
Gallocatechin	+	+
Prodelphinidin B	+	+
Uralenneoside	-	+

Table 4. (Continued.)

Esculin (Esculetin-6-O-glucoside)	+	+
3-O-(p-Coumaroyl)quinic acid	+	+
Catechin	-	+
Epigallocatechin	+	+
Magnolioside (Isoscopoletin-6-O-glucoside)	+	+
Scopolin (Scopoletin-7-O-glucoside)	+	+
Esculetin (6,7-Dihydroxycoumarin)	+	+
3-O-Feruloylquinic acid	+	+
Caffeic acid	+	+
Fraxetin-O-hexoside	+	+
Ellagic acid-4,4'-di-O-glucoside	-	+
Epigallocatechin-3-O-gallate (Teatannin II)	+	+
Epicatechin	-	+
Fraxetin (7,8-Dihydroxy-6-methoxycoumarin)	+	+
4-O-(p-Coumaroyl)quinic acid	+	+
Isoscopoletin (6-Hydroxy-7-methoxycoumarin)	+	+
Gallocatechin-3-O-gallate	+	+
p-Coumaric acid	+	+
Scopoletin (7-Hydroxy-6-methoxycoumarin)	+	+
Hydroxy-dimethoxycoumarin isomer 1	+	+
Ferulic acid	+	+
Ellagic acid-4-O-glucoside	-	+
Isoferulic acid	+	-
Hydroxy-dimethoxycoumarin isomer 2	+	+
Myricetin-3-O-glucoside (Isomyricitrin)	+	+
Myricetin-3-O-rutinoside	+	+
Scoparone (6,7-Dimethoxycoumarin)	+	+
Myricetin-O-pentoside	+	+
Dihydrokaempferol (3,4',5,7-Tetrahydroxyflavanone)	+	+
Myricitrin (Myricetin-3-O-rhamnoside)	+	+
Hyperoside (Quercetin-3-O-galactoside)	+	+
Rutin (Quercetin-3-O-rutinoside)	+	+
Eschweilenol C (Ellagic acid-4-O-rhamnoside)	-	+
Quercetin-O-pentoside	+	+
Ellagic acid	+	+
Myricetin (3,3',4',5,5',7-Hexahydroxyflavone)	+	+
Quercitrin (Quercetin-3-O-rhamnoside)	+	+
Kaempferol-3-O-rutinoside (Nicotiflorin)	+	+
Naringenin (4',5,7-Trihydroxyflavanone)	+	+
Quercetin (3,3',4',5,7-Pentahydroxyflavone)	+	+
Luteolin (3',4',5,7-Tetrahydroxyflavone)	+	+
Tiliroside (6"-O-trans-p-Coumaroylastragalin)	+	+
Quercetin-3-O-methyl ether	+	+
Kaempferol-O-(p-coumaroyl)hexoside	+	+
Methoxy-trihydroxy(iso)flavanone	+	+
Kaempferol (3,4',5,7-Tetrahydroxyflavone)	+	+
Isorhamnetin (3'-Methoxy-3,4',5,7-tetrahydroxyflavone)	+	+
Apigenin (4',5,7-Trihydroxyflavone)	+	+
Chrysoeriol (3'-Methoxy-4',5,7-trihydroxyflavone)	+	+

Table 4. (Continued.)

