

Article

Secretory Structures and Essential Oil Composition in *Santolina chamaecyparissus* L. Cultivated in Northern Italy

Claudia Giuliani ^{1,2,†}, Fabrizia Milani ^{1,2,†}, Sara Falsini ³, Alberto Spada ⁴, Piero Bruschi ⁵,
Alessio Papini ³, Laura Santagostini ^{6,*}, Martina Bottoni ^{1,2} and Gelsomina Fico ^{1,2}

- ¹ Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy; claudia.giuliani@unimi.it (C.G.); fabrizia.milani@unimi.it (F.M.); martina.bottoni@unimi.it (M.B.); gelsomina.fico@unimi.it (G.F.)
- ² Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan, Via Religione 25, 25088 Toscolano Maderno, Italy
- ³ Department of Biology, University of Florence, Via Giorgio la Pira, 50121 Florence, Italy; sara.falsini@unifi.it (S.F.); alessio.papini@unifi.it (A.P.)
- ⁴ Department of Agricultural and Environmental Sciences—Production, Landscape, Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy; alberto.spada@unimi.it
- ⁵ Department of Agricultural, Environmental, Food and Forestry Science and Technology, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy; piero.bruschi@unifi.it
- ⁶ Department of Chemistry, University of Milan, Via Golgi 19, 20133 Milan, Italy
- * Correspondence: laura.santagostini@unimi.it
- † These authors contributed equally to this work.

Abstract

Santolina chamaecyparissus L. (Asteraceae), cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, Brescia, Northern Italy) of the University of Milan, was investigated adopting a multidisciplinary research approach: micromorphological and histochemical, with special attention on the secretory structures producing secondary metabolites; phytochemical, with the analysis of the essential oil (EO) composition from the air-dried, flowered aerial parts gathered once per year across two consecutive years (2021 and 2022); bio-ecological, focusing, based on literature data, on the biological activity and ecology of the main EO compounds; didactic–educational, with the *ex novo* realization of an interpretative apparatus at the study site. Two distinct types of secretory structures were described: biseriate glandular trichomes and secretory ducts, both producing an oleoresin rich in flavonoids. Phytochemical analysis revealed stable EO profiles across the two years with regards to the total number of compounds (39 vs. 40), the main chemical classes (oxygenated monoterpenes (72.67% vs. 78.61%) and monoterpenes hydrocarbons (15.06% vs. 10.48%) and the key single components (artemisia ketone, 52.74% vs. 55.67%; camphor, 13.00% vs. 16.18%). The literature data on the bio-ecology of the main compounds allowed us to confirm antimicrobial, antioxidant, and anti-inflammatory properties. Concerning the dissemination actions, the outcomes of this multidisciplinary work were integrated into a new interpretive apparatus for *S. chamaecyparissus* at the Ghirardi Botanic Garden. The research results enhance our understanding of this species, supporting its potential EO application in medicine and agriculture.

Keywords: cotton lavender; glandular trichomes; secretory ducts; essential oils; microscopy; GC–MS; botanic gardens; Open Science



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1. Introduction

Santolina chamaecyparissus L., commonly known as cotton lavender, is a small, semi-woody, tender sub-shrub with evergreen tomentose foliage, belonging to the Asteraceae family [1–4]. The stems are 10–50 cm high, erect or ascending. The non-flowering shoots are green to grey–tomentose; the flowering stems are usually simple and leafless for some distance below the *capitula*. The leaves are pinnately divided into minute ovate-oblong segments slightly fleshy, silver-gray in color. The involucre is 6–10 mm wide, hemispherical, subtruncate with lanceolate to ovate bracts, and carinate; the inner portion has a rounded, scarious apex. The flower heads are globular, 1–2 cm in diameter, consisting of disc tubular cream to bright, yellow-colored florets; the florets, each subtended by an interfloral scale, are hermaphrodite at the center of the *capitulum* and may occasionally be female on periphery. The fruits are compressed ellipsoidal achenes [4,5].

Native to the Mediterranean region, this species has long been cultivated as an ornamental plant, due to its silver-gray foliage and yellow *capitula* [6,7]. Its morphological resilience and ability to thrive in arid, nutrient-poor soils also make it suitable for xeriscaping and low-input horticultural systems [8,9]. Additionally, this species has a long history of use in Mediterranean folk medicine for a variety of purposes: for its antiseptic, insect-repellent, and digestive properties [6,9]. For example, its inflorescences are traditionally used for their anti-inflammatory properties [6], while the foliage serves as a natural insecticide and moth repellent [10,11].

Beyond its horticultural appeal, *S. chamaecyparissus* has gained increasing attention due to its rich profile of bioactive secondary metabolites. Essential oil composition studies from diverse ecotypes, including Saudi Arabia, Turkey, and Tunisia, have identified high levels of monoterpenes (e.g., artemisia ketone, 1,8-cineole, camphor) and oxygenated sesquiterpenes (e.g., β -eudesmol, β -bisabolene), with chemical variation strongly influenced by geographic origin and seasonal harvesting [1,6,12–17]. Recent works, using hydromethanolic and ethanolic extraction techniques, have further revealed the presence of phenolic acids (e.g., ferulic acid, p-coumaric acid) and flavonoids such as quercetin and rutin, compounds known for their potent antioxidant and enzyme-inhibitory activities [2,18,19].

From a pharmacological perspective, extracts and essential oils of *S. chamaecyparissus* have demonstrated broad-spectrum biological activities. These include antioxidant [6,8], anti-inflammatory [12], antidiabetic [6,19], and cytotoxic effects, particularly against HepG2 and MCF-7 cancer cell lines [12–21]. The antifungal efficacy of this species has also been substantiated in vitro against several phytopathogenic fungi, including *Fusarium oxysporum* and *Neocosmospora* spp., suggesting its potential utility in sustainable plant protection strategies [2,11,22]. Notably, *in planta* assays and preliminary in vivo evaluations in rodent models indicate a favorable safety profile, with no significant toxicity at functional doses [23].

Despite these promising bioactivities, the standardized use of *S. chamaecyparissus* in phytopharmaceuticals and biopesticides remains limited. Major obstacles include chemotypic variability due to environmental and genetic factors, and taxonomic complexity within the *S. chamaecyparissus* complex. Recent morphometric and cytogenetic studies have revealed the presence of multiple ploidy levels (diploid to hexaploid) and high karyotypic asymmetry among populations, complicating both conservation and cultivar development efforts [1,24].

The literature on the micromorphology of cotton lavender includes two key studies by El-Sahhar et al. [25,26], based on samples from Egypt. These authors examined the anatomy of the roots, stems, leaves, and inflorescences, but made only general references to the secretory structures. They described woolly hairs on the shoots, leaf blades, and petioles, without distinguishing between glandular and non-glandular *indumenta*. Furthermore,

while they noted the presence of abundant secretory ducts in the stem cortex, they did not provide detailed descriptions.

Reports on the histochemical characteristics of the secretory material are also lacking. In contrast, the literature on some congeneric species is extensive, with detailed studies on both external and internal secretory structures. These investigations focused on the morphotypes and distribution of trichomes and internal ducts in vegetative and reproductive organs, using both light microscopy and scanning electron microscopy [27–30].

