A Review of the Traditional Uses, Phytochemistry and Biological Activities of the Genus Santolina

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ABSTRACT
The genus Santolina is a taxonomically complex group of plant species widely distributed in the Mediterranean flora and used in traditional medicine since ancient times for their biological properties, including antimicrobial, anti-inflammatory, anti-spasmodic, digestive, and analgesic activities. Phytochemical investigations of Santolina species have revealed the presence of terpenoids as the main bioactive constituents of the genus. Coumarins and flavonoids were also identified. This review deals, for the first time, with information on the traditional uses, chemical profile, and biological properties of plants of the genus Santolina in order to provide input for future research prospects.

ABBREVIATIONS
GPT glutamate pyruvate transaminase
HD hydrodistillation
HSV-1 herpes simplex type 1
HSV-2 herpes simplex type 2
MIC minimum inhibitory concentration
MPLC medium-pressure liquid chromatography
NF-κB nuclear factor-kappa B
PLA1 phospholipase A1
PLA2 phospholipase A2
SFE supercritical fluid extraction


S. chamaecyparissus L., S. pectinata Lag., and Santolina viridis W. are the most widespread [1]. With increasing interest in bioactive secondary metabolites from Santolina spp., several studies related to the investigation of the phytochemical composition and biological properties of species from this genus have been carried out. In recent decades, phytochemical studies have discovered the presence of terpenoids, particularly eudesmane and germacrene sesquiterpenoids, chrysanthemane monoterpenoids [2,3], dammarane-type triterpenes [4], flavonoids [5,6], and coumarins

Introduction
The Santolina genus (family Asteraceae, tribe Anthemideae) comprises species widely distributed in the Mediterranean area. The Plant List includes 103 scientific plant names of species of this genus. However, of these, only 20 are accepted species names (San...
Santolina species | Local name | Traditional uses | Part used | Country | Ref. |
--- | --- | --- | --- | --- | --- |
S. chamaecyparissus | lavender cotton, gray santolina | antispasmodic, digestive, analgesic, anti-inflammatory, antiseptic, stimulant, and antimicrobial; to treat dermatitis | inflorescence | Mediterranean area, India | [11–14] |
S. chamaecyparissus subsp. squarrosa | manzanilla, manzanilla de monte, manzanilla basta, manzanilla de burro | to treat ophthalmological problems, headache, belly pain, stomach problems, and as digestive and depurative | inflorescence | Spain | [15,16] |
S. corsica | crespolina di Corsica | intestinal vermifuge, parasite repellent | inflorescence | Italy | [19,20] |
S. etrusca | canfora | antiparasitic | aerial parts | Italy | [21] |
S. insularis | crespolina maggiore, santolina, crespolina sarda | vermifuge and to repel insects | inflorescence | Italy | [19] |
S. insularis | crespolina insulare | sedative, febrifuge, and antitussive | leaves | Italy | [18] |
S. neapolitana | green santolina, crespolina napoletana | cough suppressant | aerial parts | Italy | [17] |
S. oblongifolia | manzanilla de Gredos | anti-inflammatory and digestive | inflorescence | Spain | [7] |
S. rosmarinifolia | green lavender cotton | antipyretic, antihypertensive, hepatoprotective, and anti-inflammatory | flower heads | Spain | [22,26] |

Table 1 Ethnomedicinal uses of Santolina species.

[6,7] in plant species of this genus. The literature data revealed that Santolina species show various biological activities, including antibacterial, antifungal, antiviral, anti-inflammatory, cytotoxic, and hepatoprotective effects [2,5,8–11].

The aim of this review is to provide a complete overview of existing knowledge on the traditional uses, chemical constituents, and biological properties of plant species from the Santolina genus.

The available information on this genus was collected from scientific databases up until November 2017. The following electronic databases were used: PubMed, SciFinder, Science Direct, Scopus, Web of Science, Wiley, ACS, Springer, and Google Scholar. The search terms used for this review included Santolina, Santolina chamaecyparissus, Santolina insularis, Santolina corsica, Santolina oblongifolia, Santolina canescens, Santolina neapolitana, Santolina pinnata, Santolina pectinata, Santolina rosmarinifolia, Santolina etrusca, Santolina semidentata, Santolina tincoria, Santolina villosa, Santolina virens, phytochemical composition, essential oils, sesquiterpenes, phenols, flavonoids, coumarins, traditional uses, activity, pharmacology, and toxicity. No limitations were set for languages. “The Plant List” (www.theplantlist.org) was used to validate the scientific names of the Santolina species. Seventy-five potentially relevant records were found, from which seven were excluded after screening the titles or abstracts.

From the available literature, two species, namely, S. chamaecyparissus and S. insularis, have been the most investigated. S. corsica, S. oblongifolia, S. canescens, S. rosmarinifolia, and S. etrusca have also been phytochemically and biologically studied. S. pectinata and S. semidentata were investigated for the chemical composition of their essential oil, but no biological studies were described.

Critical evaluation of biological studies in terms of their relation to the chemical profile is highlighted. Available information on these species allows us to provide the scientific basis for future research studies and to explore their potential therapeutic use.