Isokaempferide (3-Methoxy-4',5,7-trihydroxyflavone)	+	+
Quercetin-3,3'-dimethyl ether (3,3'-Dimethoxy-4',5,7-trihydroxyflavone)	+	+
Rhamnetin (7-Methoxy-3,3',4',5-tetrahydroxyflavone)	+	+
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	+	+
Pinocembrin (5,7-Dihydroxyflavanone)	+	+
Dihydroxy-methoxyflavone	+	+
Luteolin-7-O-methyl ether (7-Methoxy-3',4',5-trihydroxyflavone)	+	+
Quercetin-3,7-dimethyl ether (3,7-Dimethoxy-3',4',5-trihydroxyflavone)	+	+
Quercetin-3,3',5-trimethyl ether (4',7-Dihydroxy-3,3',5-trimethoxyflavone)	+	+
Methoxy-trihydroxy(iso)flavone	+	+
Bisapigenin	+	+
Genkwanin (Apigenin-7-O-methyl ether, 4',5-Dihydroxy-7-methoxyflavone)	+	+
Luteolin-3',7-dimethyl ether (4',5-Dihydroxy-3',7-dimethoxyflavone)	+	+
Quercetin-3,3',7-trimethyl ether (4',5-Dihydroxy-3,3',7-trimethoxyflavone)	+	+
Kaempferol-3,7-dimethyl ether (4',5-Dihydroxy-3,7-dimethoxyflavone)	+	+
Dihydroxy-tetramethoxy(iso)flavone	+	+
Eriodictyol (3′,4′,5,7-Tetrahydroxyflavanone)	+	-
Dihydroxykaurenal isomer 1	+	+
Quercetin-3',4',7-trimethyl ether (3,5-Dihydroxy-3',4',7-trimethoxyflavone)	+	+
Dihydroxykaurenal isomer 2	+	+
Retusin (Quercetin-3,3',4',7-tetramethyl ether)	+	+
Apigenin-4',7-dimethyl ether (4',7-Dimethoxy-5-hydroxyflavone)	+	+
Kaempferol-3,4',7-trimethyl ether (5-Hydroxy-3,4',7-trimethoxyflavone)	+	+
Hydroxykaurenal	+	+
Hydroxykauranal isomer 1	+	+
Dihydroxykauranal	+	+
Hexadecanedioic acid	+	+
Hydroxyoctadecadienoic acid	+	+
Hydroxyhexadecanoic acid	+	+
Hydroxykauranal isomer 2	+	+
Linoleic acid	+	+
Oleic acid	+	+
Lignoceric acid (Tetracosanoic acid)	+	+

+: present; -: absent.

Table 5. Antioxidant properties of methanolic extracts of leaves and twigs from three *Cistus* species.

Species	Parts	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	Chelating (mg EDTAE/g)	PBD (mmol TE/g)
Cistus creticus	Leaves	479.17 ± 1.14^{c}	632.73 ± 10.81^{d}	531.76 ± 0.53^{c}	322.71 ± 3.50^{e}	11.27 ± 0.31^{b}	3.05 ± 0.11^{ab}
	Twigs	504.29 ± 15.71^{b}	743.15 ± 20.62^{b}	592.28 ± 6.65^{b}	396.30 ± 1.16^{b}	8.79 ± 0.31^{c}	3.30 ± 0.29^{a}
Cistus salviifolius	Leaves	612.11 ± 4.03^{a}	804.66 ± 8.20^{a}	690.54 ± 17.45^{a}	459.34 ± 3.34^{a}	15.58 ± 0.42^{a}	3.48 ± 0.25^{a}
	Twigs	507.28 ± 2.90^{b}	688.76 ± 14.66^{c}	590.02 ± 13.51^{b}	373.02 ± 8.18^{c}	6.45 ± 0.35^{d}	3.48 ± 0.21^{a}
Cistus laurifolius	Leaves	55.59 ± 0.55^{e}	80.77 ± 0.26^{e}	107.12 ± 3.36^{d}	75.35 ± 0.34^{f}	11.60 ± 0.49^{b}	2.48 ± 0.03^{b}
	Twigs	459.25 ± 5.27^{d}	647.63 ± 18.42^{d}	525.64 ± 7.59^{c}	352.76 ± 2.39^{d}	7.23 ± 0.36^{d}	3.41 ± 0.27^{a}

*Values are reported as mean ± SD of three parallel measurements. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 1,1-diphenyl-2-picrylhydrazyl; CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power; PBD: Phosphomolybdenum; MCA: Metal chelating Activity; TE: Trolox Equivalent; EDTAE: EDTA equivalent. Different superscripts indicate significant differences between the tested extracts ($p < 0.05$).

