Article

In Vitro Antimicrobial Activity of Essential Oils from Sardinian Flora against *Cutibacterium* (Formerly *Propionibacterium*) *acnes* and Its Enhancement by Chitosan

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Abstract: The in vitro antibacterial activity of some essential oils from Sardinian flora, both alone and in combination with chitosan, was investigated against a strain of *Cutibacterium acnes*, a bacterium involved in pathogenesis of acne. The composition of the essential oils was determined by gas chromatography and gas chromatography/mass spectrometry. The results of this investigation demonstrated that some of the oils examined, characterised by different chemical profiles, possessed some activity against *C. acnes*. Interestingly, this antibacterial effect was enhanced by sub-inhibitory concentrations of chitosan. These observations suggest the potential application of this synergy in the development of innovative topical formulations useful in the management of acne.

Keywords: *Cutibacterium acnes*; essential oils; antimicrobial activity; *Santolina insularis*; *Juniperus oxycedrus*; chitosan

1. Introduction

Acne vulgaris is a common skin disease that can affect people of all ages, with prevalence in up to 85% of teenagers and 11% of adults in Westernized populations [1–3]. It is a chronic multifactorial inflammatory disorder of the pilosebaceous follicles, typically affecting areas with the highest density of sebaceous follicles (face, upper chest and back), and is characterised by the presence of comedones, papular and pustular eruption, purulent cysts and scars [4]. Patients with acne present excessive sebum production, excessive keratosis of excretory ducts of sebaceous glands, development of bacterial flora and release of inflammatory mediators in the skin [4]. Acne has a complex and multifactorial pathogenesis, involving different key factors: androgen-mediated stimulation of sebaceous gland activity, altered follicular keratinisation, hormonal imbalance, inflammation, immune hypersensitivity, environmental factor (season, diet) and colonisation of the bacterium *Cutibacterium acnes* (formerly *Propionibacterium acnes*) [2–6].

C. acnes is a human, gram-positive anaerobic bacterium; although it is traditionally implicated in the pathogenesis of acne, its exact role in the pathophysiology of this disease is still debated. In general, it is present in acne lesions, but it is also a major commensal of the normal skin flora, often with similar concentrations in patients with acne and in healthy subjects; moreover, some acne lesions do not contain viable bacteria [7]. On the other hand, antibiotics that reduce *C. acnes* total count on acneic skin result in clinical improvement, and the presence of antibiotic-resistant *Cutibacterium* strains is associated with reduced efficacy of these treatments [7]. These controversial data may depend on inappropriate sampling methodologies used in qualitative and quantitative studies involving the

role of *C. acnes* in acne, as recently suggested by Omer et al. [8]. Moreover, Fitz-Gibbon et al. [9] compared the skin microbiome (at the strain level and genome level) of *C. acnes* between a group of acne patients and a group of healthy individuals: although there was no statistically significant difference in the relative abundances of these bacteria in the cohorts studied, a strong association between specific bacterial ribotypes and acne was demonstrated. At present, a direct involvement of *C. acnes* appears certain; presumably it is not involved in the initiation of acne lesions, but would mediate later inflammatory events, leading to worsening of the lesions [5]. In particular, *C. acnes* stimulates the production of host antimicrobial peptides (AMP), small molecules with antimicrobial activity and immunomodulatory properties [10]; moreover, a lipase secreted by the bacterium is responsible for the hydrolysis of sebum and the subsequent release of free fatty acids, which have an irritating and proinflammatory effect [4].

Although acne vulgaris is a disease not life threatening nor physically debilitating, it poses serious psychological problem to patients, from impairment of self-esteem and self-confidence to anxiety, depression and even suicidal ideation, and its emotional impact is comparable to that of severe systemic diseases [11].