In the study presented herein, we investigated *S. chamaecyparissus* L. cultivated at the Ghirardi Botanic Garden of the University of Milan (Toscolano Maderno, Brescia, Northern Italy) under a cross-disciplinary research approach performing (i) a micromorphological and histochemical investigation on the secretory structures of the vegetative organs and the *capitula*; (ii) analyses of the EO compositions obtained from the air-dried, flowered aerial parts collected in 2021 and 2022; (iii) a literature search to deepen the relationship between the main EO components and their potential bio-ecological value. These findings, in the context of a wider Open Science project titled, “Botanic Garden factory of molecules. . .work in progress”, led to the creation of new interpretative equipment on the target species at the Botanic Garden; in this way, the scientific research outcomes are made available to the public, significantly broadening societal participation in science.

2. Materials and Methods

2.1. Plant Material

S. chamaecyparissus L. is cultivated at the Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan (Toscolano Maderno, BS). The plants were identified by Prof. C. Giuliani and Prof. G. Fico according to Pignatti et al. [31].

The target species is present at the study site as a single individual. The analyzed plant material, therefore, derived from the same specimen, from which several flowering shoots (with inflorescences at the same phenological phase of anthesis) were gathered. The samplings were performed during the blooming period in June 2021 and June 2022. Voucher specimen was labeled with the codes FIAF 39143 and deposited in the Herbarium of the Agricultural Botany Laboratories of Florence (FIAF) at the Department of Agricultural, Environmental, Food, and Forestry Science and Technology (DAGRI) of the University of Florence. Voucher specimen details: Date of collection—20 June 2021; Collection site—Ghirardi Botanic Garden, via Religione 25, Toscolano Maderno, Brescia (GPS coordinates—45.638744970229, 10.611393928835382).

2.2. Chemicals

Solvents were of gradient-grade purity and purchased from either Exacta Optech Labcenter SpA (San Prospero—MO, Italy) or VWR International (Milan, Italy). All the reagents were of reagent-grade purity, purchased from Sigma Aldrich (Merck group, Milan, Italy), Fisher Scientific Italy (Rodano—MI, Italy), or VWR International (Milan, Italy), and used as received.

2.3. Micromorphological Survey

We described the structure, the distribution pattern, and the histochemistry of the secretory structures on the vegetative and reproductive organs by means of scanning electron microscopy (SEM), light microscopy (LM), and fluorescence microscopy (FM). For each examined plant part, at least ten replicates were examined to evaluate the level of variability of the micromorphological features.

2.3.1. Scanning Electron Microscopy (SEM)

Stems, leaves, involucre bracts, interfloral scales, and florets were hand-prepared, fixed in FAA solution (formaldehyde:acetic acid:ethanol 70% = 5:5:90) for 24 h, dehydrated in an ascending ethanol series up to absolute, and critical-point dried. The samples were mounted on aluminum stubs and gold coated. Observations were performed under a Zeiss® EVO MA15 SEM (Zeiss Group, Jena, Germany) operating at 10 kV at the Interdepartmental Center for Electron Microscopy and Microanalysis Services (M.E.M.A.) of the University of Florence (Florence, Italy).

2.3.2. Light Microscopy (LM) and Fluorescence Microscopy (FM)

The micromorphological survey under LM and FM was conducted on the vegetative and reproductive organs. We used both fresh material and fixed samples included in historesin (Technovit® 7100, Heraeus Kulzer GmbH & Co. KG, Wehrheim, Germany).

For the fresh material, sections ranging from 30 to 50 µm in thickness were obtained using a vibratome and/or a cryostat. Samples were also fixed in FAA solution for 48 h at 4 °C. Subsequently, fixed samples were washed in 70% ethanol for 24 h; they were then dehydrated progressively by treatment with 80% ethanol for 2 h, 95% ethanol for 2 h, and then twice in absolute ethanol for 2 h each. Pre-embedding was then performed, first with ethanol and historesin in 1:1 ratio for one night, then with 1:2 ratio for 2 h, and in pure historesin for 3 h. Finally, the embedding was carried out in a polypropylene capsule with the addition of hardener in a ratio of 1:15 with basic resin. The historesin samples were cut in 2 µm sections with an ultramicrotome [32].

The following dyes were used [32,33]: Toluidine Blue as a general staining; Fluoral Yellow-88 for total lipids; Nile Red for neutral lipids; Nadi reagent for terpenes; Periodic Acid-Schiff (PAS) reagent for total polysaccharides; Alcian Blue for mucopolysaccharides; Ruthenium Red for pectins; Ferric Trichloride for polyphenols; Aluminum Trichloride for flavonoids. Control procedures were carried out concurrently. Observations were made with a Leitz DM-RB Fluo optical microscope (Leitz, Wetzlar, Germany) equipped with a Nikon digital camera (Nikon Europe, Campi Bisenzio, Florence, Italy).

2.4. Phytochemical Survey

2.4.1. Preparation of Essential Oils (EOs)

The plant material was air-dried at room temperature, away from direct light, for 10 days. Subsequently, the samples were weighed, ground, and placed into 4 L flasks containing purified water at a 1:10 plant material-to-water ratio. Hydro-distillation was performed using a Clevenger-type apparatus for 3 h, ensuring that the volume of the obtained oil remained constant after this period. Once collected, the EO was decanted and separated from the water, with any residual water removed using anhydrous sodium sulfate. The oil yield was calculated based on dry weight (w/w).

Given that the target species was present as a single individual at the study site, no replicates were performed for oil extraction; however, three replicates were performed during EO dilution for the GC–MS and GC–FID analyses in order to evaluate the repeatability of the measurements for determining the EO composition. The results showed only minor variations in the relative percentage of single components, which did not affect the percentage data reported.

2.4.2. GC–MS Analysis of EOs

EOs were analyzed by GC–MS using a Thermo Scientific TRACE ISQ QD Single-Quadrupole GC–MS (Thermo Fisher Scientific Inc., Monza, Italy). EO separation was performed by a capillary column VF-5ms (5% phenyl-methyl-polisiloxane, length 60 m,

0.25 mm i.d., 0.25 µm film thickness); the temperature gradient was as follows: 8 min at 50 °C, then 4 °C/min until 60 °C, then 6 °C/min from 60 °C to 160 °C, and finally 20 °C/min from 160 °C to 280 °C. Injector and detector temperatures were set to 280 °C; carrier gas He, flux 1 mL/min. The mass range detected was 50–500 *m/z*. EOs were analyzed after being diluted 1:50 with n-hexane, with an injection volume of 1 µL.

Mass spectra were analyzed using the Wiley Mass Spectra Library (version 2021), NIST Mass Spectral Search Program (NIST 18, Wiley, Hoboken, NJ, USA; Adams), and NIST Tandem Mass Spectral Library 2.3; compounds were identified by mass fragmentation and the comparison of retention indices, calculated using a C8–C30 series of n-alkanes (Sigma-Aldrich, Milan, Italy), with data stored in mass databases (NIST 18, Wiley, Hoboken, NJ, USA; Adams) [34]. Percentage values of EO components were obtained from the peak areas in the chromatogram without the use of correction factors. GC-MS chromatograms were reported in Supplementary Materials.

2.4.3. GC-FID Analysis of EOs

EOs were analyzed by a gas chromatograph Agilent 6890 N equipped with an FID detector (Agilent Technologies, Cernusco sul Naviglio, Milan, Italy). EO separation was performed by an HP5 capillary column (5% phenyl-methyl-polisiloxane, 60 m × 0.25 mm × 0.25 µm); the oven temperature gradient was as follows: 4 min at 50 °C, then 5 °C/min until 100 °C, then 10 °C/min from 100 °C to 280 °C. Injector and detector temperatures were set to 280 °C; carrier gas He, flux 1 mL/min. EOs were analyzed diluted 1:50 with n-hexane, with an injection volume of 2 µL. The percentage composition of EO was calculated using the peak normalization method.

