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ORIGINAL ARTICLE

Integrated Botanical and Phytochemical Characterization of *Santolina chamaecyparissus* L. and Evaluation of its Antimicrobial Activity

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ABSTRACT

Objectives: *Santolina chamaecyparissus* L. is an aromatic medicinal plant with reported phytochemical and biological potential. This study aimed to characterize the botanical and phytochemical features of *S. chamaecyparissus* and to perform a preliminary screening of the antimicrobial activity of its methanolic extract against selected bacterial and fungal microorganisms.

Materials and Methods: In this experimental laboratory study, the morphological and anatomical characteristics of the aerial parts were examined using standard pharmacognostic, histological, and scanning electron microscopic methods. The essential oil was obtained by hydrodistillation, and the methanolic extract was prepared by maceration with 90% methanol. Volatile constituents were analyzed by gas chromatography–tandem mass spectrometry, while phenolic compounds were identified and quantified by liquid chromatography-tandem mass spectrometry. Preliminary antimicrobial activity was screened against five bacterial and two fungal strains using the agar well diffusion method.

Results: The plant exhibited characteristic xeromorphic features, including amphistomatic and isobilateral leaves, multilayered palisade parenchyma, anomocytic stomata, and dense non-glandular trichomes. The essential oil yield was 0.46%, and 33 volatile constituents were identified. Artemisia ketone (55.96%) and camphor (20.84%) were the major components. The methanolic extract yield was 14.40%, with chlorogenic acid (6733.85 µg/g dry extract) and quinic acid (6521.37 µg/g dry extract) identified as the predominant quantified compounds. The extract showed its strongest antimicrobial activity against *Staphylococcus aureus*, with inhibition zones of 8.00, 11.00, and 11.66 mm at concentrations of 5, 10, and 20 mg/mL, respectively. More limited activity was observed against *Enterobacter hormaechei* and *Candida albicans*, while the effects against the remaining microorganisms were weak or close to baseline.

Conclusion: *S. chamaecyparissus* possesses distinctive botanical features and a chemically rich profile characterized by an artemisia ketone- and camphor-dominant essential oil and a chlorogenic acid- and quinic acid-rich methanolic extract. The preferential activity against *S. aureus* suggests selective anti-gram-positive potential. However, the limited spectrum of activity and the absence of minimum inhibitory and bactericidal concentration analyses restrict the direct interpretation of its antimicrobial efficacy. Further studies using quantitative antimicrobial methods and bioactivity-guided fractionation are warranted.

Keywords: Volatile constituents, phytochemicals, plant extracts, antimicrobial agents, plant anatomy

INTRODUCTION

Medicinal and aromatic plants continue to be a major source of structurally diverse natural products with well-recognized pharmaceutical potential. In particular, plant secondary metabolites such as terpenoids, phenolics, and alkaloids have attracted considerable attention because of their broad-

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spectrum antimicrobial properties and their potential use as alternatives or adjuncts to conventional antibiotics in the era of antimicrobial resistance.[1,2] *Santolina chamaecyparissus* L. (Asteraceae), commonly known as cotton lavender, is a small evergreen aromatic shrub native to the Mediterranean region and widely cultivated throughout Europe and other areas.[3] Ethnobotanical reports indicate that *S. chamaecyparissus* and closely related *Santolina* taxa have long been used in Mediterranean folk medicine, particularly as vermifuge and emmenagogue preparations and for digestive, dermatological, and respiratory complaints.[4,5] Previous studies have mostly addressed its essential-oil composition, selected biological activities, or micromorphological features separately. Essential-oil studies have reported variable profiles containing compounds such as camphor, 1,8-cineole, borneol, β-pinene, and artemisia ketone, while biological studies have mainly focused on antioxidant, anti-inflammatory, antibacterial, antifungal, and cytotoxic activities. In particular, the essential oil of this species has demonstrated antibacterial and antifungal effects against selected clinically relevant microorganisms.[3,4,6,7] However, integrated pharmacognostic and phytochemical assessments combining morphological, anatomical, chemical, and antimicrobial evaluations remain limited. Additionally, significant variation in essential oil profiles among different plant populations and organs suggests that phytochemical expression may be influenced by structural and developmental factors.[4,6] Recent evidence has shown that *S. chamaecyparissus* possesses distinct secretory structures, including biseriate glandular trichomes and secretory ducts, which may play a direct role in the production and accumulation of bioactive metabolites.[8] Therefore, this study aimed to provide an integrated pharmacognostic and phytochemical evaluation of *S. chamaecyparissus* by characterizing its morphological and anatomical features, analyzing the volatile profile of its essential oil and the phenolic composition of its methanolic extract, and performing a preliminary screening of antimicrobial activity of the methanolic extract against selected bacterial and fungal strains.