The approach to acne management depends on different factors (e.g., severity, age of the patient); a topical combined treatment (with benzoyl peroxide, retinoids, antibiotics) is recommended for mild to moderate acne, while the use of systemic therapies (with antibiotics, retinoids, oral contraceptives, androgen receptor blockers) is reserved for moderate to severe or refractory cases of acne [12–14]. However, the development of antibiotic resistance, the cost of the conventional therapies and their side effects have led to search for alternative remedies, including plants, plant extracts and phytomolecules [15–18]. In particular, some essential oils exhibited an interesting antibacterial activity against *C. acnes* [19–21]. The aim of the present study was to assess the in vitro inhibitory effect of some essential oils obtained from Sardinian plants against a strain of *C. acnes*. Moreover, we investigated an eventual synergistic interaction between some of these essential oils and chitosan, a partially deacetylated chitin of great biological interest characterized by multifunctional properties.

2. Materials and Methods

2.1. Materials

Stock solutions of the essential oils were prepared by dissolving them in PEG-200 in order to obtain a concentration of 100 mg mL^{-1} (10% *w/v*); solutions were then sterilized by filtration using sterile membrane filters (Sartorius, pore size 0.22 μm) and stored at $-20\ ^\circ$C until use. Chitosan (molecular weight 190–310 kDa; deacetylation degree 75–85%; viscosity Brookfield, 1% solution in acetic acid 200–800 cps; manufacturer values) was supplied by Aldrich (Milwaukee, WI). Its aqueous solution was prepared by dissolving chitosan in hydrochloric acid 0.1 mol L^{-1} at 1% w/vol and by evaporating the resulting solution to dryness in a Rotavapor R110 (Buchi, Flawil, Switzerland) at 70 $^\circ$C under vacuum; the residual was then redissolved in 100 mL of Milli-Q water and sterilised by filtration through 0.2 μm Sartorious filters. The pH of this solution, evaluated with a pH-meter Hanna 8417, was 2.35 $\pm$ 0.04.

Propionibacterium acnes ATCC 6919, Brain Heart Infusion Broth (BHI), Brain Heart Infusion Agar (BHA) and defibrinated horse blood were supplied by Thermo Fisher Scientific (Rodano, Italy). Anaerobic conditions were obtained in the Anaerobic Plus System with Gas Generating Kit H$_2$/CO$_2$ (Oxoid, Milano, Italy).

2.2. Plant Material and Isolation of Essential Oil

Except for the *Lavandula officinalis* L. essential oils, purchased from Flora (Lorenzana, Pisa, Italy), the other essential oils were extracted in our laboratory. Aerial flowering plant materials of different botanical species were obtained from several locations during the months May and June 2016 during the same vegetative stage (anthesis), while *Myrtus communis* L. leaves were gathered in April; fruits

from *Juniperus oxycedrus* L. ssp. *macrocarpa* (Sibth & Sm.) Balland and *Citrus limon* var. *pompia* Camarda were collected in December and June, respectively. We collected aerial parts and fruits from all parts of the plants (top, sides and basis) to ensure that the samples were representative of the plants they came from. Before being processed, plant specimens were cleaned from any foreign matter. All species were identified by Prof. M. Usai, and a voucher specimen was deposited in the SASSA Herbarium of Department of Chemistry and Pharmacy, University of Sassari under collective numbers that will be reported below.

Fruits of *Juniperus oxycedrus* L. ssp. *macrocarpa* (Sibth & Sm.) Balland were collected in December from plants growing in Cala Sarraina (Aglientu) (41°05′00″ N 8°58′00″ E) (SASSA N°16529), while biomass (aerial parts) of *Achillea maritima* (L.) Ehrend. & Y.P. Guo was collected in June from plants growing in Poltu Biancu (Badesi) (40°57′33″ N–08°50′20″ E) (SASSA N°1196 bis). Fruits of *Citrus limon* var. *pompia* Camarda were collected in March from plants growing in Siniscola (40°34′24 N 9° 41′49 E) (SASSA N°231 septies). Leaves of *Myrtus communis* L. were collected in April (before blooming) from plants growing near Iglesias (39°18′41″ N 8°32′06″ E) (SASSA N°514). Aerial parts of *Santolina insularis* (Fiori) Arrigoni were collected in June, at blooming time, in Gennargentu massif (Bruncu Spina 1.829 m) 40°00′56.72″ N 9°18′14.29″ E (SASSA N°732). *Thymus capitatus* (L.) Hoffmanns. & Link was collected in June at blooming time in San Michele in Plaiano, near Sassari (40°47′24″ N 8°31′4″ E) (SASSA N°1078). Moreover, in June, we collected aerial parts of blooming *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso in four different stations of Sulcis (South-Western Sardinia). Station 1 is located 4 km from Iglesias (39°18′41″ N 8°32′06″ E). Station 2 is located 10 km from Carbonia (39°09′52″ N 8°31′16″ E), Station 3 is located in Portoscuso area (39°12′26″ N 8°22′51″ E) and Station 4 is located 19 km from Oristano (39°54′12″ N 8°35′33″ E) (SASSA cumulative N°729).