in line with Lukas et al.'s findings (2021); the authors of that study observed that the white and whitish-pink-flowered clade (*C. salviifolius* = 264 mg TE/g dry weight) displayed higher antioxidant activity than the purple-flowered clade (*C. creticus* = 170 mg TE/g dry weight). Additionally, the antioxidant activity of different extracts correlated well with their TPC. Compounds like hexahydroxydiphenoyl-glucose, gallocatechin, GA catechin, m-3-O-rhamnoside, and rutin(quercetin-3-O-rutinoside) were responsible for the antioxidant activity of *Cistus* species rather than punicalagin derivatives (moderate activity), other quercetin glycosides (weak activity), or myricetin glycosides (not active) (Lukas et al., 2021; Nur Onal et al., 2023). Nevertheless, the three investigated *Cistus* species exerted potent antioxidant activity and can be considered a valuable source of antioxidant agents.

4.4. Enzyme inhibitory activity

The methanolic extracts of the leaves and twigs of the three *Cistus* species were evaluated for their capacity to inhibit the AchE, BChE, Tyr, α-amylase, and α-glucosidase enzymes. The results are shown in Table 6. The twigs of the three *Cistus* species and the leaves of *C. salviifolius* displayed a comparable inhibitory effect against the AChE enzyme (2.48–2.51 mg GALAE/g; $p \geq 0.05$). In comparison, the leaves of the other two species were not effective. Interestingly, all organs of the three species exerted remarkable anti-BChE activity (5.59–10.50 mg GALAE/g) in the following descending order: *C. laurifolius* twigs > *C. salviifolius* twigs > *C. creticus* leaves = twigs > *C. salviifolius* leaves > *C. laurifolius* leaves. A previous study on the cholinesterase inhibitory activity was performed on essential oils of *C. creticus* and *C. salviifolius* leaves. The results revealed that the oil of the two species exerted potent anti-BChE activity (IC_{50} values of 29.1 and 34.2 µg/mL). In contrast, only the latter plant's oil showed anti-AChE activity (IC_{50} 58.1 µg/mL). It was suggested that phenolics and terpenes played an essential role in the neuroprotective effect of these species (Loizzo et al., 2013). In the current study, among all extracts, *C. laurifolius* twigs had the highest enzyme inhibitory activity against Tyr. Its leaves revealed the least activity (73.15 and 50.45 mg KAE/g, respectively), while the twigs of the other two species, in addition to the leaves of *C. salviifolius*, showed comparable anti-Tyr effects (70.91–71.37 mg KAE/g; $p \geq 0.05$). A prior study revealed that the hydromethanolic extract from the aerial parts of C. *salviifolius* significantly inhibited the Tyr enzyme (61%) at a concentration of 50 µg/mL (Chiocchio et al., 2018). Concerning the enzymes influencing the blood glucose level, the best α-amylase inhibitory activity was recorded from the *C. salviifolius* twigs (0.67 mmol ACAE/g), followed by its leaves and both organs of *C. creticus,* which exerted a similar effect (0.65 mmol ACAE/g). The two organs of *C. salviifolius* displayed comparable α-glucosidase inhibitory activity (1.06 and 1.10 mmol ACAE/g, $p \geq 0.05$), followed by the twigs of the other two *Cistus* species (1.05 and 1.04 mmol ACAE/g; $p \geq 0.05$). The antidiabetic activity of these *Cistus* species was previously reported. The aqueous and methanolic extracts from the aerial parts of *C. salviifolius* were previously found to exert α-glucosidase (IC_{50} 0.95 and 8.47 µg/mL, respectively) and α-amylase (IC_{50} 217.10 and 597.10 µg/mL, respectively) inhibitory activity (Sayah et al., 2017). The ethanolic extract of *C. laurifolius* leaves displayed an α-glucosidase inhibitory effect (IC50 = 6.3 μg/mL) and a dose-dependent inhibitory effect on α-amylase (Orhan et al., 2013).