MATERIALS AND METHODS

Plant material

Aerial parts of *S. chamaecyparissus* were collected during the flowering period on 11th June of 2025 from the Medical and Aromatic Plants Garden of the Faculty of Pharmacy, Inonu University, Malatya, Türkiye (Figure 1A). The taxonomic identity of the species was confirmed by Botanist Prof. Dr. Turan Arabacı, and a voucher specimen was deposited in the herbarium of Inonu University Faculty of Pharmacy under voucher number RO2501 (Figure 1B). The plant material was cleaned, shade-dried at room temperature, and powdered before further analyses.

Morphological and anatomical investigations

For morphological evaluation, dried aerial parts of *S. chamaecyparissus* were examined macroscopically using standard pharmacognostic criteria. Diagnostic characters were documented photographically and compared with previously published descriptions and the book of Flora of Turkey and the East Aegean Islands (Grierson).[9] For anatomical analysis, representative samples from stems and leaves were fixed in 70% ethanol and manually dissected. This workflow is consistent with routine plant histological approaches widely used in anatomical characterization and with previous anatomical work on *S. chamaecyparissus* and related taxa.[8] Photographs were taken using a Leica DM1000 light microscope equipped with a Leica DFC290 digital camera. For SEM examination, dried leaf pieces were mounted on stubs using double-sided adhesive tape and coated with gold using a BALTEC SCD-050 sputter coater. SEM observations of the leaf surface were then performed using a Leo Evo 40 scanning electron microscope at the Inonu University Scientific and Technological Research Center, Malatya, Türkiye.

Essential oil isolation

The powdered dried aerial parts (100 g) of *S. chamaecyparissus* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. After completion of distillation, the obtained essential oil was collected and stored in sealed amber vials at 4 °C until analysis.

GC–MS/MS analysis of the essential oil

The volatile profile of the essential oil was analyzed at the Research Laboratory Application and Research Center of Iğdır University (Iğdır, Türkiye) using an Agilent Technologies 7000 GC–MS Triple Quad system coupled to a 7890 GC and 7693 autosamplers. Chromatographic separation was achieved on an HP-5ms capillary column [(5%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm × 0.25 µm]. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injection volume was 1 µL in split mode (50:1), using hexane as the solvent. The septum purge flow was set at 3 mL/min. The GC transfer line temperature was maintained at 250 °C, and the quadrupole temperature was set at 150 °C. The oven temperature program was as follows: initial temperature 60 °C, held for 4 min; increased at 4 °C/min to 220 °C; and held for 10 min, giving a total run time of

Figure 1. (A) General view of *Santolina chamaecyparissus*, (B) the herbarium specimen.

54 min. Mass spectrometric detection was performed with an inlet temperature of 250 °C and a scan range of 35–550 amu. Compound annotation was based on comparison of EI mass spectra with the NIST MS library and, where applicable, supported by comparison with literature retention index data commonly used in essential-oil GC–MS analysis.[10] No authentic standards were analyzed; therefore, compound identities should be regarded as putative/tentative annotations based primarily on EI-MS library matching supported, where available, by literature RI agreement.

Preparation of the extract

The powdered dried aerial parts of *S. chamaecyparissus* (30 g) were extracted with 90% methanol at a 1:10 (w/v) plant material-to-solvent ratio. Extraction was performed at room temperature under continuous magnetic stirring for 24 h and repeated three times. The combined filtrates were concentrated, and the solvent was completely removed under reduced pressure using a rotary evaporator. The resulting dry extract was stored at 4 °C until further use.

LC–MS/MS analysis of phenolic compounds

The LC–MS/MS analysis for phenolic antioxidant profiling of *S. chamaecyparissus* methanol extract (SME) was performed at the Atatürk University Eastern Anatolia High Technology Application and Research Center.[11] For analysis, the dried methanolic extract was dissolved at a concentration of 1 mg/mL in the extraction solvent. Accordingly, quantitative results were normalized to dry extract weight and expressed as µg/g of dry extract. Chromatographic analyses were carried out using an Agilent Technologies 1290 Infinity UPLC system (Palo Alto, CA, USA) equipped with an autosampler (Agilent 1260 Infinity G1329B ALS), a high-pressure binary pump (Agilent 1260 Infinity G1312B, 600 bar), a degasser (Agilent 1260 Infinity G4225A HIP), and a thermostatted column compartment (Agilent 1290 Infinity TCC G1316C). Separation of phenolic constituents was achieved on a Zorbax SB-C18 column (3.5 µm, 100 mm × 4.6 mm), maintained at 30 °C. The mobile phase consisted of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid, and elution was performed using a gradient program. Phenolic compounds were identified and quantified according to retention behavior and mass spectral data in comparison with reference standards and previously validated chromatographic conditions.