The essential oil samples were obtained from fresh materials by hydro-distillation for 4-5 h using a Clevenger-type apparatus. The extraction yield was calculated in mL of oil for 100 g of fresh material. The reached yields are reported in Figure 1.

Differences between yields may be explained considering that essential oils were obtained from different parts of plants. *T. capitatus* flowering aerial parts gave the higher yield (1.08%), while *Juniperus oxycedrus* L. ssp. *macrocarpa* fruits gave the lower yield (0.14%). All the other samples, except *Achillea maritima* aerial part (0.52%), gave a yield varying from 0.38% to 0.45%. The oils were kept refrigerated in sealed vials at −20 °C until used.
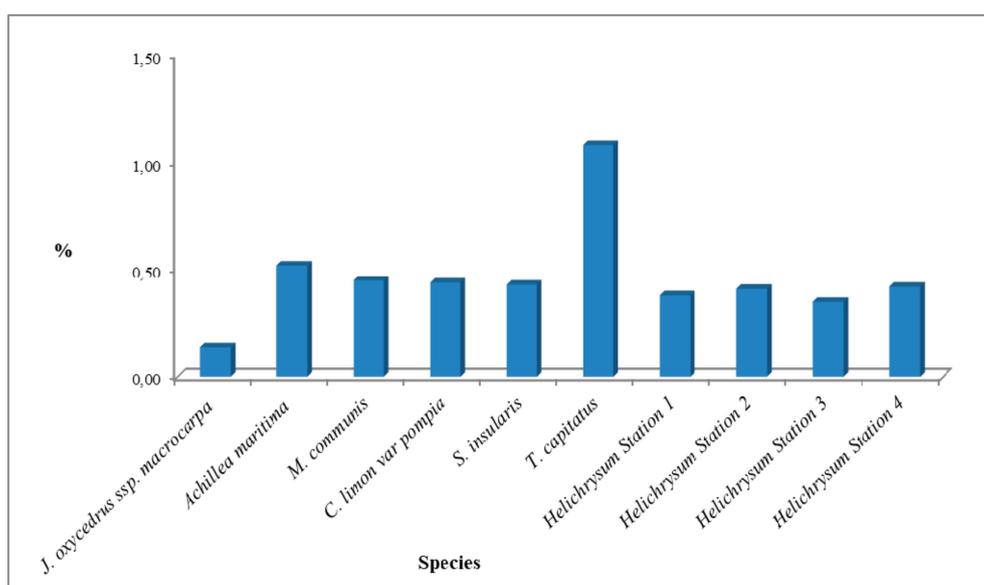

Figure 1. Yields of essential oils belonging to different species.

2.3. Oil Analyses and Quantification

GC (Gas chromatography)—Three replicates of each sample were analysed using a Hewlett-Packard Model 5890A GC equipped with a flame ionization detector and fitted with a 60 m × 0.25 mm, thickness 0.25 µm ZB-5 fused silica capillary column (Phenomenex). Injection port and detector temperature were 280 °C. The column temperature was programmed from 50 °C to 135 °C at 5 °C/min (1 min), 5 °C/min up 225 °C (5 min), 5 °C/min up 260 °C and held for 10 min.