4.5. Antimicrobial activity

The MIC values of extracts from *Cistus* species against bacteria, yeasts, and dermatophytes, determined using the broth microdilution method, are detailed in Tables 7–9. All extracts demonstrated antimicrobial activity at concentrations ranging from 1.562 to 200 µg/mL. Specifically, the extracts from samples 9-CSL, 10-CST, and 12-CLT were the most effective and exhibited the lowest MIC values. These extracts were particularly effective

Table 6. Enzyme inhibitory properties of methanolic extracts of leaves and twigs from three *Cistus* species.

Species	Parts	AChE (mg GALAE/g)	BChE (mg GALAE/g)	Tyrosinase (mg KAE/g)	Amylase (mmol ACAE/g)	Glucosidase (mmol ACAE/g)
Cistus creticus	Leaves	na	6.94 ± 0.91^{bc}	65.54 ± 4.60^{b}	0.65 ± 0.04^{ab}	0.99 ± 0.05^{b}
	Twigs	2.57 ± 0.02^{a}	6.94 ± 0.92^{bc}	71.37 ± 1.13^{ab}	0.65 ± 0.03^{ab}	1.05 ± 0.01^{ab}
Cistus laurifolius	Leaves	Na	5.59 ± 0.23^{c}	50.45 ± 1.35^{c}	0.60 ± 0.02^{b}	0.78 ± 0.03^{c}
	Twigs	2.50 ± 0.05^{a}	10.50 ± 1.57^{a}	73.15 ± 0.48^{a}	0.60 ± 0.02^{b}	1.04 ± 0.02^{ab}
Cistus salviifolius	Leaves	2.51 ± 0.11^{a}	6.19 ± 0.31^{c}	70.91 ± 2.00^{ab}	0.65 ± 0.01^{ab}	1.06 ± 0.02^{a}
	Twigs	2.48 ± 0.03^{a}	9.28 ± 0.65^{ab}	70.93 ± 1.32^{ab}	0.67 ± 0.01^{a}	1.10 ± 0.01^{a}

**Values are reported as mean ± SD of three parallel measurements. AChE: acetylcholinesterase; BChE: butyrylcholinesterase; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different superscripts indicate significant differences between the tested extracts ($p < 0.05$).

against *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 15442), with MICs ranging from 6.25–12.5 µg/mL (GM, 9.92 µg/mL) and 6.25–12.5 µg/mL (GM, 7.87 µg/mL), respectively. In contrast, *Bacillus subtilis* (PeruMycA 6) and *Salmonella typhi* (PeruMyc 7) showed limited sensitivity to most extracts (Table 7).

The study also found that gram-positive bacteria were generally more susceptible to *Cistus* spp extracts than gram-negative bacteria. Notably, gram-negative strains such as S. typhi (PeruMycA7) exhibited less sensitivity to these plant extracts than gram-positive strains. These findings align with other studies suggesting that gram-positive bacteria are generally more vulnerable to various plant extracts (Álvarez-Martínez et al., 2021; Koohsari et al., 2015).

Moreover, the 9-CLS and 10-CST extracts were effective against *Candida parapsilosis* (YEPGA 6551) and *Candida albicans* (YEPGA 6184), with MIC values ranging from 25–50 µg/mL and a geometric mean (GM) of 39.68 µg/mL (Table 8). All tested extracts were also effective in inhibiting the growth of dermatophytes. Among them, *Arthroderma quadrifidum* (CCF 5792), *Arthroderma tonsurans* (CCF 4834), and *Auxarthron ostraviense* (DB7) were the most susceptible, with MIC values between 31.50 and 39.68 µg/mL (Table 9).

For comparison, the MIC values for standard antibiotics such as ciprofloxacin, fluconazole, and griseofulvin against *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were consistent with established ranges (CLSI, 2008b). While conventional antibiotics typically have MICs ranging from 0.01 to 10 µg/mL, plant-derived compounds are often classified as antimicrobials when their MICs range from 100 to 1000 µg/mL. However, it is erroneous to claim positive activity at excessively high concentrations. According to Rios et al. (2005), a significant MIC for antimicrobial activity should be below 100 µg/mL for plant extracts and below 10 µg/mL for isolated compounds. Taguri et al. (2006) proposed that MIC values under 400 µg/mL indicate a strong antimicrobial effect, values ranging from 400–800 µg/mL indicate a moderate impact, and values above 800 µg/mL indicate a weak effect.