2.5. Scientific Dissemination

In order to engage the public of the Ghirardi Botanic Garden into the research outcomes obtained herein, and, under the policy of the Open Science, a novel interpretative outdoor facility was created for the target species at the study site.

An ad hoc coordinated graphic was planned in order to design an appealing label layout reflecting the multi-step research approach: macromorphological (with the production of original line drawing and brief information on the macroscopic diagnostic features), micromorphological (with photos and information on the secretory structures responsible for the productivity in secondary metabolites), phytochemical (with data on the main EO components), and bio-ecological (regarding their potential ecological roles or biological activities).

3. Results

3.1. Micromorphological Survey

The non-glandular *indumentum* and the external and internal glandular structures observed on the examined plant parts of *S. chamaecypacissus* displayed a general uniformity concerning morphotypes and distribution patterns in all the replicates. Their distribution pattern on the examined plant parts is shown in Table 1.

On the vegetative and reproductive organs examined, a single morphotype of protective hairs was observed (Table 1; Figure 1a,c,d). They were elongated, simple, multicellular, uniseriate, and pointed at the apex; the basal cells generally protruded from the plant epidermis and were isodiametric, while the distal ones were increasingly smaller in diameter as they approach the apical tip. The length of the protective hairs was variable: the shortest were only sporadically straight and erect, whereas the longest were generally flexuous, curved, and oriented at different angles with respect to the plant epidermis, until appearing completely close to it. They are ubiquitous on the vegetative organs, being particularly

abundant on the whole leaf abaxial side while being preferentially located along the median rib of the adaxial one (Figures 2b,c,e,f and 3c).

Table 1. Distribution pattern of the non-glandular and biseriate glandular trichomes, along with the secretory ducts, on the examined vegetative and reproductive organs of *S. chamaecyparissus*.

	Stem	Leaf		Involucral Bract		Interfloral Scales		Tubular Floret	
		Adax	Abax	Adax	Abax	Adax	Abax	Ovary	Corolla
non-glandular trichomes	++	++	+++	—	+	—	+	—	—
biseriate glandular trichomes	±	+++	+++	—	++	++	++	—	++
secretory ducts	++		++		++		++	—	—

Symbols: (–) missing, (±) sporadic, (+, ++, +++) increasing presence of trichomes.

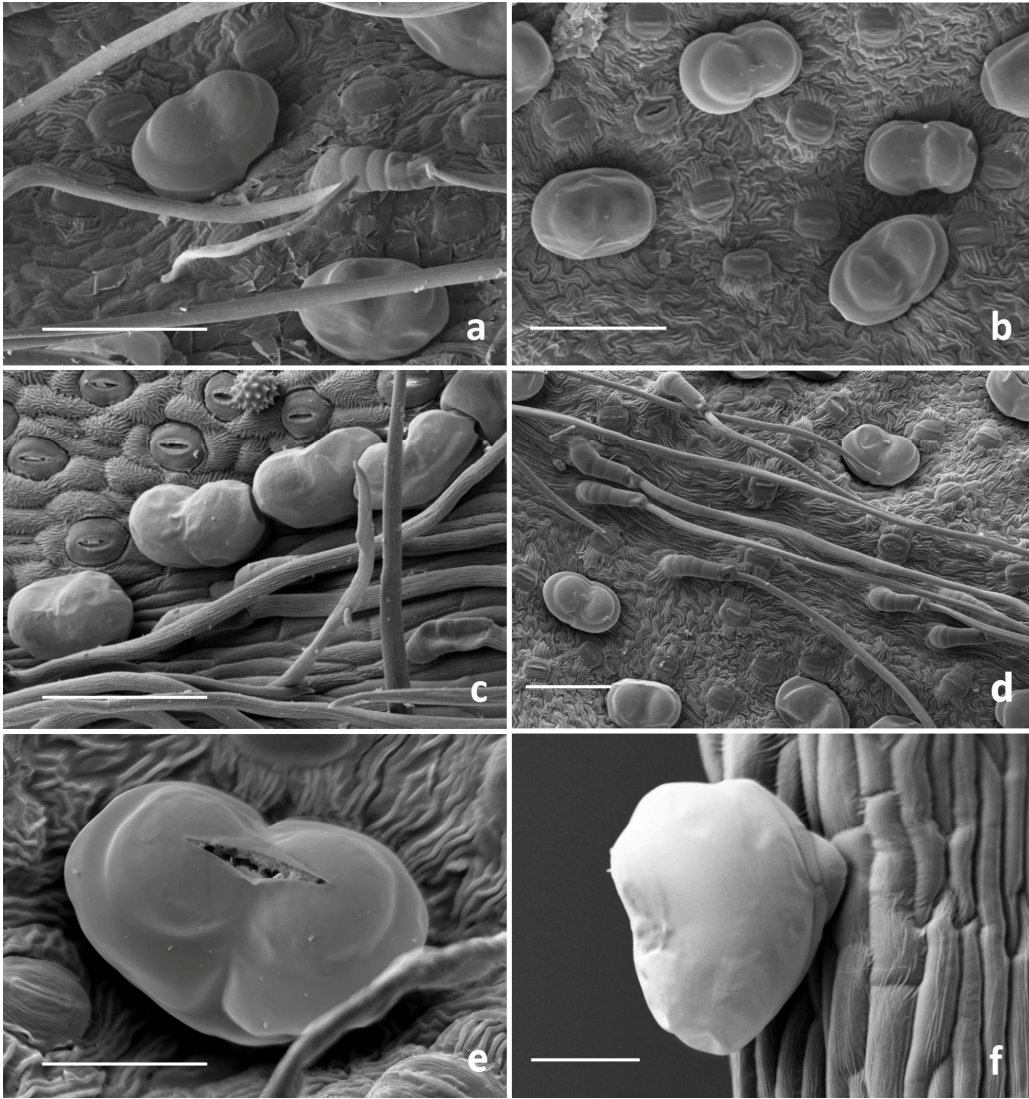


Figure 1. SEM micrographs showing the non-glandular trichomes and the biseriate glandular trichomes observed on the vegetative and reproductive organs of *S. chamaecyparissus*. (a–d) Sunken biseriate glandular trichome with a small central cuticle opening on the vegetative organs. (e) Sunken biseriate glandular trichome with the rupture of the cuticle along a straight line of weakness. (f) Protruding biseriate glandular trichomes on the corolla abaxial lobes of the tubular florets. Scale bars: 50 µm (a–d); 30 µm (e,f).