Traditional Uses

For a long time, several plant species have been used for medicinal purposes. Among Santolina species, S. chamaecyparissus, S. etrusca, S. insularis, S. neapolitana, and S. oblongifolia have been reported in traditional medicine. A summary of their traditional use is presented in Table 1. S. chamaecyparissus has been inserted in the Ayurvedic system of medicine in India for the treatment of liver diseases. Moreover, the yellow inflorescences of S. chamaecyparissus are widely used in Mediterranean traditional medicine for their analgesic, anti-inflammatory, antispasmodic, antiseptic, and antimicrobial properties [11,12]. Moreover, this plant, commonly called “lavrader” or “gray santolina”, is employed for the treatment of numerous kinds of dermatitis, as a stimulant, and as a stomachic [13,14]. In the traditional medicine of Spain, S. chamaecyparissus subsp. squarrosa was used as a substitute for Artemisia granatensis, “manzanilla real”, for the treatment of ophthalmological problems, headache, belly pain, stomach problems, and as a digestive and depurative [15,16]. This species is locally named “manzanilla de monte”, “manzanilla basta”, and “manzanilla de burro” [16].

Savo et al. [17] reported the use of S. neapolitana (= S. pinnata subsp. neapolitana) against cough in the Amalfi Coast (Campania, Southern Italy). A decoction of the leaves of S. insularis, known in Italy as “crespolina maggiore”, has been used as a sedative, febrifuge, and antitussive [18]. The whole plant was used as an intestinal vermifuge against horse strongyloidsis and as a parasite repellant [19,20]. Another Santolina species used in Italy as an intestinal vermifuge and parasite repellent is S. corsica [19,20]. S. etrusca (“canfora”) is known in Italy for its traditional uses. This is an endemic species growing in the gravel beds of rivers, clayey, and arid hills, and is found exclusively in Central Italy, particularly in Northern Latium, Tuscany, and Umbria. Its antiparasitic activity has been reported [21]. In the traditional medicine of Spain, S. oblongifolia, known as “manzanilla de Gredos”, is used as a digestive...
sedative and tonic, and for the treatment of rheumatism and menstrual disorders [7]. This species is known for its mild flavor and sweetness, and for its beneficial effects on the digestive tract [7].

S. rosmarinifolia, known as “green lavender cotton”, is a dwarf perennial shrub mainly distributed in the Iberian Peninsula and South France. S. rosmarinifolia flower heads (fresh or dried) are used in traditional preparations for their antihypertensive, anti-inflammatory, antipyretic, and hepatoprotective properties [22].

### Phytochemicals

Phytochemical investigations on Santolina species revealed the presence of several classes of constituents. Eudesmane-type sesquiterpenes [23–27], germacrane-type sesquiterpenes [2, 23–26], dammarane-type triterpenes [4], acetylene heterocycles [4, 28, 29], spiroketalenol ether-type acetylenes [4, 24, 27–29], flavonoids [5, 6], and coumarins [6, 7, 27] have been identified as common secondary metabolites from the genus. Several Santolina species have been studied for their essential oil composition. S. chamaecyparissus is one of the most investigated Santolina species. A different chemical composition, depending on its origin, was evidenced by several researchers (Table 2) [1, 30–35]. The HD of the flower heads of S. chamaecyparissus from Tunisia has allowed for obtaining an oil rich in 1,8-cineole (12.94%), β-eudesmol (10.49%), terpinene-4-ol (6.97%), γ-cadinene (6.55%), spathulenol (5.80%), camphor (5.27%), germacrene D (5.03%), and myrtenol (4.26%) [35]. The oil of S. chamaecyparissus obtained by HD of the air-dried aerial parts collected in Turkey is characterized mainly by monoterpenes (81% of the total oil) [34]. Oxygenated monoterpenes predominated over the monoterpenes hydrocarbons. The main constituents are artemisia ketone (38.1%), camphor (11.7%), β-phellandrene (9.2%), α-bisabolol (6.6%), and myrcene (4.3%). Artemisia ketone is also the dominant constituent of the essential oil of S. rosmarinifolia from India [36]. Other abundant compounds are 1,8-cineole (15.6%), myrcene (14.2%), germacrene D (8.8%), sabinene (4.5%), and terpinin-4-ol (2.9%). Artemisia ketone (15.6%) together with α-amorphene (12.11%), β-phellandrene (10.63%), β-myrcene (7.42%), and nootkatone (6.97%) were identified as the main compounds of the oil from Syria [37]. The composition of this oil greatly differed from the oil of S. chamaecyparissus collected in Algeria [38].

![Table 2 The main volatiles of the essential oils from Santolina species.](image-url)
fact, Djeddi et al. [38] showed camphor (31.1%), cubenol (17.0%), p-cymene (8.3%), and sabinene (4.0%) as the most abundant constituents. Instead, as in the oil from India, the essential oil of _S. chamaecyparissus_ collected in France [33] and in Italy [39] showed artemisia ketone as the dominant constituent. Monoterpene hydrocarbons (29.5%) and oxygenated monoterpenes (36.81%) are the main classes of the essential oil of _S. chamaecyparissus_ from Italy [39], in which, besides artemisia ketone (28.24%), the other abundant constituents are β-phellandrene (12.78%), myrcene (8.02%), and sabinene (7.65%).