Antimicrobial activity

The methanolic extract of *S. chamaecyparissus (*SME) was subjected to preliminary antimicrobial screening against seven test microorganisms, including five bacteria—*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 976, *Enterobacter hormaechei* ATCC 700323, and vancomycin-resistant *Enterococcus* (VRE) and two fungi, *Aspergillus* sp. and *Candida albicans* ATCC 14053. The concentrations of 5, 10, and 20 mg/mL were selected to provide a graded concentration range for preliminary screening and to allow assessment of concentration-related changes in inhibition-zone diameters. Mueller–Hinton agar (MHA) was used for bacterial assays, whereas potato dextrose agar was used for fungal assays. Use of MHA for bacterial susceptibility testing and agar-based screening methods for plant-derived substances is consistent with standard antimicrobial testing practice.[12,13] Before testing, stock cultures were reactivated, and microbial suspensions were adjusted to 0.5 McFarland turbidity. Then, 100 µL of each suspension was spread evenly onto the corresponding agar surface. Wells of 4 mm diameter were made aseptically in the inoculated agar using a sterile cork borer, and the test samples were introduced into the wells. Plates were subsequently incubated under appropriate conditions for each microorganism, and antimicrobial activity was evaluated by measuring the diameter of inhibition zones around the wells. The agar well diffusion assay, 0.5 McFarland inoculum standardization, and approximately 4 mm agar depth/well-based screening are all widely used in antimicrobial evaluation of plant extracts and essential oils.[12,13]

Statistical analysis

Antimicrobial assays were performed in triplicate, and inhibition zone diameters were expressed as mean ± standard deviation. For microorganisms showing measurable inhibition above baseline, differences among the three extract concentrations (5, 10, and 20 mg/mL) were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

Ethics committee approval

This study was conducted exclusively using plant material and did not involve humans, human data, human biological materials, or experimental animals. Therefore, ethics committee approval was not required.

Informed consent

Informed consent was not required because the study did not involve human participants or identifiable personal data.

RESULTS

Yields of essential oil and methanol extract

The hydrodistillation of the aerial parts of *S. chamaecyparissus* yielded a pale-yellow essential oil with a percentage yield of 0.46% (w/w). The methanolic extraction process, performed via maceration, resulted in a dry extract (SME) with a yield of 14.40% (w/w).

Morphological and anatomical characterization

The morphological evaluation of *S. chamaecyparissus* confirmed its characteristic appearance as a perennial, silvery-gray subshrub. The capitula was terminal, solitary, and discoid, with bright yellow tubular florets and no ray florets. The stem surface was densely tomentose, contributing to the silvery-gray appearance of the aerial parts (Figure 2A and B). Leaves collected from different stem nodes showed the characteristic segmented morphology of the species (Figure 3). Examination

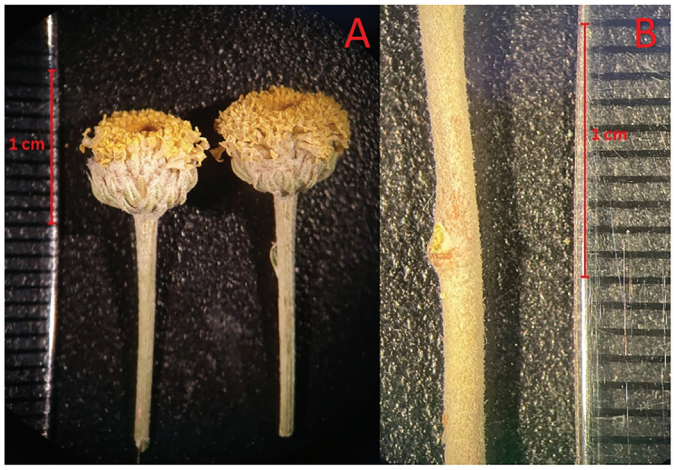

Figure 2. Morphological characteristics of *Santolina chamaecyparissus*: (A) discoid capitula and tubular flowers, (B) stem surface.

Figure 3. Morphological appearance of the leaves of *Santolina chamaecyparissus* from different nodes.

of the reproductive structures demonstrated tubular florets originating from both the peripheral and central regions of the capitulum. Longitudinal dissection of the florets revealed the ovary and corolla organization, while the mature cypselae lacked a pappus (Figure 4A–C). Scanning electron microscopy of the adaxial leaf surface revealed a densely pubescent surface (Figure 5). Higher-magnification observations demonstrated anomocytic stomata surrounded by densely interlaced non-glandular trichomes (Figure 6A and B). Detailed examination of the mature cypselae showed an oblong-obovoid shape, distinct longitudinal ribs, and the absence of a pappus (Figure 7A and B).

Anatomical examination of the stem revealed a typical eustelic organization. A well-developed collenchyma layer was located beneath the epidermis, and the vascular bundles contained clearly distinguishable primary and secondary xylem elements (Figure 8A–C). The leaf exhibited an isobilateral structure with multilayered palisade parenchyma on both surfaces. The midrib region contained a well-defined vascular system, and anomocytic stomata were observed in the epidermis. The leaves were amphistomatic and covered by a dense indumentum composed predominantly of non-glandular trichomes (Figure 9A–C).