The samples (0.1 µL each), generally analysed without dilution (using 2,6-dimethylphenol as internal standard), were injected using a split/splitless automatic injector HP 7673 and using helium as carrier gas. Several measurements of peak areas were performed with a workstation with a threshold set at 0 and peak width at 0.02. The quantization of each compound was expressed as absolute weight percentage using internal standard and response factors. The detector response factors (RFs) were determined for key components relative to 2,6-dimethylphenol and assigned to other components on the basis of functional group and/or structural similarity, since oxygenated compounds have lower detectability by F.I.D. than hydrocarbons. The standards were >95% also, and actual purity was checked by GC. Several response factor solutions were prepared that consisted of only four or five components (plus 2,6-dimethylphenol) to prevent interference from trace impurities.

It is known that the oxygenated compounds have a lower sensitivity than the hydrocarbons to F.I.D. We have calculated the response factor using a standard mixture of α-pinene, α-terpineol, neral, geranial, geranyl acetate and caryophyllene; in this mixture terpenes accounted for 92% of the mixture, aldehydes 5% and alcohols, esters and sesquiterpenes 1% each. In our analysis we obtained that the RF of hydrocarbons was equal to 1, while for alcohols it was 0.80 and for esters 0.71. For this reason, we have multiplied the experimental data obtained for the following correction factors: hydrocarbons for 1, aldehydes and ketones for 1.24, alcohols for 1.28 and esters for 1.408.

GC/MS (Gas chromatography/Mass): MS analyses were carried out with an Agilent Technologies model 7820 A connected with a MS detector 5977E MSD (Agilent) and using the same conditions and column described above. The column was connected to the ion source of the mass spectrometer. Mass units were monitored from 10 to 900 AMU at 70 eV. In the identification procedure we considered only the peaks from 40 to 900 AMU. The identification of compounds was based on comparison of their retention times with those of authentic samples and/or by comparison of their mass spectra with those of published data [22,23] or on the interpretation of the EI-fragmentation of the molecules.

2.4. Antimicrobial Activity of Essential Oils and Chitosan

Antimicrobial activity of essential oils against *C. acnes* was determined by evaluating their Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration (M.B.C.) values. M.I.C. values were evaluated in 96-well round-bottom microtiter plates by a broth microdilution technique, as described in [24]. Briefly, two-fold dilutions of the stock solution of the oil, ranging from 4 mg mL^{-1} to 0.063 mg mL^{-1}, were prepared in triplicate in BHI; control wells contained only liquid medium and liquid medium with the highest concentration of PEG-200 used. As positive control, erythromycin (from 12.5 to 0.012 µg/mL; mother solution 2 mg/mL in distilled water) was used. Microplates were inoculated with about 1×10^4 bacteria/well incubated at 35 °C for 72 h in anaerobic conditions. After incubation, plates were visually checked for bacterial growth and the M.I.C.s were defined as the lowest concentration at which no growth was observed. PEG-200 had no inhibitory effect on the growth of microorganisms at all concentrations tested. To determine the M.B.C. aliquots of 10 µL of medium from each well with no visible growth were subcultured onto Blood Agar Sheep plates, which were then incubated at 35 °C for 24 h; MBC was defined as the lowest concentration at which no growth was detectable. Unless otherwise specified, all assays were performed in triplicate.

The M.I.C. and M.B.C. values of chitosan solution against *C. acnes* were determined as described above; for M.I.C. evaluation, chitosan solution was subjected to two-fold serial dilutions (2–0.0039 mg/mL) in BHI (whose pH value did not change after chitosan solution addition).

2.5. Antibacterial Activity of M. communis, S. insularis and J. oxycedrus Essential Oils in the Presence of Sub-Inhibitory Concentrations of Chitosan

The antimicrobial activity of some of the oils tested (*M. communis*, *S. insularis* and *J. oxycedrus*), characterized by a well-documented antioxidant activity potentially helpful in acne, were subjected to further investigation. MIC and MBCs of these essential oils against *C. acnes* were evaluated, as previously described, in the presence of sub-inhibitory concentration of chitosan (0.031 mg/mL, corresponding to one-half of the MIC value of chitosan). The results of these experiments are reported in Table 1.