Grosso et al. [40] compared the essential oil of _S. chamaecyparissus_ (Spain) obtained by SFE with the essential oil obtained by HD, and found 1,8-cineole to be the most abundant component (7–48%) and 25–30% for SFE and HD, respectively, followed by camphor (8–14 and 7–9% for SFE and HD, respectively), borneol (2–11 and 7–8% for SFE and HD, respectively), terpinen-4-ol (1–4 and 6–7% for SFE and HD, respectively), and terpinolene (1–7 and 1–4% for SFE and HD, respectively). In previous studies, some _S. chamaecyparissus_ subspecies from Spain were described to contain 1,8-cineole (2–18%), artemisia ketone (0.1–28%), camphor (trace–43%), borneol (1–28%), copaenol (trace–15%), allo-alpha-dendrene (19%), and cubenol (1–17%) as major constituents [12, 30]. Three works reported the chemical profile of the essential oil from _S. corsica_, a perennial shrub growing in the rocky places of Corsica (France) and Sardinia (Italy). The essential oil of _S. corsica_ collected in Corsica was dominated by monoterpene hydrocarbons. Artemisia ketone (20.0%), β-phellandrene (14.4%), myrcene (11.7%), santolinatriene (8.2%), 1,8-cineole (4.4%), and β-pinene (4.3%) were identified as the main compounds [41].

Except for artemisia ketone, found with a percentage of 0.1%, myrcene (34.6%), santolinatriene (13.5%), and β-phellandrene (11.7%) were found to be the most abundant compounds in the Corsican essential oil in a study by Liu et al. [8]. Otherwise, the essential oil from _S. corsica_ collected in Sardinia (Italy) showed camphor (18.5%), artemisia ketone (12.97%), borneol (7.41%), α-madadrene (5.55%), and muurolene (4.63%) as the main constituents [20]. 3,3,6-Trimethyl-1,5-heptadien-4-one (21.1%), 10-H-cyclopentyl-1,1,7-trimethyl-4-methyldecahydro azulene (12.7%) cineole (9.01%), camphene (8.47%), bornyl acetate (6.35%), and borneol (4.23%) were the main constituents of the essential oil of _S. insularis_ collected in Sardinia (Italy) [20]. A different composition with myrcene (14.8–17%), β-phellandrene (8–9%), ar-curcumene (6–10%), and trans-β-terpineol (5–6%) as the main constituents was reported by Cherchi et al. [42] for the essential oils obtained from the aerial parts of _S. insularis_ collected in the same Italian region (Sardinia).

More recently, Gnvi et al. [43] studied four _S. insularis_ samples from Sardinia (Italy). Artemisia ketone, cis-chrysanthenol, myrcene, β-phellandrene, β-pinene, and santolinatriene were identified as the most abundant constituents. However, different percentages of these main compounds were reported. This work showed remarkable chemical variation in the terpenoid profile and a consistent genomic difference in the 55S-rRNA spacer regions that led to identifying four chemotypes of _S. insularis_ grouped into two ecotypes. The analysis of these data showed a high variability in the composition and content of monoterpene and sesquiterpenes of _S. insularis_ essential oils from the same geographical area. Two isomeric irregular sesquiterpenes, 3,9-dimethyl-6-isopropyl-2(E),7(E),9-decatrien (1) and 3,9-dimethyl-6-isopropyl-2(Z),7(E),9-decatrien (2), were isolated from the essential oil obtained by HD from the leaves of _S. corsica_ (France) [Fig. 1 and Table 3] [44]. The oil was subjected to flash column chromatography on silica gel, affording a nonpolar and a polar fraction. The polar fraction was purified by column chromatography on silica gel using pentane with increasing amounts of diethyl oxide as the eluent. Compounds 1 and 2 were isolated from subfractions using pentane/diethyl ether (95/5) as the eluent. Their structure was elucidated by using 1D and 2D NMR spectroscopy. The essential oil of _S. pectinata_ contained β-eudesmol, nerolidol, spathulenol, α-cadinol, γ-eudesmol, and elemol as the major constituents [45]. The essential oil of _S. semidentata_, an endemic species of Spain, was characterized by the presence of β-eudesmol, nerolidol, spathulenol, α-cadinol, τ-cadinol, γ-eudesmol, and elemol as the major components [45].

The essential oil of _S. neapolitana_ obtained by HD from aerial parts collected in Italy with a yield of 0.30% (v/v) was characterized by 41 constituents [46]. γ-Muurolene (31.9%), α-pinene (15.5%), and borneol (9.4%) were the most abundant compounds. Myrcene (12.0%), 1,8-cineole (11.1%), terpinen-4-ol (9.9%), and sabine (6.7%) were the main constituents of the essential oil of _Santolina ligustica_ aerial parts [47]. _S. ligustica_ is an endemic _Santolina_ species that grows in Eastern Liguria (Italy), generally on ophiolitic substrates. Other compounds found in good amounts were myrtanol (4.7%), γ-terpinene (4.3%), β-pinene (4.2%), and bisabolol (3.1%).

Flamini and Cioni [48] evaluated the seasonal variation of the components of the essential oil of inflorescences and fertile and sterile branches of another _Santolina_ species from Italy, _S. etrusca_. The obtained results showed that the extraction yields generally increased from November to June, while decreasing in the months of August and September. Taking into account the variability of the oil during the whole year, the most abundant compounds belong to the class of monoterpene hydrocarbons, mainly, β-pinene, myrcene, and sabine, and to the class of oxygenated monoterpenes, mainly 1,8-cineole. Twenty-six compounds characterized...
the essential oil obtained by the flowers of *S. africana* collected in Algeria. The oil showed monoterpene hydrocarbons (27.56%) as the main class of constituents, followed by sesquiterpenes hydrocarbons (26.89%). The main compounds were acenaphthene (25.23%), calarene (21.54%), and ocimene (17.44%) [49]. Instead, Zaiter et al. [50], analyzing the essential oil of *S. africana* within the same country of origin (such as Algeria), reported β-pinene (12.78%), 1,8-cineol (10.02%), myrcene (6.94%), curcumene (7.96%), spathulenol (5.96%), and β-eudesmol (14.58%) as the main components.