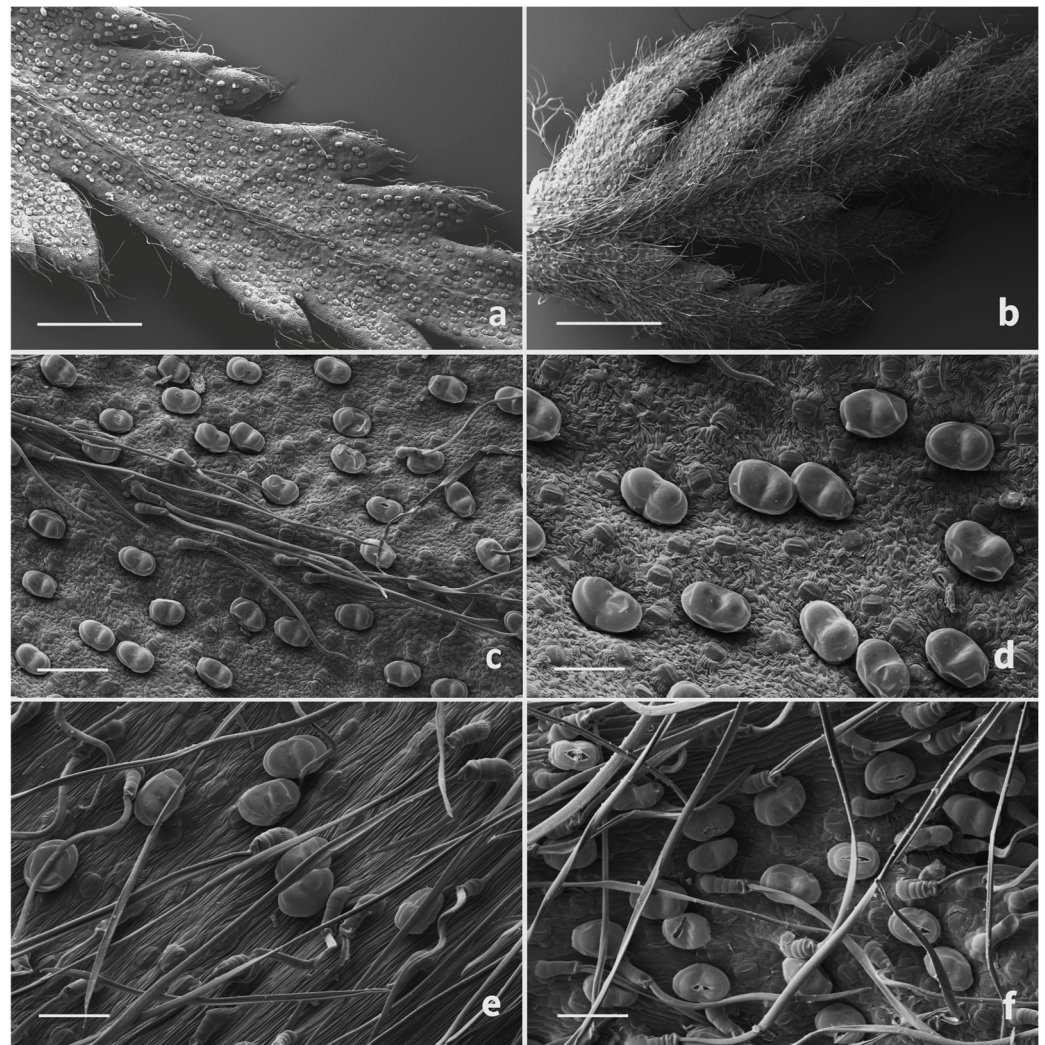


Figure 2. SEM micrographs showing the leaves of *S. chamaecyparissus*. (a,b) General views of the leaf adaxial (a) and abaxial (b) sides. (c,d) Details of the leaf adaxial sides. (e,f) Details of the leaf abaxial sides. Scale bars: 500 μ m (a–c); 50 μ m (d–f).

As regards the secretory structures, two different types were observed: external glandular trichomes and internal secretory ducts (Table 1; Figures 1–4).

3.1.1. Glandular Trichomes

The glandular trichomes were represented by a single morphotype: multicellular and biseriate. Each consisted of five pairs of cells: two basal cells, two stalk cells, and six cells forming the secretory head. These trichomes were sunken in a depression, which made the basal and the stalk cells hidden and challenging to observe (Figure 1e). They occurred on all the organs examined (Figures 1 and 2, Table 1). Their presence was sporadic on stems, but common and uniform across leaf surfaces, both on the interveinal regions and on the ovate–oblong foliar segments (Figure 1a–f). The secreted material accumulated in the subcuticular space, originated by the detachment of the cuticle from the top cells, and it is extruded through breaks in the cuticle caused by the internal pressure (Figure 1e).

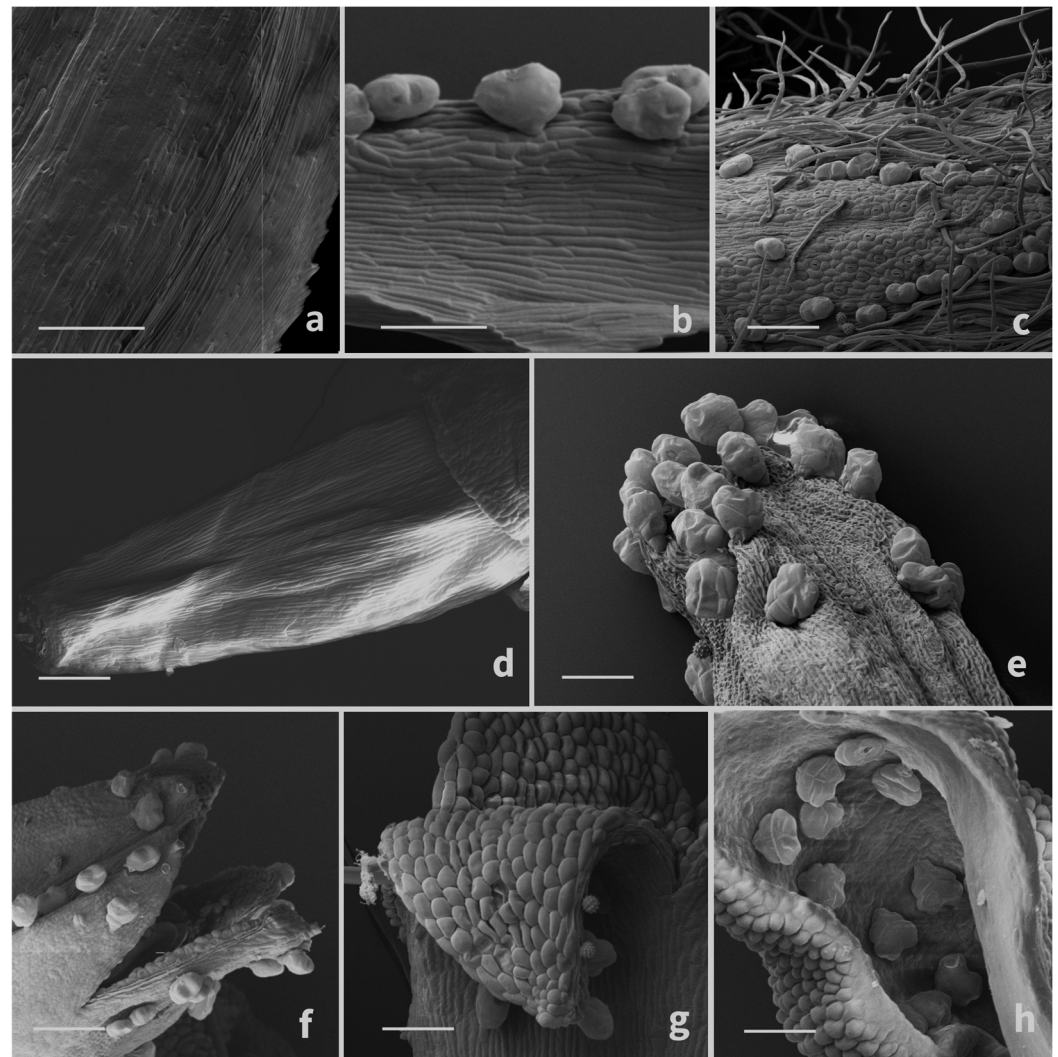


Figure 3. SEM micrographs showing the reproductive organs of *S. chamaecyparissus*. (a,b) Details of the adaxial (a) and abaxial (b) sides of the involucre bracts. (c) Details of the abaxial side of the interfloral scale (d–h) Tubular floret: details of the ovary (d) and of the apical portions (e–h) of the corolla at different phenological stages of anthesis. Scale bars: 500 μm (a); 200 μm (c); 100 μm (d–f); 50 μm (b,g,h).