Table 1. Minimum Inhibitory Concentration (M.I.C.) of *Myrtus communis*, *Santolina insularis* and *Juniperus oxycedrus* essential oils against *Cutibacterium acnes* in the presence of a sub-inhibitory concentration of chitosan (M.I.C. chitosan against *C. acnes* = 0.063 mg/mL) (mean of 3 experiments).

	e.o. *M. communis*	e.o. *S. insularis*	e.o. *J. oxycedrus*
MIC of essential oil	>4 mg/mL	1 mg/mL	1 mg/mL
MIC of essential oil + 0.031 mg/mL chitosan	1.5 mg/mL	0.063 mg/mL	0.094 mg/mL

3. Results

3.1. Essential Oils Characterization

Uncoloured, yellow and blue liquid oils are the result of the hydrodistillation of the aerial parts, lives and fruits of the different used species. Overall, numerous compounds were identified for every sample, representing from a minimum amount of 88.97% to a maximum of 99.73% of the total composition. One hundred eighty-one compounds were detected and quantified by GC and GC/MS methods in the studied oils. All these constituents were identified on the basis of their mass spectra, retention indices and/or by comparison with authentic compounds [22,23]. The analytical data are the average of three injections. Each one of the eleven samples shows a very peculiar chromatographic profile. The complete characterization of the essential oils is reported in Table S1 (Supplementary File).

Among the analyzed plants we observed that the fruits of *Juniperus oxycedrus* L. ssp. *macrocarpa* (Sibth & Sm.), collected in December, were characterized by an essential oil in which the main constituent is represented by a very higher percentage of pinene, 77.63% on 91.54% of identified compounds. With the exception of germacrene D (3.5%), all the other constituents are present in concentration <1%. No other essential oil among those investigated exhibits a similar chromatographic profile.

From the aerial parts of *Achillea maritima* (L.) Ehrend. & Y.P. Guo an essential oil with a very stable dark blue colour was obtained, in which some constituents in high concentration could be identified. The blue colour is due to azulenes, in particularly to chamazulene (0.38%). Yomogi alcohol reached the concentration of 22.83%, artemisia alcohol 17.18% and camphor 20.82%. Other remarkable constituents were camphene (5.38%), artemysil acetate (5.28%) and crysantenone (3.38%). With the exception of camphor, all the other mentioned compounds were not found in the other analysed oils.

We also analysed a commercial essential oil of *Lavandula officinalis* L. that has a concentration of 32.01% of linalool, 28.65% of linalyl acetate, 7.4% of camphor and 5.07% of linalyl acetate.

The essential oil obtained from flamed of *Citrus limon* var. *pompia* Camarda fruits was colorless and characterized by the presence of 88.23% of limonene followed by a 2% of myrcene; typical constituents of the oils of *Citrus* species, such as neral, geranial and geraniol (1.15%, 1.56% and 1.23% respectively), were also identified. All the other constituents were present in very low concentration.

The essential oil extracted from *M. communis* leaves had as principal constituents α-pinene (52.72%), 1,8-cineole (16.11%) and limonene (6.53%).

The aerial parts of *Santolina insularis* (Fiori) Arrigoni gave a yellow essential oil rich in artemisia ketone (24.61%) and β-phellandrene (18.87%), which were the main constituents. Other constituents in

high concentration were santolina triene (9.32%), found only in this essential oil, 1S-pinene (7%) and myrcene (5.15%).

Thymus capitatus (L.) Hoffmanns. & Link, as many *Thymus* species, was characterized by a high carvacrol concentration (57%); other important constituents were o-cymene (8.65%), γ-terpinene (6.92%), 3.7% of myrcene and α–thujene.