Santolindiaceteline (28.5%), camphor (12.5%), myrcene (5.6%), and β-phellandrene (5.4%) were the main constituents identified in the essential oil of *S. canescens* from Spain, obtained by HD [51]. Other compounds found in a good amount were *allo-
aromadendrene (4.4%), β-caryophyllene (4.1%), borneol (4.0%), α-terpineol (3.5%), and germacrene D (3.0%). A previous study reported camphor as the main constituent of the essential oil of *S. canescens*, followed by 1,8-cineole, β-pinene, myrcene, sabi-nene, ar-curcumene, and β-eudesmol [45].

A total of 41 compounds were identified in the essential oil from the flower heads of *S. rosmarinifolia* from Romania, which were rich in oxygenated monoterpenes and oxygenated sesqui-terpenes. β-Eudesmol (13.5%), 1,8-cineole (12.9%), camphor (8.0%), borneol (5.1%), ar-curcumene (4.8%), terpinen-4-ol (4.5%), and spathulenol (4.4%) were the main constituents [52].

In an evaluation of the seasonal variation of the essential oil from the aerial parts of *S. rosmarinifolia* subsp. *rosmarinifolia* collected in Spain, β-phellandrene (14.4–27.6%), β-pinene (17.0–26.5%), limonene (2.7–5.2%), and myrcene (0.3–15.3%) were identified as the main constituents [53]. Moreover, it was found that the oil concentration showed a positive correlation with precipitation and a negative correlation with temperature. Specifically, the monoterpenes 1,8-cineole, limonene, and β-phellandrene correlated negatively with temperature, while capillene showed a positive correlation with precipitation. The other compounds did not show any manifest trend.

The aerial parts of *S. pinnata* collected in Tuscany (Italy) were characterized by the presence of seven flavonoids, namely, apigenin (3), chrysoeriol (4), luteolin (5), luteolin-7-glucoside (6), kaempferol (7), pectolinarigenin (8), and nepe- tin (9), and two coumarins, namely, scopoletin (10) and fraxetin (11) [6]. One new [1α,10β-epoxy-7αH-germacr-4(15)-ene-2β5α,6β-triol] (12) and four known germacrane derivatives [4β,5α-epoxy-7αH-germacr-1(10)E-ene-2β,6β-diol] (13), its 2-acetate 14, 7αHgermacra-1(10)E,4(15)-diene-2β,5α,6β-triol (15), and its 2-acetate 16 were isolated from the aerial parts of *S. pinnata* subsp. *neapolitana* [Fig. 3] [54]. Compounds 13–16 have also been previously isolated from *S. chamaecyparissus* [25].

Three eudesmane sesquiterpenoids, (1R,2R,5R,6R,7S,10S)-eudesma-4(15)-en-1,2,6-triol (17), (1R,2S,5R,7S,10S)-1,2,6-trihydroxyeudesma-4-en-3-one (18), and (1R,2R,7S,10S)-eudesma-3,5-dien-1,2-diol (19), and the trans-chrysanthemyl monoterpe-noid (2R,3R,4R)-5-chrysanthemen-1,4-diol (20) have been isolated from the acetone extract of the defatted aerial parts of *S. insula-ris* from Italy [Fig. 4] [3]. Specifically, the acetone extract was subjected to MPLC, affording two fractions that were further purified by HPLC.

A new monocyclic sesquiterpenic alcohol, elegansidiol (21), was isolated from the hexane extract of *S. elegans* [Fig. 5] [55].
From the aerial parts of *S. chamaecyparissus* subsp. *squarrosa*, collected in Sierra Nevada (Granada, Spain), four new sesquiterpenes, (4E,9Z)-6β-acetoxy-7αH-germacra-4,9-diene-1,2β-diol (22), (E)-6β-acetoxy-7αH-germacra-4,10(14)-dienes-1,2β-diol (23), (E,6β)-acetoxy-7αH-germacra-1(10),4-diene-2β-ol (24), and 6β-acetoxy-5βH,7αH,10βMe-eudesm-4(15)-ene-1α,2β-diol (25) (▶ Fig. 5), were isolated [23]. The air-dried aerial parts of *S. chamaecyparissus* subsp. *squarrosa* were subjected to maceration by using t-butylmethyl ether as the solvent. A portion was defatted by precipitation in methanol at a low temperature. The defatted extract was subjected to column chromatography by using the mixture hexane-t-butylmethyl ether-ETOAc of increasing polarity as the eluent. New sesquiterpenes with a germacrane skeleton were isolated from the aerial parts of *S. rosmarinifolia* subsp. *canescens* [24]. The air-dried and powdered aerial parts of *S. rosmarinifolia* subsp. *canescens*, collected in Sierra Nevada (Granada, Spain), were extracted by the Soxhlet apparatus with hexane as the solvent. After removal of the solvent, the obtained residue was dissolved in chloroform. This mixture was added to methanol at 50°C, allowed to cool to room temperature, then further cooled at −10°C for 24 h, yielding an insoluble fraction that was purified by column chromatography. The six main fractions collected were subjected to repeated separations by silica gel chromatography. The isolated compounds were (E)-7αH-germacra-1(10),4(15)-dien-5α,6β-diol (26), 4β,5α-epoxy-7αH-germacra-1(10)-en-1β,6β-diol (27), (E)-6α,11-dihydroxy-7αH-germacra-4,10(14)-dien-1-one (28), (E)-7αH-germacra-4,10(14)-dien-1α,6α,11-triol (29), (E,7αH-germacra-4,10(14)-dien-1β,6α,11-triol (30), (E)-7αH-germacra-4,10(14)-dien-1α,6β-diol (31), (1α,4E)-7αH-germacra-1(10),4-dien-6β-ol (32), and shiromool (33) (▶ Fig. 6). The aerial parts of *S. insularis* collected in Italy were sequentially extracted with n-hexane and acetone. The acetone extract was subjected to MPLC to afford, after purification by HPLC, 11 germacrene sesquiterpenes, four of which are new [2]. The new compounds are (2R,5R,6R,7S)-germacra-1(10)E,4(15)-dien-5-hydroperoxy-2,6-diol (34), (2R,5R,6R,7S)-germacra-1(10)E,4(15)-dien-5-hydroperoxy-2,6-diol-2-ace- tate (35), (1R,2R,4S,5S,6R,7S)-4,5-epoxygermacra-9Z-en-1,2,6-triol (36), and (3R,6R,7S)-3,6-dihydroxygermacra-4(5)E,10(14)-dien-1-one (37) (▶ Fig. 7).