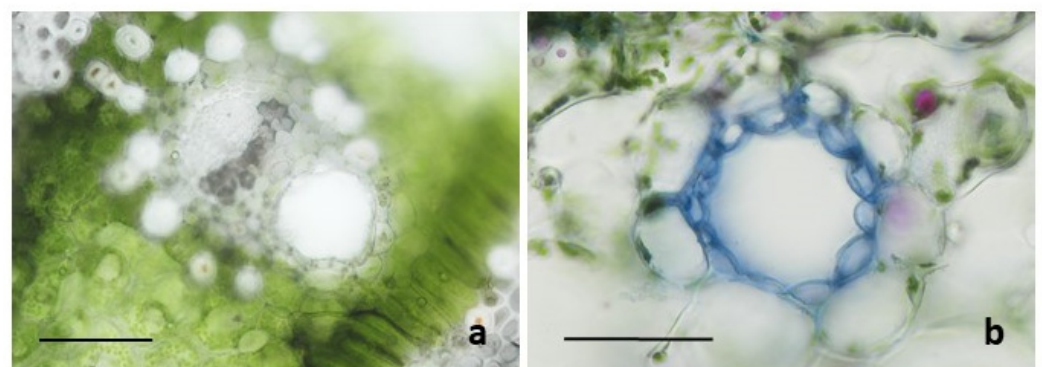


Figure 4. LM micrographs showing the secretory ducts of *S. chamaecyparissus*. (a) Transverse section of the leaf with details of a secretory duct associated with a conduction element. (b) Details of a secretory duct bounded by a single layer of flattened secreting cells. Scale bars: 100 μm .

They also occurred on the *capitulum* parts, where the basal cells generally projected above the surface of the surrounding epidermal cells (Figure 1f). These hairs were located

on the involucre bracts, on the interfloral scales, and on the corolla (Figure 3). On the bracts, they were few, located preferably on the edges of the abaxial surface (Figure 3a,b); on the involucre scales, they were more abundant and partly hidden among protective trichomes (Figure 3c). Concerning the corolla, they were absent or sporadic on the tube and abundant on the abaxial side of each of the apical lobes (Figure 3e–h). The ovary appeared hairless (Figure 3d).

3.1.2. Secretory Ducts

The ducts consisted of a lumen bounded by a single/double layer of flattened secreting cells (Figure 4a,b).

Secretory ducts occurred in all vegetative plant parts (Table 1). In the stem, they were located within the cortical parenchyma, generally parallel to the veinal system. They were elongated and appear unbranched in the longitudinal view. In the leaves, too, the ducts ran parallel to the conduction elements (Figure 4a) and no anastomosis could be observed.

In the *capitula*, ducts occurred in the involucre bracts and interfloral scales (Figure 4b), but they were absent in the corolla. In these floral parts, ducts presented a larger lumen in comparison to the vegetative organs and extended along the longitudinal axis.

3.1.3. Histochemistry of the Secretory Material

To assess the histochemical profiles of the secreted material of the biseriate glands and of the internal ducts, we applied specific dyes. The complete results revealed the chemical consistency of the secretory products in both the glandular structures, regardless of the distribution pattern on the vegetative and reproductive organs (Table 2; Figure 5).

Table 2. Results of the histochemical tests on the biseriate glands and secretory ducts of the vegetative and reproductive organs of *S. chamaecyparissus*.

Stainings	Target-Compounds	Biseriate Glands	Secretory Ducts
Fluoral Yellow-088	Total lipids	++	++
Nile Red	Neutral lipids	++	++
Nadi reagent	Terpenoids	++	++
PAS reagent	Total polysaccharides	—	—
Ruthenium Red	Acid polysaccharides	—	—
Alcian Blue	Muco-polysaccharides	—	—
FeCl ₃	Polyphenols	++	++
AlCl ₃	Flavonoids	+	+

Symbols: (—) negative response; (+) positive response; (++) intensely positive response.

The material produced by both secretory structures stained intensively in response to all the applied lipophilic dyes, namely, those to total lipids and neutral lipids (Table 2 and Figure 5e). Nadi's reagent showed drops of varying colors from blue to pale violet in the subcuticular space of the glandular trichomes and in the ducts (indicating the presence of an oleoresin (Figure 5a,c–e); material dark red in color, compatible with the resiniferous nature of the secretion was occasionally observed within the lumen of the duct. The positive reactions to Ferric Trichloride and Aluminum Trichloride demonstrated the presence of phenolic compounds, in particular flavonoids (Table 2; Figure 5b,f). The histochemical tests for polysaccharides and muco-polysaccharides gave negative results in both structures.

3.2. Phytochemical Survey

The chemical compositions of the EOs obtained from the flowered aerial parts of *S. chamaecyparissus* across the two years monitored are reported in Table 3. The obtained oil yields ranged from 0.42% in 2021 up to 0.48% in 2022.

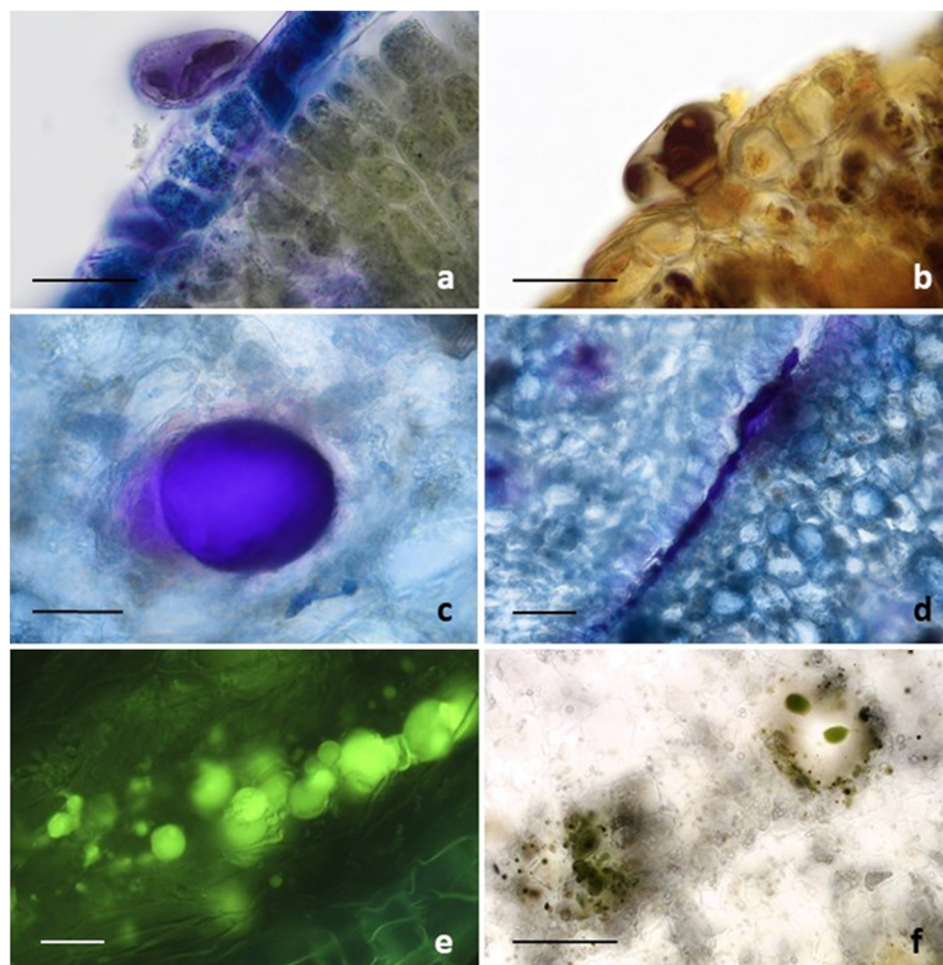


Figure 5. LM micrographs showing the histochemical results on the secretory materials of the biseriate trichomes and ducts of *S. chamaecyparissus*. (a,b) Glandular biseriate trichomes: (a) Nadi reagent; (b) Ferric Trichloride test. (c–f) Secretory ducts: (c,d) Nadi reagent; (e) Fluoral Yellow-088 test; (f) Ferric Trichloride test. Scale bars: 100 μm (d–f); 50 μm (a–c).