Four samples of *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo and Giusso were collected in four stations located in South-West of Sardinia. The four essential oils obtained were very different each other; in particular the samples coming from Station 1 (Iglesias) and Station 2 (Carbonia) were characterized by an unusual concentration of γ-curcumene (more than 28%). The essential oil obtained from plants collected in Station 3 (Portoscuso) presents many constituents with concentration among 1 and 2% and the main constituents are represented by γ-curcumene (12.22%), linalool (11.47%), 5-eudesmen-11-ol (10.35%) and nerolidol (5.07%). The oil from station 4 (Oristano) showed the chromatographic profile expected for this species growing in middle and North Sardinia and is very rich in geranyl acetate (33.6%).

3.2. Antimicrobial Activity of the Essential Oils against C. acnes

The results of the antibacterial screening of the essential oils examined are summarized in Table 2. The oils showed different levels of inhibition; the essential oil obtained from *T. capitatus* exhibited the highest activity against *C. acnes*, with MIC and MBC values of 0.25 and 0.5 mg/mL respectively. *C. lemon* var. *pompia* and *Lavandula* essential oils demonstrated significantly lower activity (MIC values 4 mg/mL), while *C. acnes* growth was not affected by *M. communis* oil at the highest concentration tested (MIC >4 mg/mL). Other essential oils presented moderate activity against *P. acnes*, with MIC values ranging from 1 to 2 mg/mL. The MIC of the reference standard erythromycin against *C. acnes* was 0.024 μg/mL.

Table 2. Minimum inhibitory concentration (M.I.C.) and minimum bactericidal concentration (M.B.C.) of ten essential oils against *Cutibacterium acnes* ATCC 6919 (mean of 3 experiments, with the exceptions of *S. insularis*—mean of 7 experiments- and *J. oxycedrus*—mean of 5 experiments) (n.d. = not determined).

Essential Oil	M.I.C.	M.B.C.
Thymus capitatus	0.25 mg/mL	0.5 mg/mL
Santolina insularis	1 mg/mL	2 mg/mL
Helichrysum microphyllum subsp. *tyrrhenicum* (Station 1)	1 mg/mL	2 mg/mL
Helichrysum microphyllum subsp. *tyrrhenicum* (Station 2)	2 mg/mL	4 mg/mL
Helichrysum microphyllum subsp. *tyrrhenicum* (Station 3)	1 mg/mL	n.d.
Achillea maritima	2 mg/mL	4 mg/mL
Juniperus oxycedrus	1 mg/mL	2 mg/mL
Citrus limon var. *pompia*	4 mg/mL	>4 mg/mL
Lavandula officinalis	4 mg/mL	n.d.
Myrtus communis	>4 mg/mL	>4 mg/mL

3.3. Antibacterial Activity of M. communis, S. insularis and J. oxycedrus Essential Oils in the Presence of Sub-Inhibitory Concentrations of Chitosan

In our experimental conditions, MIC and MBC values of chitosan against *C. acnes* resulted 0.063 and 0.25 mg/mL respectively. When evaluated in presence of a concentration of chitosan of half the MIC (0.031 mg/mL), the antimicrobial activity of some of the essential oils examined was significantly enhanced (Table 2). In particular, *M. communis* essential oil, that alone did not show any inhibitory activity at the highest concentration tested (4 mg/mL), became active (MIC 1.5 mg/mL) when combined with chitosan. Moreover, the presence of sub-inhibitory concentrations of chitosan significantly improved the antibacterial properties of essential oils of *S. insularis* (whose MIC was reduced by 1 mg/mL to 0.063 mg/mL) and *J. oxycedrus* (whose MIC value decreased from 1 to 0.094 mg/mL).

4. Discussion

Among the essential oils investigated in the present study, *T. capitatus* oil showed the most marked inhibitory activity against *C. acnes*; this result is not unexpected, because the activity of essential oils from other *Thymus* species against *P. acnes* has been reported [20,24]. On the other hand, this paper documents for the first time the antimicrobial activity towards *C. acnes* of essential oils obtained from plant typical of Sardinian flora; this is interesting, because essential oils are currently being investigated, amongst other plant-derived products, for their application in acne management [16].