Biological Properties

Several *Santolina* species have been studied for their biological properties. The antimicrobial, antifungal, antiviral, and anti-inflammatory activities were mainly investigated. However, most of the literature data concern in vitro studies (▶ Table 4). Few studies are performed by using in vivo models.

Antibacterial and antifungal

In the last years, the search for new antibiotics has accelerated. There are two main reasons. Nearly every antibiotic used today is based on a discovery of more than 30 years ago. At the same time, multidrug-resistant bacteria have been observed with increasing frequency over the past several decades. Many plants have been used for their antimicrobial properties due to their constituents, including phenolic compounds and essential oils. The essential oil of several *Santolina* species was subjected to investigations against different gram-positive bacteria, gram-negative bacteria, and fungi. The essential oil of *S. corsica* was tested in vitro for its antimicrobial properties by using the agar diffusion method against two gram-positive bacteria (*Staphylococcus aureus* and *Listeria innocua*) and four gram-negative bacteria (*Campylobacter jejuni*, *Enterobacter aerogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*) [8]. The essential oil inhibited the growth of *S. aureus* (14.7 mm) and, especially, the growth of *C. jejuni* (39 mm). A moderate antimicrobial activity was found against *L. innocua*.
(9.5 mm), while the growth of *E. aerogenes*, *E. coli*, and *P. aeruginosa* was not inhibited. In order to identify the compounds responsible for this activity, fractions were tested. An interesting antimicrobial activity of a lyratol-rich fraction (84%) was observed against *C. jejuni* (90 mm) and *S. aureus* (19 mm), suggesting that lyratol could be the main responsive of the antimicrobial properties of *S. corsica*.

A bactericidal action was recognized for *S. corsica* essential oil, which rapidly inhibited the cell viability of *S. aureus* (MIC of 5 mg/mL) [56]. After treatment with the MIC value and 8 times the MIC value of *S. corsica* essential oil, no lytic effect was observed. The cell wall and the cytoplasmic membrane are involved in the activity of *S. corsica* essential oil. In fact, invaginations of the plasmic membrane with thickenings of the cell wall and aggregations of the cytoplasmic contents were detected in *S. aureus* treated with the *S. corsica* essential oil at the MIC value.

A study reported on the antimicrobial activity of the essential oil *S. rosmarinifolia* flower heads [51]. The essential oil showed good activity against the gram-positive bacteria *Bacillus cereus*, *S. aureus*, and *Sarcina lutea*, and a minor activity against the fungus *Candida albicans* and the gram-negative *E. coli*. The strongest activity was reported against *S. aureus* with MIC and MBC values of 0.3 and 0.6 µL/mL, respectively. The antimicrobial effects of *S. chamaecyparissus* essential oil were studied in different works. The oil of *S. chamaecyparissus* from Algeria was tested by the agar disc diffusion method against the gram-negative bacteria *Bordetella bronchiseptica*, *E. coli*, *Klebsiella pneumonia*, and *P. aeruginosa*, the gram-positive bacteria *Enterococcus faecalis*, *Micrococcus luteus*, *S. aureus*, and *Staphylococcus epidermis*, the fungus *C. albicans*, and the yeast *Saccharomyces cerevisiae* [38]. The essential oil strongly inhibited the growth of *C. albicans* and *K. pneumonia*. Interesting results were also obtained with the essential oil of *S. chamaecyparissus* collected in Tunisia [35]. This essential oil