The most complex EO profile, characterized by the highest number of total compounds, was obtained in 2022, with 40 components accounting for 98.06%; 39 compounds (97.57% of the total) were found in 2021. An overall quali-quantitative overlap was documented between the two examined profiles, since all the compounds present in 2021 were also isolated in 2022 in comparable relative amounts. The dominant compound classes in all the EOs analyzed were oxygenated monoterpenes (72.67%, 78.61%) and monoterpene hydrocarbons (15.06%, 10.48%), followed by oxygenated sesquiterpenes (5.43%, 5.80%) and by sesquiterpene hydrocarbons (3.28%, 1.98%); non-terpenic derivatives were present in very low amounts, with percentages ranging from 1.13% to 1.19% (Table 3).

The main identified compounds were artemisia ketone (12, 52.74% and 55.67%, respectively) and camphor (16, 13.00% and 16.18%), followed by α -bisabolol (40, 4.05% and 4.49%), camphene (4, 3.45% and 3.42%), β -myrcene (7, 3.30% and 0.92%), α -curcumene (35, 3.04% and 1.79%), and 4-terpineol (20, 2.94% and 1.97%). β -Pinene (6), 3,6-Heptadien-2-ol,2,5,5-trimethyl (8), sabinene (5), and β -phellandrene (11) were found with relative percentages ranging between 1.00% and 1.79% over the two years monitored. The remaining 29 compounds were found with percentage values lower than 1.00%, except santolina triene, found in 2022 oil with a percentage of 1.25%.

Table 3. GC–MS profiles of the essential oils obtained from the flowered aerial parts of *S. chamaecyparissus* collected in 2021 and 2022.

N.	Type	LRI	Compound	Relative Abundance (%)	
				2021	2022
1	MH	890	santolina triene	0.97	1.25
2	MH	920	2,5,5-trimethyl-1,3,6-heptatriene	0.18	0.21
3	MH	931	α -pinene	0.59	0.49
4	MH	949	camphene	3.45	3.42
5	MH	970	sabinene	1.58	0.77
6	MH	976	β -pinene	1.79	1.12
7	MH	990	β -myrcene	3.30	0.92
8	MO	1008	3,6-heptadien-2-ol,2,5,5-trimethyl	1.60	1.99
9	MH	1033	3-carene	-	0.08
10	MO	1034	santolina alcohol	0.64	0.55
11	MH	1038	β -phellandrene	1.06	1.00
12	MO	1079	artemisia ketone	52.74	55.67
13	MO	1092	artemisia alcohol	0.37	0.40
14	MH	1102	<i>p</i> -cymenene	0.18	0.34
15	NH	1105	amyl isovalerate	0.51	0.66
16	MO	1156	camphor	13.00	16.18
17	MO	1172	lavandulol	0.35	0.25
18	MO	1176	pinocarvone	0.25	0.16
19	MO	1183	endo-borneol	0.07	0.09
20	MO	1190	4-terpineol	2.94	1.97
21	MO	1197	<i>p</i> -cymen-8-ol	0.81	0.69
22	MO	1199	cryptone	0.28	0.32
23	MO	1205	myrtenol	0.77	0.63
24	MO	1224	carveol	0.03	0.02
25	MO	1230	methyl thymol	0.49	0.48
26	NH	1234	2-butenic acid, 3-methyl-, 3-methyl-2-butenyl ester	0.10	0.07
27	NH	1238	3-methyl-2-butenyl tiglate	0.11	0.16
28	NH	1250	cuminaldehyde	0.27	0.17
29	MO	1260	piperitone	0.02	0.03
30	MO	1284	phellandral	0.20	0.14
31	NH	1384	benzyl valerate	0.15	0.13
32	SH	1387	β -elemene	0.12	0.13
33	SH	1420	β -caryophyllene	0.02	0.01
34	SH	1448	β -farnesene	0.10	0.04
35	SH	1480	α -curcumene	3.04	1.79
36	SO	1539	italicene ether	0.28	0.17
37	SO	1561	nerolidol	0.65	0.68
38	SO	1587	caryophyllene oxide	0.15	0.19
39	SO	1658	bisabolol oxide II	0.29	0.27
40	SO	1691	α -bisabolol	4.05	4.49
Oil Yields (%)				0.42	0.48
Monoterpene hydrocarbons (MH)				15.06	10.48
Oxygenated monoterpenes (MO)				72.67	78.61
Sesquiterpene hydrocarbons (SH)				3.28	1.98
Oxygenated sesquiterpenes (SO)				5.43	5.80
Aromatic esters (AE)				1.13	1.19
Total identified				97.57	98.06

The main common compounds are highlighted in grey color. LRI = Linear Retention Index, experimentally obtained on a VF-5MS column using a C₇–C₃₀ mixture of *n*-alkanes.

3.3. Scientific Dissemination

The research outcomes reported in the “Micromorphological survey” and “Phytochemical survey” sections were used to design and create an original interpretative and iconographic apparatus for the target species at the Ghirardi Botanic Garden (Toscolano Maderno, Brescia, Italy). Specifically, the left side of the figure presents an original line drawing, specially prepared to highlight the macroscopic diagnostic features of the plant under study (Figure 6). On the right side, a vertical sequence from bottom to top illustrates the main macroscopic traits of the plant, the microscopic characteristics of the secretory structures, the two major EO compounds, and information regarding their potential bio-ecological roles (Figure 6).