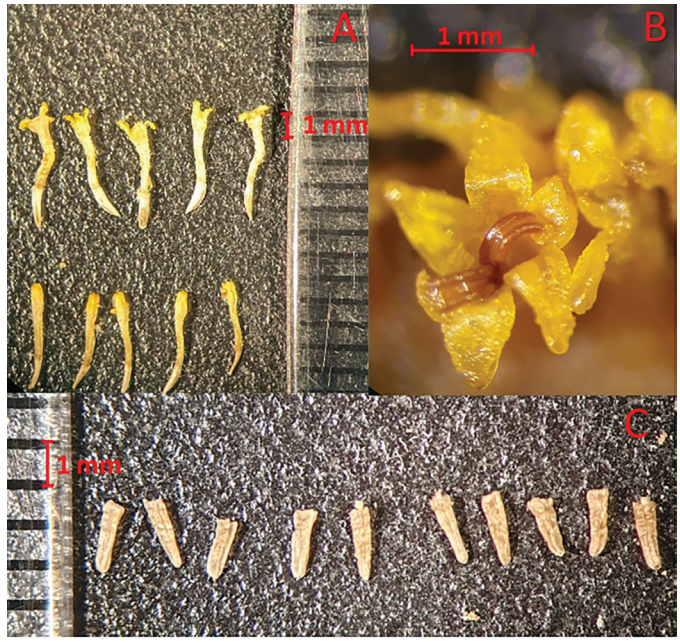

Figure 4. Reproductive morphology of *Santolina chamaecyparissus*: (A) tubular florets from the peripheral and central regions of the capitulum, (B) longitudinal dissection of a floret showing the ovary and corolla structure, (C) mature cypselae demonstrating the characteristic oblong shape and the absence of a pappus.

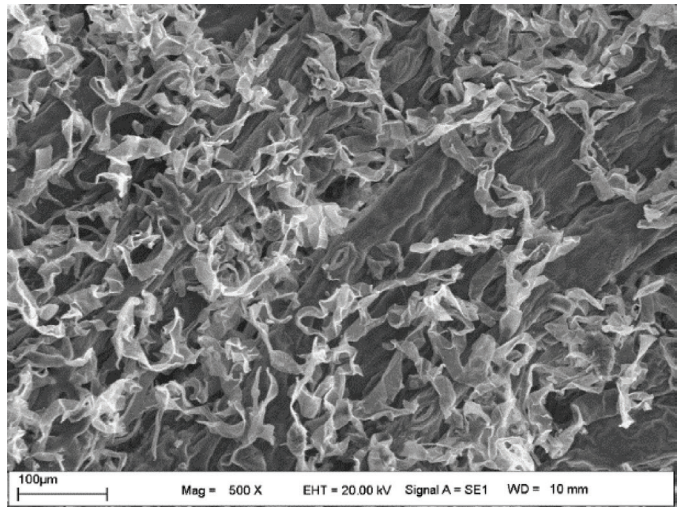

Figure 5. Scanning electron micrographs of the adaxial leaf surface of *Santolina chamaecyparissus*.

Phytochemical composition of the essential oil

The volatile profile of *S. chamaecyparissus* essential oil, analyzed by GC–MS/MS, led to the identification of 33 compounds, representing the majority of the total oil composition (Table 1). The essential oil was characterized by a high content of ketones and monoterpenoids. The major constituent was found to be artemisia ketone (55.96%), followed by camphor (20.84%). Other notable components included camphene (4.79%), benzylacetone (2.36%), and santolina triene (2.03%). The presence of these oxygenated monoterpenes defines the characteristic aromatic profile of the species.

LC–MS/MS analysis of phenolic compounds

The phenolic profile of the methanol extract (SME) was characterized using LC–MS/MS, focusing on antioxidant compounds (Table 2). A total of 35 phenolic compounds were screened. The analysis revealed that chlorogenic acid (6733.85 µg/g dry extract) and quinic acid (6521.37 µg/g dry extract) were the most abundant quantified compounds.

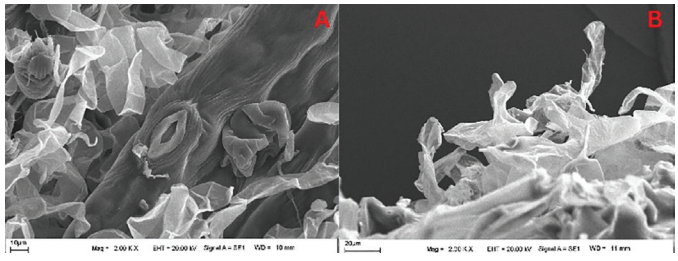

Figure 6. Scanning electron micrographs of the adaxial leaf surface of *Santolina chamaecyparissus*: (A) high-magnification view showing an anomocytic stoma, (B) dense indumentum consisting of interlaced non-glandular trichomes.