### Table 4 Investigated bioactivities of Santolina species.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Species</th>
<th>Test/bacteria/fungi/yeast/cell line</th>
<th>Sample</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflamatory</td>
<td><em>S. chamaecyparissus</em></td>
<td>PLA2-induced mouse paw edema</td>
<td>methanol extract</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td><em>S. chamaecyparissus</em></td>
<td>carrageenan paw edema in rats</td>
<td>chloroform extract</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td><em>S. chamaecyparissus</em></td>
<td>inhibition of PLA1</td>
<td>methanol extract</td>
<td>[60]</td>
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<tr>
<td></td>
<td><em>S. insularis</em></td>
<td>croton oil-induced dermatitis in mouse ears</td>
<td>methanol extract</td>
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<td><em>S. oblongifolia</em></td>
<td>adjuvant carrageenan-induced inflammation, ACI, model using Wistar male rats</td>
<td>hexane, dichloromethane, ethyl acetate, and methanol extracts</td>
<td>[61]</td>
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<td></td>
<td><em>S. oblongifolia</em></td>
<td>ionophore-stimulated mouse peritoneal macrophages</td>
<td>hexane, dichloromethane, ethyl acetate, and methanol extracts</td>
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<td></td>
<td><em>S. viscosa</em></td>
<td>NF-kB, IL-6, IL-8, TNF-α, PGE2</td>
<td>petroleum ether, ethyl acetate, and methanol extracts</td>
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<td><em>S. chamaecyparissus</em></td>
<td>C. albicans</td>
<td>essential oil</td>
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<td><em>S. corsica</em></td>
<td>C. jejuni, <em>E. aerogenes</em>, <em>E. coli</em>, L. innocua, <em>P. aeruginosa</em>, <em>S. aureus</em></td>
<td>essential oil</td>
<td>[8, 56]</td>
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<td><em>S. etrusca</em></td>
<td>S. ferax</td>
<td>aqueous and methanol extracts</td>
<td>[57]</td>
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<td><em>S. rosmarinifolia</em></td>
<td>B. cereus, C. albicans, <em>E. coli</em>, S. aureus, S. lutea</td>
<td>essential oil</td>
<td>[51]</td>
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<td>Antiviral</td>
<td><em>S. insularis</em></td>
<td>HSV-1 and HSV-2</td>
<td>essential oil</td>
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<td></td>
<td><em>S. insularis</em></td>
<td>HSV-1</td>
<td>liposome-incorporated essential oil</td>
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<td>Cytotoxic</td>
<td><em>S. chamaecyparissus</em></td>
<td>MCF-7, HCT116, A549, HepG2 human tumor cell lines</td>
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<tr>
<td>Hepatoprotective</td>
<td><em>S. canescens</em></td>
<td>carbon tetrachloride-induced hepatotoxicity in Wistar rats model</td>
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was investigated against seven strains of fungi (three dermatophytes, Epidermophyton floccosum, Microsporum canis, and Trichophyton rubrum; one opportunistic pathogenic yeast, C. albicans; and three hyphalmycetes, Aspergillus fumigatus, Scopulariopsis brevicaulis, and Scytalidium dimidiatum), two gram-positive bacteria (E. faecalis and S. aureus), and four gram-negative bacteria (E. coli, P. aeruginosa, Proteus mirabilis, and Citrobacter freundii) [35]. Except for C. albicans, the inhibition of the fungi growth rate varied in the range of 73.0–89.25% in the presence of 500 µg/mL S. chaemecyparissus essential oil. E. floccosum was the most sensitive to the flower head essential oil (89.25%). The MIC values varied in the range from 500 to 1000 µg/mL. Generally, S. chaemecyparissus oil showed antibacterial activity against all bacterial strains with MIC values in the range of 0.625–10 µg/mL. In the agar diffusion method, the gram-positive bacterium E. faecalis (inhibition zone of 26 mm) was the most susceptible microorganism to the action of the flower head essential oil. S. chaemecyparissus essential oil from India showed potent antifungal activity against C. albicans, with MICs from 62.5 to 125 µg/mL of broth [9]. Clotrimazole was used as a positive control. The drug showed an MIC value of 3.125–6.25 µg/mL. When the mixture of S. chaemecyparissus oil and clotrimazole at the D4 dilution (31.25 and 3.125 µg/mL for the essential oil and clotrimazole, respectively) was tested, the MIC was effective in controlling C. albicans. The S. chaemecyparissus oil was also effective in vivo in controlling experimental vaginal candidiasis. This activity was comparable to that of clotrimazole. The essential oil of S. africana demonstrated activity against several microorganisms (standard strains Bacillus subtilis, E. faecalis, E. coli, P. aeruginosa, Proteus vulgaris, S. aureus, and S. epidermidis, clinical strain K. pneumonia, and fungi Aspergillus flavus, Aspergillus niger, and C. albicans), with an inhibition zone medium diameter in a concentration-dependent manner [49]. The inhibitory activity was in the range of 7.0–20.15 mm. The highest inhibition zone was found for B. subtilis at 8 × 10^3 µg/mL. Saprolegniosis is a very common mycosis of animals that live in fresh and mesohaline water. The aqueous and methanol extracts of S. etrusca aerial parts were studied for their potential antimicrobial properties against Saprolegnia ferox [57]. The most active extract was the aqueous extract that exhibited an MIC value of 2%. The methanol extract showed an MIC of 1%.

Overall, studies on Santolina species as antimicrobial agents are of interest. Some of the essential oils obtained by Santolina species showed comparable activity to widely used drugs such as clotrimazole. For this reason, an urgent need of animal and human studies to determine their effectiveness in whole-organism systems, with particular reference to their potential toxicity, are required in order to clarify if these phytochemicals could be used in therapy or in lead compounds for the development of more efficacious and safer antimicrobial products. Moreover, in addition to tests and classic bacteriostatic and bactericidal activities, it is also important to investigate extracts and pure compounds against alternative bacterial targets, such as host-directed targets, pathogenesis, and virulence.