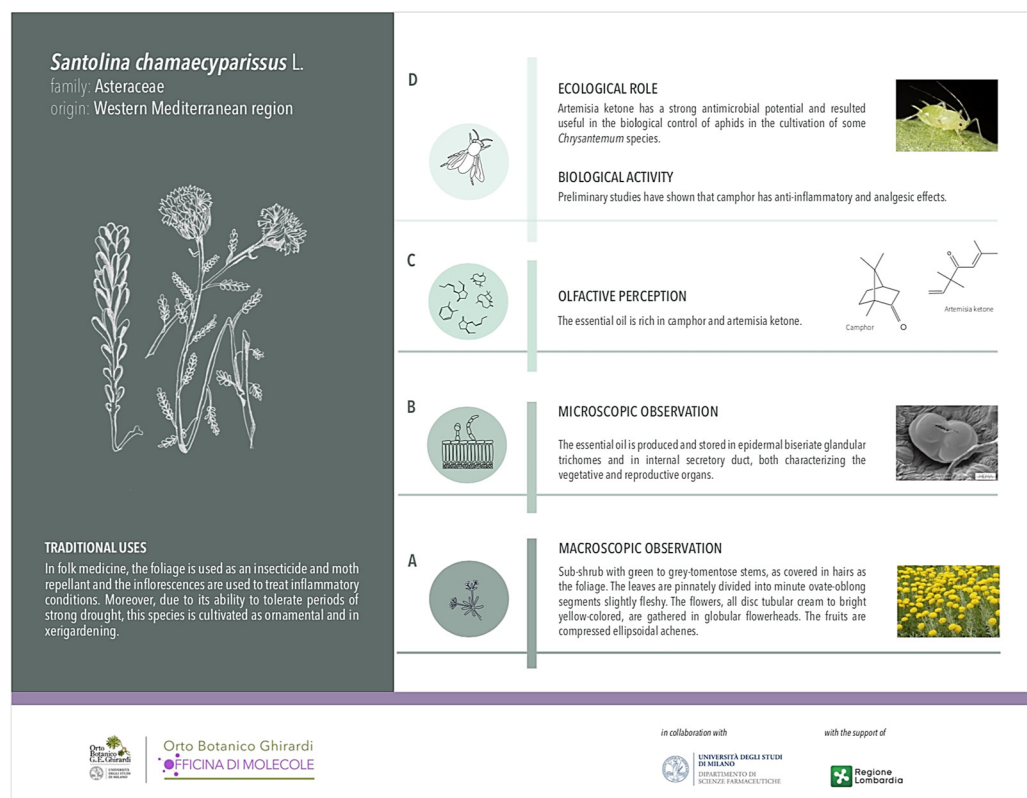


Figure 6. New labeling of *S. chamaecyparissus* L. at the Ghirardi Botanic Garden (University of Milan, Toscolano Maderno, Brescia, Italy). This species was studied under multiple levels: A. Macroscopic observation: description of the macroscopic diagnostic features; B. Microscopic observation: description of the secretory structures; C. Olfactive perception: characterization of the essential oil profile; D. Biological activity and Ecological Role: evaluation of the bio-ecology of the main essential oil components.

4. Discussion

4.1. Micromorphological Survey

The micromorphological survey on *S. chamaecyparissus* together with the definition of the histochemical profile of the secreted material represent significant novel contributions in the panorama of the current literature.

External and internal secretory structures of different types are commonly found in all plant parts of Asteraceae species, though their main variability has been documented at the leaf level [35,36]. In the Anthemideae tribe, in particular, secretory ducts and biseriate glandular trichomes co-occurred [35], as reported in several *Santolina* species [26–29,37].

Concerning the glandular trichomes observed on the target species, they belong to the vesicular morphotype, consistent with the recent proposed updated nomenclature for the leaf glandular *indumentum* in Asteraceae [35].

It was peculiarly recognized by its shorter base and the more compact whole structure compared to the stipitate morphotype (characterized by an elongated and narrow biseriate body and a wider biseriate apex), not observed in the target species and only occasionally described in *Santolina impressa* [30].

The vesicular morphotype was widespread across the entire plant epidermis of the target species, except for the adaxial side of the involucre bracts and the ovary. This distribution pattern was consistent with the observation of the previously examined congeneric species [26–29,35], apart from the corolla. In the latter, indeed, a common trichome distribution in longitudinal rows was documented on the corolla tube, in contrast to the target species, on which they were absent or sporadic on the tube, being abundant only on the apical lobes.

The main classes of compounds found in the secreted material were in accordance with the histochemical results obtained by previous authors for the glandular trichomes of *S. insularis* [37], *S. leucantha* [27,28], *S. ligustica* [29], and *S. impressa* [30]. The histochemical results indeed indicated that trichomes synthesized an oleoresin, along with a phenolic fraction.

In our samples, a similar histochemical profile was also documented for internal secretory ducts, with the production of essential oils and polyphenols. In the previously investigated congeneric species [26–29,37], on the contrary, resiniferous material dominated the secretory products, along with a polyphenolic fraction. Therefore, the available literature data on congeneric species were exclusively consistent to our results concerning the productivity in phenolic compounds.

4.2. Phytochemical Survey

This work represents the first contribution characterizing the EO composition of samples grown in Italy. The EO profiles obtained from the flowered aerial parts of *S. chamaecyparissus* in the two consecutive monitoring years, 2021 and 2022, showed a high quali-quantitative overlay, with regards to the total number of compounds (39 vs. 40), the main chemical classes, and the key single compounds.

The dominant compound classes were oxygenated monoterpenes (72.67% vs. 78.61%) and monoterpene hydrocarbons (15.06% vs. 10.48%), followed by oxygenated sesquiterpenes (5.43% vs. 5.80%). The main identified compounds were artemisia ketone (12), camphor (16), followed by α -bisabolol (40), β -myrcene (7), camphene (4), α -curcumene (35), and 4-terpineol (20).

The scientific literature on the characterization of cotton lavender EO composition is extensive and referred to samples from different provenances: European countries (France, Spain, Greece, Poland, Serbia) [17,38–43], Turkey [14], North Africa (Egypt, Algeria, Tunisia) [15,19,25,26], Saudi Arabia [23], Syria [16], India [44], and China [20].

The aerial parts were the most studied plant material; however, it has never been specified whether the harvesting period corresponded or not to the developmental phase of anthesis, apart for the Egyptian samples (collected both at the bud-stage and at the full blooming stage) [25]. Only three previous publications specifically focused on the flowerheads (samples from Serbia, Spain, and Tunisia) [19,28,39,43]. In the Tunisian samples, roots, properly divided from inflorescences, were also the target of the study [19].

The drying process resulted as the most common conservation procedure and hydro-distillation in a Clevenger type apparatus, the most usual extraction technique.

The oil yields varied significantly between 0.06% and 2.10% (the highest value referred to the analyses of the EOs obtained from fresh leaves from Syria [11]): this variability is likely due to factors such as plant part used, conservation methods, and geographical origin ([43] and literature therein). As for the complexity of the EO composition, the literature indicates profiles typically containing between 34 and 71 identified compounds [14,25,36–49]. Some works reported over 100 constituents, though not all were identified ([43] and literature therein).

Compared to previous studies, the EO composition of the target species exhibited marked chemical polymorphism. The dominant class of compounds consistently included oxygenated monoterpenes. Other notable constituents included oxygenated sesquiterpenes and monoterpene hydrocarbons [43], as also observed in the present samples.

With regard to the main key compounds, a critical review of the literature enabled the identification of several distinct chemotypes ([43] and references therein):

- ✓ Camphor-rich profiles, such as the one observed in the present study, were also reported in Turkish samples (17.7%) [14];
- ✓ Artemisia ketone-dominant profiles, where this compound was the major (as in our samples) or second major component, were found in specimens from Serbia, Spain, India, Greece, and France [17,38–41,43,44];
- ✓ Mixed artemisia ketone and camphor profiles, resembling our findings, characterized the Tunisian samples [19];
- ✓ β -phellandrene-rich profiles, in which this compound was a major constituent (present in our samples only in trace amounts: ~1.0–1.5%), were reported in samples from China [20] and Poland [42];
- ✓ 1,8-cineole-dominant profiles, which differed significantly from our results (compound not detected), were observed in plants from Tunisia [19] and Egypt [25,26];
- ✓ Profiles with *trans-p*-mentha-2,8-dienol, detected exclusively in Saudi Arabian samples [23], suggested a clearly distinct chemical profile compared to those reported elsewhere.

The other main compounds occurring in our samples (α -bisabolol (40), β -myrcene (7), camphene (4), α -curcumene (35), and 4-terpineol (20)) were variously distributed in the EO profiles present in the literature, and it is not possible to identify trends that can lead to associations with the geographical origin.