Some of the essential oils deriving from Sardinian wild plants examined in this investigation possess an antimicrobial activity of interest. As far as *T. capitatus* is concerned, it is likely that its high content of carvacrol justify its activity. Carvacrol, or cymophenol, is a phenolic monoterpene present in the essential oils of oregano and thyme. It is the substance that imparts to oregano its characteristic odor. It is a regioisomer of thymol and its antimicrobial activity is well-known [25,26]. In essential oils of *Helichrysum* (Station 1 and 3) linalool, a compound with anti-inflammatory activity [27], 5-eudesmen-11-ol, an oxygenated sesquiterpene, and γ-curcumene have been reported. These compounds are normally found in *Helichrysum* essential oil which shows an antimicrobial activity as reported in [28]. Moreover, Rossi et al. [29] demonstrated that the essential oil obtained from endemic plants of Corsica is more effective on the Gram-positive bacterium *S. aureus* than on the Gram-negative strains *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

Essential oil sample from *J. oxycedrus* contained a very high concentration of α-pinene, which possesses anti-inflammatory and antibacterial activities, as reported in [30]. The large amount of this compound could explain the antibacterial activity observed in our investigation.

The activity of *S. insularis* is probably due to the presence of artemisia ketone, β-phellandrene as main constituents; Radulović et al. [31] demonstrated that artemisia ketone has a stronger free radical scavenging effect and a stronger antimicrobial activity when compared to other well-known monoterpenes. Moreover β-phellandrene is reported to exhibit antifungal and antibacterial activities, according to [32]. In the study, it was stated that nerolidol, sabinene and β-phellandrene was reported to possess antibacterial and antifungal activity.

To complete our investigation, we studied an eventual synergistic interaction between some of these essential oils and chitosan, a partially deacetylated chitin with multifunctional properties, including bioadhesivity, biodegradability and bio-compatibility, which make it an attractive biomaterial for pharmaceutical purposes; in addition to the abovementioned properties, chitosan possesses interesting antimicrobial and antioxidant activities. The exact mechanism of the antimicrobial activity of chitosan is still unknown; it depends on intrinsic factors of the polymer (molecular weight, positive charge density, chelating capacity, physical state), environmental factors (pH, ionic strength, temperature) and microbial factors (cell age, microbial species) [33]. In particular, chitosan demonstrated in vitro antimicrobial activity, dose- and pH-dependent, against two common cutaneous pathogens, *Staphylococcus aureus* and *C. acnes* [34]. Moreover, some studies demonstrated that the combination of chitosan and essential oils results in an increase of the oil antimicrobial efficacy [35–37]. Some of the essential oils tested (*M. communis*, *J. oxycedrus*, and *S. insularis*), exhibiting an inhibitory activity moderate or even no activity against *C. acnes*, still possess a well-documented antioxidant activity [38–40]. It is well known that in acne the reactive oxygen species (ROS) produced by neutrophils play a critical role in the irritation and destruction of the follicular wall and are responsible for the progression of acne [41]; therefore, essential oils with antioxidant activity could have beneficial effects in acne management. Consequently, essential oils with good antioxidant activity but little antimicrobial effect can be combined with chitosan to improve their potential utility as multifunctional ingredients of topical anti-acne formulations. Actually, our experimental data showed that the combination of sub-inhibitory concentrations of chitosan (0.031 mg/mL, namely half of the minimum inhibitory concentration) with essential oils of *M. communis*, *J. oxycedrus* and *S. insularis* significantly improved their antibacterial activity against *P. acnes*, bringing unexpected chances to use them as adjuvants in the complex clinical situation of acne.

5. Conclusions

Our investigation has been focused on the evaluation of the in vitro inhibitory effect of some essential oils obtained from Sardinian plants against a strain of *C. acnes*, with the purpose to re-evaluate traditional ethnobotanical resources. On the basis of the well-known antioxidant activity and of the antibacterial activity against *C. acnes* of some of the oils investigated, and considering the synergism chitosan-essential oil, it seems appropriate to consider them for further formulative and pharmacological investigations.

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