**Antiviral activity**

The only Santolina species that was investigated for its antiviral activity was S. insularis. In particular, the essential oil of S. insularis was studied against HSV-1 and HSV-2 [10]. Infections by HSV-1 and HSV-2 are among the most common viral infections in humans. When HSV-1 and HSV-2 were exposed to the S. insularis essential oil for 1 h at 37°C, a concentration-dependent inhibition of plaque formation was observed. A 50% inhibition was observed at 0.88 and 0.7 µg/mL for HSV-1 and HSV-2, respectively. The inactivation of both HSV-1 and HSV-2 depends on the length of exposure to S. insularis essential oil. In fact, a higher inhibition was detected when HSV-1 and HSV-2 were preincubated for 2 h at the same temperature of 37°C (50% inhibition at 0.31 and 0.26 µg/mL for HSV-1 and HSV-2, respectively). Moreover, the inactivation of HSV-1 was more efficient than HSV-2 when viruses were preincubated for 15 min before adsorption (50% inhibition at 6.39 and 7.66 µg/mL for HSV-1 and HSV-2, respectively). In attachment tests, a 50% inhibition was demonstrated in respect to the untreated controls at concentrations > 30 µg/mL for both viruses. These values were higher than those obtained with the controls of viruses preincubated (for 2 h at 4°C) in the presence of the same concentrations of S. insularis oil, showing that attachment was not affected and the activity was primarily due to the direct effects on the virion. Overall, obtained results indicated that S. insularis essential oil is effective in inactivating HSV-1 and HSV-2. The inactivation is time and temperature independent. No differences were detected in the plaque reduction assays when cells were treated with the oil before virus adsorption.

The way in which S. insularis essential oil acts is unique in that no natural products are able concomitantly to inactivate the virus and inhibit cell-to-cell virus spread. Moreover, it is of interest that the IC50 values of S. insularis essential oil against both HSV-1 and HSV-2 is comparable to those reported for approved drugs for the treatment of HSV infections, such as acyclovir and ganciclovir. Further studies are necessary to investigate the effectiveness of the essential oil in animal models infected with HSV, and to isolate phytochemicals responsible for the antiviral activity.

Successively, Valenti et al. [58] prepared, characterized, and investigated the in vitro antiviral activity of liposome-incorporated S. insularis essential oil (multilamellar and unilamellar vesicles obtained from hydrogenated soya phosphatidylcholine and cholesterol). The anti-HSV-1 properties were studied by plaque and yield reduction tests. The S. insularis essential oil could be incorporated in high amounts in the prepared liposomes. The essential oil inactivated HSV-1. This activity is mainly due to direct virucidal effects. The S. insularis oil was more active than the liposome-incorporated essential oil. The ED50 values were considerably lower when cells were preincubated with the oil before the adsorption of the virus. These data suggested an intracellular mechanism for the activity of the oil of S. insularis. Importantly, liposomal S. insularis essential oil was nontoxic in the range of concentrations tested in the study.

Despite the recent progress made in immunization and drug development, many infections represent a serious health problem since there are no vaccines and efficient antiviral therapies, which are often surrounded by the generation of viral mutants. Consequently, the search for new antiviral drugs is a hot topic of research. Taking into account that plants are an excellent source of phytochemicals with a broad range of bioactivity, including antiviral, the research of natural compounds with this property will be...
enhanced. In this context, S. insularis represents a promising species that requires much attention.

Anti-inflammatory activity

Studies were carried out in order to validate the traditional uses of some Santolina species as anti-inflammatory agents. Some extracts of S. chamaecyparissus were the object of study for evaluation of their potential anti-inflammatory activity. Giner et al. [59] proved the anti-inflammatory properties of the chloroform extract of S. chamaecyparissus against carrageenan paw edema in rats. The anti-inflammatory activity of the methanol extract of S. chamaecyparissus was demonstrated in different experimental models [60]. In particular, this extract inhibited PLA1 activity in vitro at 1 mg/mL. No lipoxigenase inhibitory effects were found.

Sala et al. [11] reported the activity of the methanol extract of S. chamaecyparissus against PLA2-induced mouse paw edema. An inhibition of the edema with percentages of 55 and 60% at 30 and 60 min, respectively, was evidenced. This activity is comparable to the reference drug cyproheptadine with 65 and 66% inhibition at 30 and 60 min, respectively. After fractionation by using solvents with different polarities (hexane, dichloromethane, ethyl acetate, and butanol), only the dichloromethane extract was active against the PLA2 in vitro test with a percentage of inhibition of 48%. Moreover, it was shown to reduce the edema induced by 12-O-tetradecanoylphorbol-13-acetate and arachidonic acid in a multidose test.

Fractionation of the dichloromethane extract gave eight fractions, three of which were active with inhibition percentages against PLA2 activity ranging from 47 to 62%. From the active fractions, four purified sesquiterpenes and one flavone, spathulenol, (7S)-4β,5α-epoxygermacr(10)E-en-2β,6-diol, (7S)-germacra-4(15)Z,9dien-1α,2β,5α,6β-tetraol, (7S)-germacra-1(10)E,4(15)-dien-2β,5α,6β-triol, and nepetin, were isolated. Only the fraction from which nepetin was isolated maintained an inhibition range of 50%, including the flavonoid with a percentage of 52%.

Hexane, dichloromethane, ethyl acetate, and methanol extracts obtained from the air-dried aerial parts of S. oblongifolia by means of a Soxhlet extractor were investigated for their potential anti-inflammatory activity targeting NF-κB and other proinflammatory mediators (IL-6, IL-8, TNF-α) or PGE2 in monocytes. A weak inhibitory activity was found against NF-κB, while in tests against the cytokines, a number of species, including S. viscosa, inhibited TNF-α (10 µg/mL).