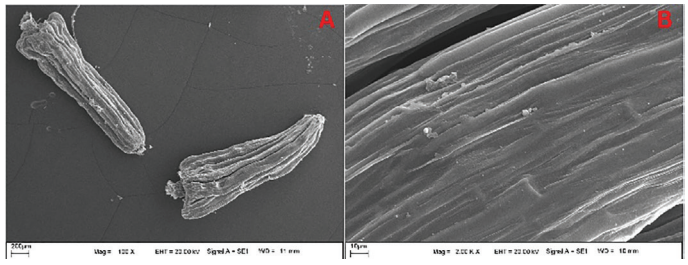

Figure 7. Detailed seed morphology of *Santolina chamaecyparissus*: (A) general view of mature cypselae, (B) close-up showing the oblong-obovoid shape, longitudinal ribs, and the distinct absence of a pappus.

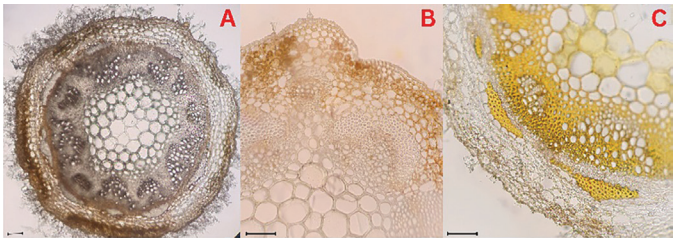

Figure 8. Anatomical structure of the *Santolina chamaecyparissus* stem (transverse section): (A) general view of the eustelic arrangement, (B) detailed view of the cortex and the supportive collenchyma layer beneath the epidermis, (C) vascular bundles showing primary and secondary xylem elements (scale bar 50 µm).

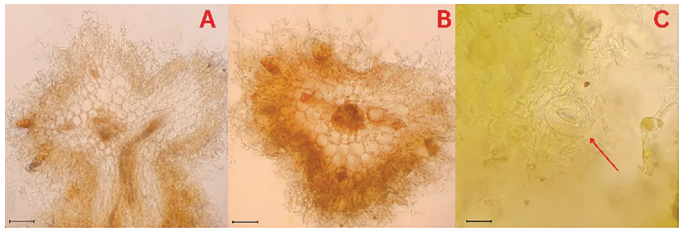

Figure 9. Leaf anatomy and micromorphology of *Santolina chamaecyparissus*: (A) transverse section illustrating the isobilateral mesophyll and multilayered palisade parenchyma, (B) magnified view of the midrib region with vascular tissues, (C) the anomocytic stomata (scale bar 50 µm for A,B; 25 µm for C).

Table 1. Chemical composition of the essential oil of *Santolina chamaecyparissus* L. analyzed by GC–MS/MS.

No	RT	RI (NIST Lib.)	Compound	%
1	7.92	908	Santolina triene	2.03
2	8.46	925	Tricyclene	0.25
3	8.56	926	2,5,5-trimethyl-1,3,6-heptatriene	0.27
4	8.85	929	1R-α-pinene	0.81
5	9.37	952	Camphene	4.79
6	9.77	962	Benzaldehyde	0.10
7	10.22	974	Sabinene	0.23
8	10.35	979	β-pinene	0.91
9	10.81	991	β-myrcene	0.47
10	10.84	994	2-methyl-2-bornene	0.30
11	11.10	998	Yomogi alcohol	1.99
12	12.06	1025	p-cymene	0.27
13	12.24	1031	β-phellandrene	1.75
14	12.64	1043	Lavender lactone	0.25
15	13.49	1062	Artemisia ketone	55.96
16	13.74	-	Not identified	0.22
17	14.21	1084	Artemisia alcohol	1.02
18	14.49	1095	α-pinene oxide	0.75
19	14.97	1107	Hotrienol	0.29
20	16.54	1145	Camphor	20.84
21	17.27	1167	Endo-borneol	0.75
22	17.92	1213	Myrtenol	0.50
23	18.40	1218	Benzylacetone	2.36
24	18.99	1228	Not identified	0.24
25	19.78	1236	2-butenoic acid, 3-methyl-, 3-methyl-2-butenyl ester	0.09
26	22.78	1331	Silphiperfol-5-ene	0.13
27	24.71	1396	Benzyanyl	0.16
28	27.70	1483	α-curcumene	0.19
29	28.36	1500	Pentadecane	0.26
30	30.03	1564	Not identified	0.17
31	30.75	1581	Caryophyllene oxide	0.11
32	32.71	1655	Bisabolol oxide II	0.54
33	33.45	1684	α-bisabolol	0.97

Note: Compound identification was based on comparison of EI mass spectra with the NIST MS library. Low-abundance peaks with similar library matches were reported as tentative annotations unless confirmed by authentic standards.

Table 2. Identification and quantification of phenolic compounds in the methanolic extract of *Santolina chamaecyparissus* using LC–MS/MS.