The chemical polymorphism of *S. chamaecyparissus* EOs was indeed affected by the climatic and environmental conditions, cultivation technique, time of collection, developmental stage, and the infraspecific status of *taxa* [41]. Indeed, regarding this last point, the subspecies to which the investigated samples belonged was never indicated, with the exception of specimens from Spain, belonging to *S. chamaecyparissus* subsp. *squarrosa* [46].

Concerning the literature information on the biological activities recognized for the target species' EOs, analgesic, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, cytotoxic, anticancer, and antidiabetic properties have been found [6,11]. Moreover, they have also potential for application in organic agriculture and for the development of biopesticides, due to their insecticidal, fungicidal, and herbicidal properties [42].

The EO profiles obtained over two consecutive years demonstrate a high stability, with consistent compound identities and relative abundances. This pattern contrasts with the wide chemotypic variation often reported for Mediterranean populations of the same species. Notably, the dominant constituents (artemisia ketone, camphor, α -bisabolol, and β -myrcene) are well-documented for their insecticidal, antimicrobial, and anti-inflammatory properties [42], underscoring the potential biological activity of this chemotype. The stable chemical composition may reflect localized genetic or ecological influences that constrain variability. A slight increase in oil yield and component richness observed in

2022 suggests possible modulation by annual climatic differences or phenological stages at harvest. These findings align with broader trends in EO-producing *taxa*, where abiotic conditions subtly influence secondary metabolite output. Despite minor fluctuations, the overall chemotypic profile remained robust, indicating its reliability for pharmacological or agricultural applications. This stability enhances the value of the regional chemotype for targeted bioactivity exploration and sustainable EO sourcing.

Since a high chemical variability is reported in the literature, and Italian samples have never been investigated before, we evaluated the potential biological activity and the ecological roles of the EOs examined herein; we particularly focused on its key compounds: camphor (16), artemisia ketone (12), α -bisabolol (40), β -myrcene (7), camphene (4), α -curcumene (35), and 4-terpineol (20).

Camphor exhibits a range of biological activities, including anti-inflammatory and analgesic [50]; indeed, it is useful in treating skin irritations [51] and other inflammatory conditions, and can act as a pain reliever, potentially by desensitizing sensory nerves [52]. It is also known for its antitussive effects [53,54]. As to the ecological role, camphor has shown insecticidal and antimicrobial activity against various Gram-positive and Gram-negative bacteria, as well as fungi like *Candida albicans* [55–57].

Artemisia ketone displays antioxidant activity and antimicrobial properties, against various fungi and bacteria [58]. In addition, the combination with camphor seemed to have a more significant impact on certain antifungal activities [57].

α -Bisabolol can reduce inflammation and it is an antioxidant. It also has neuroprotective, analgesic, anticancer, and anti-genotoxic effects [59]. As to the ecology, this compound exerts a multifaceted ecological role, encompassing allelopathic and antimicrobial effects [60].

β -Myrcene possesses analgesic, sedative, antioxidant, anti-inflammatory, anticancer, and antidiabetic properties [61]. In addition, this compound can attract natural enemies of herbivores, such as parasitic wasps, creating a beneficial plant–herbivore–natural enemy interaction. In some cases, it may attract pollinators or seed dispersers, facilitating plant reproduction [62].

Camphene exhibits anticancer, antioxidant, anti-inflammatory, and hypolipidemic (cholesterol-lowering) effects. It is also being investigated for its potential antiviral properties and its ability to protect against myocardial ischemia [63]. Camphene acts as a plant defense mechanism by exhibiting allelopathic effects and deterring herbivores and pathogens; for instance, camphene has shown larvicidal and ovicidal activity against *Helicoverpa armigera*, a pathogen for many important cultivated crops [64].

4-Terpineol and α -curcumene both exhibit a range of biological activities, including anti-inflammatory and anticancer effects [65,66]. They also have shown potential in natural defense mechanisms for plants, protecting them from microbial infections and oxidative stress [65,66].

Moreover, *S. chamaecyparissus* can be added to food, condiments, and beverages to improve flavor and enhance their properties [43].

Oxygenated monoterpenes, such as artemisia ketone (13), are responsible for herbal fragrance, while monoterpene hydrocarbons with compounds like camphene (4) and α -myrcene (7) contribute to woody and spicy notes [6,17,41]. Oxygenated sesquiterpenes, such as nerolidol (37), bisabolol oxide II (39), and α -bisabolol (40), shift toward more floral and sweet notes [6,17,41]. Aromatic esters, such as benzyl valerate, remained at minimal levels across the years, suggesting that they play a negligible role in the oil's overall fragrance.

In light of this evidence, the traditional uses of the target species based on its anti-inflammatory and sedative properties, as well as insecticide and moth repellent appear

to be validated by the scientific literature regarding bio-ecological activity. In addition, based on the composition of the EO analyses herein, it is reasonable to suggest that the investigated EOs may possess therapeutic properties like those discussed here. Finally, compounds present in our EO profile suggest a potential use in agriculture as a biopesticide and in the food sector as a flavoring agent.

In conclusion, the essential oil of *S. chamaecyparissus* from Italy consistently demonstrates the dominance of oxygenated monoterpenes, particularly artemisia ketone and camphor. These compounds are central to its therapeutic properties, especially the antimicrobial and anti-inflammatory effects. The presence of oxygenated sesquiterpenes further enhances its therapeutic potential, aligning with current trends in EO research.

4.3. Scientific Dissemination

The newly designed and original labeling of the target species at the Ghirardi Botanic Garden is crucial for both research and public engagement. Labels typically include information on scientific and common names, botanical family, origin, and sometimes additional data. This certainly ensures accurate identification and provides valuable educational information for visitors. However, the new interpreting outdoor facility goes beyond this concept, since visitors' knowledge will further be enriched with the results of the scientific research conducted just on the plant individuals preserved at the Botanic Garden. In this way, the public enhances its consciousness on the plant heritage at the study site, understanding (i) the therapeutic potential or the ecological role of the main secondary metabolites produced; (ii) the diagnostic macroscopic and microscopic traits by means of appealing iconographic contents. Moreover, this additional label also improves the analogous interpretative proposals already present at the Botanic Garden for other previously investigated species, in order to realize a thematic pathway within the ongoing overall project [67–69].

5. Conclusions

This multidisciplinary work on *S. chamaecyparissus* led to the following considerations:

- (i) The micromorphological approach, combining digital light and fluorescence microscopy with scanning electron microscopy, allowed us to describe for the first time the non-glandular *indumentum* and the glandular structures of both the vegetative and reproductive organs; specifically, our survey demonstrated a great affinity regarding the micromorphology and the distribution pattern of the secretory structures between the target species and the congeneric *taxa* known in literature, whereas the histochemistry proved to be divergent, with the homogeneous production of oleoresin and polyphenols in both the glandular hairs and the internal ducts.
- (ii) The characterization of the EO profiles from Italian samples represented an element of novelty in the panorama of the literature. The EOs composition proved to be repeatable in the analyzed samples and confirmed the dominance of oxygenated monoterpenes, with artemisia ketone and camphor as key single components. The literature data on the bio-ecology of these two compounds, along with other major molecules, supported the potential of the Italian oil in the therapeutical and agricultural fields.
- (iii) The scientific results were combined into the creation of an appealing interpretative outdoor facility for the target species at the study site; this strategy falls within the Open Science policies, aimed at promoting practices that make scientific research more transparent, accessible, and collaborative.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11101184/s1>, GC-MS chromatograms of the essential oils.

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