4.6. Cytotoxic effects

The cytotoxic effect of methanolic extracts from the three *Cistus* species was evaluated against human hepatocarcinoma (HepG2), murine macrophages (RAW 264.7), and normal mouse bone marrow stromal (S17) cell lines. Extracts were tested at 100 µg/mL, and the results are expressed as a percentage of cellular viability (%) relative to the control containing 0.5 % DMSO (Table 10). The leaves of *C. laurifolius* exerted the highest cytotoxic effect against the three tested cell lines, with remarkable cytotoxicity toward S17 (cell viability = 21.5%). Its effect against the other two cell lines was comparable (cell viability of RAW = 44.6% and HepG2 = 41%). The cell viability of S17 was also reduced upon treatment with extracts of *C. salvifolius* leaves (34.8%) and twigs (37.9%), as well as that of *C. laurifolius* twigs (36.9%). However, the leaf and twig methanolic extracts of *C. creticus* were ineffective against S17 and HepG2 cell lines and showed some toxicity toward the RAW cell line (cell viability = 68.8% and 74.9%). For a drug to be cytotoxic, cell viability should not exceed 70% (ISO 10993–5:2009(E)). Thus, the methanolic extract of *C. creticus* leaves was a promising candidate for further toxicity tests as it was not toxic to normal cells and exhibited considerable toxicity toward the RAW cell line. Phenolics like punicalagin (Subkorn et al., 2021), quercetin, kaempferol, isorhamnetin, and their derivatives (Davì et al., 2023), ellagitannin, (Liberal et al., 2019) exert significant

Table 7. Minimal inhibitory concentrations (MICs) of the tested extracts against bacteria isolates.

	MIC (µg/mL)			
	Escherichia coli (ATCC 10536)	*Pseudomonas aeruginosa* (ATCC 15442)	*Bacillus subtilis* (PeruMycA 6)	*Salmonella typhi* (PeruMycA 7)
MeOH extracts				
7-CCL	15.75	9.92	125.99	79.37
8-CCT	9.92	15.75	62.99	125.99
9-CSL	9.92	31.50	39.68	125.99
10-CST	7.87	9.92	62.99	158.74
11-CLL	39.68	62.99	62.99	>200
12-CLT	7.87	15.75	31.50	>200
Ciprofloxacin (µg/mL)	31.49	125.99	125.99	79.37

*MIC values are reported as geometric means of three independent replicates (n = 3).
MIC range concentrations are reported within brackets.

Table 8. Minimal inhibitory concentrations (MICs) of the tested extracts against yeast isolates.

	MIC (µg/mL) *		
Yeast strain	*Candida tropicalis* (YEPGA 6184)	*Candida albicans* (YEPGA 6379)	*Candida parapsilosis* (YEPGA 6551)
MeOH extracts			
7-CCL	158.74	>200	125.99
8-CCT	79.37	125.99	62.99
9-CSL	>200	>200	**39.68**
10-CST	62.99	**39.68**	>200
11-CLL	>200	>200	>200
12-CLT	158.74	>200	>200
Fluconazole (µg/mL)	2	1	4

Table 9. Minimal inhibitory concentrations (MICs) of *Cistus monspelialis* and *C. parviflorus* extracts against dermatophyte isolates.