Compound	RT	Response	Concentration (µg/g dry extract)
Quinic acid	2.417	17169	6521.3748
Fumaric acid	3.971	92	33.0137
Gallic acid	5.519	26	ND
Pyrogallol	6.928	5	ND
Keracyanin chloride	10.393	21	11.8677
Cyanidin-3-o-glucoside	10.539	3	25.2668
Chlorogenic Acid	10.947	178257	6733.8453
Peonidin-3-o-glucoside	10.973	3	ND
Catechin	10.993	5	ND
4-OH-benzoic acid	11.406	213	ND
Caffeic acid	11.491	324	ND
Epicatechin	11.544	4	ND
Epigallocatechin gallate	11.611	6	ND
Vanillic acid	11.637	21	ND
Vitexin	11.699	11	ND
Hesperidin	11.726	1487	104.1124
Syringic acid	11.823	3	ND
Ellagic acid	11.934	4	ND
Naringin	12.060	113	ND
p-Coumaric acid	12.275	12	ND
Ferulic acid	12.461	5	ND
Rosmarinic acid	12.497	55	25.6033
Vanillin	12.551	15	45.0932
Taxifolin	12.600	137	ND
Myricetin	12.686	15	ND
Sinapic acid	12.808	6	ND
Resveratrol	12.924	4	ND
Luteolin	13.356	2980	<LOQ
Quercetin	13.427	20	ND
Apigenin	13.966	5109	<LOQ
Naringenin	13.994	55	ND
Isorhamnetin	14.180	393	ND
Chrysin	15.228	8	ND
Galangin	15.662	1	ND
Curcumin	16.033	2	ND

RT, retantion time; < LOQ, detected below the limit of quantification; ND, not detected.

Other detected phenolics included hesperidin (104.11 µg/g), vanillin (45.09 µg/g), fumaric acid (33.01 µg/g), rosmarinic acid (25.60 µg/g), and cyanidin-3-O-glucoside (25.27 µg/g). Several other flavonoids and phenolic acids, such as luteolin and apigenin, were detected but remained below the quantification limits in this specific extract.

Antimicrobial activity

The preliminary antimicrobial screening of the SME was evaluated against a panel of seven pathogenic microorganisms using the agar well diffusion method (Table 3). The largest inhibition zones were observed against *S. aureus*, with inhibition zones of 11.66 mm, 11 mm, and 8 mm at concentrations of 20 mg/mL, 10 mg/mL, and 5 mg/mL, respectively. Smaller inhibition zones were recorded against *E. hormaechei* (6.33 mm at 20 mg/mL). For the fungal strains, the extract showed activity against *C. albicans* (5.66 mm at 20 mg/mL), while its effect on *Aspergillus* sp. was equivalent to the minimum measurable zone (5 mm). The extract showed limited or baseline inhibition (5

Table 3. Preliminary agar well diffusion screening of *Santolina chamaecyparissus* methanolic extract against selected microorganisms.

Microorganism	20 mg/mL	10 mg/mL	5 mg/mL	NC	PC
Escherichia coli	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	NI	17.66 ± 1.15
Pseudomonas aeruginosa	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	NI	17.00 ± 1.00
Staphylococcus aureus	11.66 ± 0.58[a]	11.00 ± 1[ab]	8.00 ± 1.73[b]	NI	21.00 ± 1.00
VRE	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	NI	15.33 ± 0.58
Enterobacter hormaechei	6.33 ± 0.58[a]	6.00 ± 0.00[a]	5.66 ± 0.58[a]	NI	17.00 ± 1.00
Candida albicans	5.66 ± 1.15[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]	NI	15.33 ± 0.58
***Aspergillus* sp.**	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	NI	16.66 ± 0.58

PC, positive control; gentamicin 10 µg for bacteria and nystatin 100 IU for *Candida albicans;* NC, negative control; NI, no inhibition.
Different superscript letters within the same row indicate statistically significant differences among extract concentrations according to Tukey's HSD test ($p < 0.05$).

mm) against *E. coli*, *P. aeruginosa*, and VRE across the tested concentrations.

Among the microorganisms showing measurable inhibition, a significant concentration-dependent difference was observed only for *S. aureus* (one-way ANOVA, F = 7.923, p = 0.021). Tukey's post-hoc analysis showed that the inhibition zone at 20 mg/mL was significantly greater than that at 5 mg/mL (p = 0.023), whereas the differences between 20 and 10 mg/mL and between 10 and 5 mg/mL were not statistically significant. No significant differences among concentrations were observed for *E. hormaechei* ($p = 0.296$) or *C. albicans* ($p = 0.422$).