The methanol extract of S. insularis leaves was studied for its chemical profile and topical anti-inflammatory activity by using croton oil-induced dermatitis in the mouse ear [5]. Six flavonoids, namely, cirsimaritin, hispidulin, luteolin, luteolin 7-O-β-D-glucopyranoside, nepetin, and rhhamnocitrin, and one new xanthone, (E)-3-(6-[(E)-3-hydroxy-3-oxo-1-propenyl]-9-oxo-9Hxanthen-2-yl)-2-propanoic acid, were isolated and tested. All compounds were able to inhibit croton oil-induced ear edema. The most active, after topical application, was luteolin (0.3 µmol/cm²), that led to a 62% reduction of edema, while indomethacin (0.3 µmol/cm²), used as a reference compound, led to a 59% reduction of edema. Cirsimaritin, hispidulin, nepetin, and rhhamnocitrin showed lower anti-inflammatory activity compared to luteolin. Taking into account that all these flavonoids are methoxylated, the lack of one of the free hydroxyl groups determined a decrease of activity. After topical application, xanthone showed only a 20% reduction of edema.

Cytotoxic activity

Few studies reported the cytotoxic activity of Santolina species. The essential oil of S. chamaecyparissus was investigated against MCF-7, HCT116, A549, and HepG2 cancer cell lines [66]. The highest cytotoxic activity was found against HepG2 cancer cells.

Eleven germacrane sesquiterpenes were isolated from the acetone extract of the aerial parts of S. insularis and tested for their potential cytotoxic activity against the human colon carcinoma
cell line Caco-2 [2]. The highest activity was found for compound 36, with an IC₅₀ value of 1.1 μM. These results encourage research towards the study of the antiproliferative activity of other Santolina species and isolated constituents.

Other properties

A study was conducted to investigate possible protective effects of the essential oil of S. canescens and its main constituent santolindiacetylene on carbon tetrachloride (CCl₄)-induced hepatotoxicity in an experimental Wistar rat model [67]. Determination of GPT serum levels is a useful indicator of hepatocellular damage. In fact, in the presence of damage to the cell membrane, some cytoplasmatic enzymes such as GPT are released into the bloodstream. In the employed model, a dose of carbon tetrachloride produced a rise in GPT levels and lipid peroxides in the liver. The protective effects of S. canescens essential oil and santolindiacetylene were demonstrated by their ability to prevent this increase. In both groups treated with the essential oil or santolindiacetylene, levels of GPT were clearly lower than in the group treated only with CCL₄. Moreover, no significant differences between the results obtained with S. canescens and santolindiacetylene and those obtained with silymarin (known as a hepatoprotective agent, used as a positive control) were found. The interest in the essential oils as biocides is growing because the long-term uses of synthetic insecticides lead to the accumulation of residues and produce adverse effects on human health and ecosystems. In this context, the biocidal properties of S. chamaecyparissus were investigated [68]. Specifically, the objects of the study were the nematocidal (Meloidogyne javanica), ixodidic (Hyalomma lusitanicum), insect antifeedant (Leptinotarsa decemlineata, Myzus persicae, Spodoptera littoralis, and Rhoposiphum padi), and phytotoxic effects (Lolium perenne and Lactuca sativa). The essential oil of S. chamaecyparissus demonstrated strong antifeedant effects against R. padī and H. lusitanicum while showing moderate activity against L. decemlineata and S. littoralis. Moreover, moderate phytotoxic activity against the leaf growth of L. perenne was found.

Conclusions and Perspectives

This review summarized the uses in traditional medicine and the main phytochemicals and biological properties of species of the genus Santolina.

Some Santolina species are traditionally used in different countries for their antispasmodic, digestive, analgesic, anti-inflammatory, astringent, stimulant, and antimicrobial properties. To a certain extent, some traditional uses have been scientifically validated and supported by biological studies. In particular, results obtained on the antimicrobial effects of Santolina species showed a good correlation with the reported traditional uses. However, according to literature information, only a few species of this genus have been extensively investigated for the evaluation of their chemical profile. Among isolated constituents, terpenes (mainly germacrene and eudesmane derivatives) are the most representative compounds. Several new sesquiterpenes have been identified in S. insularis, S. chamaecyparissus subsp. squarosa, S. pinnata subsp. neapolitana, S. rosmarinifolia subsp. canescens, and S. insularis. These constituents may be considered chemotaxonomic markers of the genus. However, phytochemical investigations on more Santolina species are required in order to confirm the possibility to use these molecules as taxonomic markers.

Although phytochemical and biological studies on Santolina species have received considerable interest, some gaps are still noteworthy. Firstly, most of the studies are aimed at evaluating the nonpolar constituents of Santolina species and not at characterizing the polar compounds, such as flavonoids, identified in S. insularis and S. pinnata. Secondly, studies mainly focused on four Santolina species, S. chamaecyparissus, S. insularis, S. corsica, and S. oblongifolia. Therefore, a comprehensive investigation of other species is necessary. Thirdly, various biological activities of the extracts and pure compounds were mainly investigated by using in vitro tests and less were carried out by in vivo models. Therefore, there are few reported data focused on toxicity, side effects, and clinical efficiency. Fourthly, the few pharmacological studies are still insufficient to determine the effects and validate the uses of Santolina species in traditional medicine. Therefore, more detailed studies are required 1) to investigate Santolina species that have never been chemically and biologically studied, 2) to analyze the potential toxicity of Santolina extracts and/or essential oils, 3) to identify the pharmacokinetics and pharmacodynamics of bioactive compounds isolated from the most promising extracts/essential oils, and 4) to develop systems to increase the efficacy and safety of Santolina-derived products.

Conflict of Interest

The authors declare no conflicts of interest.

References


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