	MIC (µg/mL)*					
Dermatophyte	*Trichophyton mentagrophytes* (CCF 4823)	*Trichophyton tonsurans* (CCF 4834)	*Arthroderma quadrifidum* (CCF 5792)	*Arthroderma insingulare* (CCF 5417)	*Trichophyton mentagrophytes* (CCF 5930)	*Auxarthron ostraviense* DB7
MeOH extracts						
7-CCL	125.99	125.99	62.99	79.37	79.37	62.99
8-CCT	>200	125.99	79.37	62.99	158.74	125.99
9-CSL	125.99	62.99	39.68	39.68	79.37	62.99
10-CST	>200	62.99	31.50	>200	125.99	79.37
11-CLL	>200	79.37	62.99	62.99	158.74	39.68
12-CLT	158.74	39.68	158.74	62.99	158.74	125.99
Griseofulvin (µg/mL)	2.52	0.198	>8	>8	3.174	3.17

Table 10. Cytotoxicity of methanolic extracts of leaves and twigs from three *Cistus* species.

	Parts	RAW	HepG2	S17
0.5 % DMSO		87.7 ± 5.5	99.7 ± 5.7	99.3 ± 7.2
Cistus creticus	Leaves	68.8 ± 1.9	102 ± 9	112 ± 10
	Twigs	74.9 ± 2.4	91.2 ± 7.7	117 ± 10
Cistus laurifolius	Leaves	44.6 ± 2.3	41.0 ± 0.9	21.5 ± 0.4
	Twigs	56.9 ± 2.4	76.7 ± 4.1	36.9 ± 0.3
Cistus salvifolius	Leaves	60.7 ± 2.3	86.6 ± 5.5	34.8 ± 1.6
	Twigs	63.3 ± 2.7	86.2 ± 3.9	37.9 ± 0.9

Extracts were tested at 100 µg/mL, and results are expressed as a percentage of cellular viability (%) relative to the control containing 0.5 % DMSO. Values represent the mean ± standard error of the mean.

cytotoxicity. Thus, more studies using different extraction methods and solvents, as well as fractionation of crude extracts to alleviate the antagonistic effect (if present), are recommended to collect more information on the cytotoxic properties of the three *Cistus* species. Indeed, many studies have revealed the cytotoxic effect of these three species on other cell lines. For example, recent studies showed that extracts from *C. laurifolius* inhibited human cervical adenocarcinoma cells, human muscle rhabdomyosarcoma cells, and mouse fibrosarcoma cells (Wehi164) (Soydam Aydın et al., 2021), A549, DU-145, PNT-1A, MDA-MB231, CRL-4010, and HCT-116 (Budak et al., 2022), pancreatic MIA PaCA-2 (Guzelmeric et al., 2023) and breast MCF-7 (Yücel et al., 2024) cancer cell lines. The flower bud extract of *C. salvifolius* was shown to exert higher toxicity than the leaf against OVCAR and MCF-7 ovarian cancer cells (El Euch et al., 2015). *C. creticus* revealed a cytotoxic effect against 14 lines of human leukemic (Dimas et al., 1998), human prostate (Vitali et al., 2011), and cervical cancer (HeLa), breast cancer (MDA-MB-453), and melanoma (FemX) (Skorić et al., 2012) cell lines.

5. Conclusion

This study is an in-depth investigation of the chemical profile and biological activity of *C. creticus, C. laurifolius,* and *C. salviifolius* grown in Türkiye. Extracts contained variable metabolites like flavonoids, tannins, coumarins, phenolic acids, and fatty acids, with the leaves of *C. salviifolius* accumulating the highest total phenolic and flavonoid contents. The three *Cistus* species' antioxidant, enzyme inhibitory, and cytotoxic activities varied according to species and plant part. The three plants possess potent antioxidant activity, with the highest effect recorded from the leaves of *C. salvifolius*. They also showed enzyme inhibitory activity with remarkable cholinesterase and tyrosinase inhibitory activity exerted by C. *laurifolius* twigs and α-glucosidase and α-amylase inhibition by *C. salvifolius*. Among the tested extracts, *C. creticus* leaves were considered the most promising candidate for anticancer research. These leaves exhibited considerable toxicity toward the RAW cell line and were not toxic to normal cells (S17). These findings reinforce the potential of *Cistus* species as an essential source of bioactive compounds for different pharmaceutical, cosmetic, and food applications. The quantification and isolation of bioactive compounds, as well as their mechanism of action, are recommended.

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