DISCUSSION

The present study provides an integrated pharmacognostic and phytochemical evaluation of *S. chamaecyparissus,* showing that its anatomical organization, volatile profile, polar phenolic composition, and antimicrobial behavior are closely interconnected. The anatomical findings indicate that *S. chamaecyparissus* exhibits a pronounced xeromorphic organization, characterized by its greyish-tomentose indumentum and specialized leaf morphology. These features align with the diagnostic descriptions in the Flora of Turkey, which identifies the species by its ± vermiform leaves with numerous oblong, obtuse segments, typically arranged in four ranks. In addition, the taxonomic complexity of the *S. chamaecyparissus* species complex has been emphasized in recent nomenclatural studies, supporting the importance of voucher-based identification and detailed morphological characterization in pharmacognostic investigations.[14] Such structural adaptations, particularly the amphistomatic leaf structure and dense non-glandular indumentum observed here, are consistent with adaptation to dry Mediterranean environments. In pharmacognostic terms, these traits are not only useful for species characterization, distinguishing it from related taxa by its homogamous, discoid capitula and 3–4-angular achenes as noted in regional floristic records,[9] but may also reflect structural strategies that support tolerance to high irradiance and water limitation. This interpretation is in line with previous reports emphasizing the importance of protective and secretory structures in aromatic Asteraceae and in *S. chamaecyparissus* in particular.[8] In aromatic Mediterranean plants, such protective and secretory structures may also contribute to the production and retention of volatile metabolites, which have been proposed to participate in ecological responses to high temperature, intense solar radiation, and other environmental stresses. Accordingly, the anatomical profile observed here provides a relevant structural background for understanding the phytochemical richness of the species.

The volatile profile obtained in the present study further supports this interpretation. The essential oil was clearly dominated by oxygenated monoterpenes, with artemisia ketone (55.96%) and camphor (20.84%) as the principal constituents. This general pattern agrees with previous reports indicating that *S. chamaecyparissus* often produces oils rich in oxygenated monoterpenes, although the relative abundance of the major constituents is highly variable among populations, plant organs, developmental stages, and extraction procedures.[3,4,6,15,16] For example, Indian material analyzed by Garg et al.[15] contained artemisia ketone as the main component, but with lower abundance (approximately 32%), together with substantial levels of 1,8-cineole and myrcene, indicating a related but not identical profile to the present sample. Likewise, cultivated and wild Spanish materials examined by Pérez–Alonso and Velasco–Negueruela[16] showed marked interpopulation heterogeneity and supported the existence of chemically distinct groups within the taxon. More recent work from Serbia also confirmed a chemically variable oil, again with artemisia ketone among the dominant components but accompanied by a different sesquiterpene background.[17] Taken together, these comparisons indicate that the present material can reasonably be interpreted as an artemisia ketone/camphor-dominant chemotype within the known chemical plasticity of the species rather than as an anomalous population.

This variability is also important from a pharmacognostic standpoint. In *S. chamaecyparissus*, literature does not support a single invariant oil profile; instead, it points to a species complex with substantial phytochemical breadth.[3,4,6] Such variation is not unexpected in aromatic plants, where environmental factors, ontogeny, plant part selection, and extraction methodology can alter the balance between monoterpenes and sesquiterpenes.

The non-volatile profile of the methanolic extract adds a second, complementary phytochemical layer to this picture. In

the present study, chlorogenic acid and quinic acid were by far the most abundant quantified constituents, while hesperidin, rosmarinic acid, vanillin, and cyanidin-3-O-glucoside occurred at much lower levels. This pattern suggests that the methanol extract is enriched primarily in polar phenolic acids rather than being dominated by flavonoids alone. Earlier studies on the polar chemistry of *S. chamaecyparissus* likewise showed that the species contains a chemically diverse phenolic fraction, including luteolin derivatives, lonicerin, apigenin glycosides, and other unusual polar constituents, with the butanolic fraction displaying markedly higher total phenolic content and stronger antioxidant activity than the chloroform fraction.[18] By contrast, a recent ethanolic extract study identified ferulic acid and p-coumaric acid as the dominant quantified phenolics.[19] The difference from our results likely reflects differences in extraction solvent, plant material, and analytical target panel rather than contradiction. In other words, the available evidence consistently supports *S. chamaecyparissus* as a phenolic-rich species, but the exact dominant polar metabolites appear to be method- and sample-dependent. The volatile and non-volatile profiles should be interpreted as distinct phytochemical fractions. The essential oil was characterized by artemisia ketone and camphor, whereas the methanolic extract was dominated by chlorogenic acid and quinic acid and contained additional minor phenolic constituents. Because only the methanolic extract was subjected to antimicrobial screening, no inference regarding the antimicrobial contribution of artemisia ketone, camphor, or other essential-oil constituents can be made from the present data. Oxygenated monoterpenes such as camphor are frequently linked to membrane-disruptive effects, whereas chlorogenic-acid-rich polar extracts may contribute to antioxidant and auxiliary antimicrobial actions.[12,13,18,19] Nevertheless, a careful distinction should be maintained between the essential oil and the methanol extract. In the present study, the antimicrobial assay was performed with the methanol extract, not with the isolated essential oil. Therefore, although the essential-oil profile strengthens the phytochemical importance of the species, it should not be used as direct proof of the activity observed in the agar well diffusion test. The observed inhibition-zone responses may reflect contributions from multiple constituents of the methanolic extract; however, the contribution of individual compounds and potential interactions among them were not investigated in the present study.

The agar well diffusion findings should be interpreted as preliminary screening data rather than as quantitative evidence of antimicrobial potency. Within these experimental conditions, the methanol extract produced its largest inhibition zones against *S. aureus*, while the effects against *E. coli*, *P. aeruginosa*, VRE, and *Aspergillus* sp. were weak or close to baseline, and only modest inhibition was recorded for *Enterobacter hormaechei* and *C. albicans*. This trend is biologically plausible and agrees with the broader literature showing that plant-derived phytochemicals often act more efficiently against gram-positive bacteria than against gram-negative bacteria, partly because the outer membrane of gram-negative organisms forms an additional permeability barrier.[2,12,13] The preferential susceptibility of *S. aureus* in the present study is therefore reasonable and suggests a preferential inhibition pattern toward *S. aureus* under the present screening conditions; however, this observation requires confirmation by quantitative broth dilution methods. At the same time, comparison with previous *Santolina* studies shows that the magnitude and spectrum of activity can differ substantially. Djeddi et al.[6] reported strong activity of the essential oil from Algerian material, especially against *Klebsiella pneumoniae* and *C. albicans*, together with camphor/cubenol-rich chemistry and anatomical evidence for oil localization in both endogenous and exogenous secretory sites. Other reports have also described substantial antimicrobial or antifungal effects for *S. chamaecyparissus* essential oils, including activity against *Candida* spp. and other microorganisms.[4,7] Our results are clearly more moderate, but this difference is not surprising because the tested matrix here was a methanolic extract rather than the essential oil, and the applied bioassay was agar well diffusion rather than a broth microdilution design. Accordingly, the lower zones observed in our study should not be interpreted as evidence that the species is weakly bioactive overall; rather, they indicate that under the present extraction and assay conditions, the polar extract expresses selective and limited antibacterial activity, with its clearest effect directed toward *S. aureus*. This distinction between matrices is especially important for future work. The literature suggests that *S. chamaecyparissus* can yield biologically active preparations in both volatile and polar fractions, but the responsible constituents are not necessarily the same. Essential-oil studies emphasize monoterpenes and sesquiterpenes, whereas polar-extract studies highlight phenolic acids, flavonoids, and glycosides.[3,4,6,7,17-19] Our study contributes to this picture by showing, in a single voucher-identified sample, that an artemisia ketone/camphor-rich essential oil coexists with a chlorogenic acid/quinic acid-rich methanolic fraction. That integrated chemical characterization is valuable because it broadens the pharmaceutical-botanical profile of the species beyond the essential-oil literature that has historically dominated the field.

Study limitations

Several limitations, however, should be explicitly acknowledged. First, because antimicrobial testing was carried out only with the methanol extract, no direct correlation can yet be made between the essential-oil constituents and the observed inhibition zones. Second, antimicrobial testing was limited to agar well diffusion, which is suitable for preliminary screening but does not provide quantitative MIC or MBC values. Consequently, the present inhibition-zone findings cannot establish the antimicrobial potency, bacteriostatic, or bactericidal efficacy of the methanolic extract and require confirmation using standardized broth microdilution methods. Finally, although chlorogenic acid and quinic acid were the major quantified phenolics, the observed activity may reflect contributions from multiple constituents; however, potential additive or synergistic interactions were not investigated in the present study.

CONCLUSION

Overall, the present findings strengthen the pharmaceutical-botanical relevance of *S. chamaecyparissus*. The species examined here combines xeromorphic structural adaptation with a chemically layered metabolite profile, consisting of an essential oil dominated by artemisia ketone and camphor and a methanolic extract dominated by chlorogenic and quinic acids. In the preliminary agar well diffusion screening, the methanolic extract produced its largest inhibition zones against *S. aureus*; however, this finding should be considered exploratory and does not establish antimicrobial potency. Quantitative MIC/MBC testing is required before definitive conclusions regarding antimicrobial efficacy can be drawn. Future work should therefore focus on MIC/MBC-based antimicrobial testing, bioactivity-guided fractionation of the methanol extract, separate testing of the essential oil, and direct evaluation of isolated major constituents and defined combinations in order to clarify which fraction best explains the biological potential of this species.

Ethics

Ethics Committee Approval: This study was conducted exclusively using plant material and did not involve humans, human data, human biological materials, or experimental animals. Therefore, ethics committee approval was not required.

Informed Consent: Informed consent was not required because the study did not involve human participants or identifiable personal data.

Footnotes

Authorship Contributions

Concept: Ş.R.O.D., Design: Ş.R.O.D., Data Collection or Processing: Ş.R.O.D., A.D., Analysis or Interpretation: Ş.R.O.D., H.S., Literature Search: Ş.R.O.D., A.D., Writing: Ş.R.O.D., A.D., H.S., T.A.

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