



Annual variation in yield, chemical composition, antioxidant and antibacterial activity of rockrose (*Cistus ladanifer* L.) essential oils

Veronika Chaloupková^{a,b,*}, Irene Mediavilla^a, Raquel Bados^a,
Markéta Houdková^c, Johana Rondevaldová^c, Luis Saúl Esteban^a

^a Centre for the Development of Renewable Energies - Centre for Energy, Environmental and Technological Research CEDER-CIEMAT, Autovía de Navarra A-15, Salida 56, 42290, Lubia, Spain

^b Department of Agricultural Production. School of Agricultural, Food and Biosystems Engineering, Universidad Politécnica de Madrid (UPM), Madrid, Spain

^c Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýčka 129, 165 21, Prague-Suchbát, Czech Republic

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ABSTRACT

The fluctuation in yield, composition, antibacterial and antioxidant activity of the essential oil (EO) of rockrose (*Cistus ladanifer* L.) obtained from two locations in central Spain was analysed and compared over the course of one year. Fifteen kilograms of *C. ladanifer*, collected monthly or fortnightly from two sites with different plant age (7 and 12 years), underwent steam-distillation. GC (FID)-MS analyses were done to determine the volatile profile of EOs. Obtained EO yields varied significantly ($P < 0.001$) throughout the year, ranging between 0.03 and 0.19% w/d.w. (± 0.05) with the highest yields ($>0.1\%$) observed from September to February. The younger population exhibited higher EO yields than the older one. Both rockrose populations displayed a high content of α -pinene (40.4–62.0%); the other predominant constituents included *allo*-aromadendrene + *trans*-pinocarveol, limonene, bornyl acetate and viridiflorol. The higher EO yields coincided with a higher content of α -pinene and a lower content of the other components. Furthermore, EOs' antibacterial potential was tested against *Bacillus cereus*. Only a weak antibacterial potency (512–1024 $\mu\text{g/mL}$) was revealed, with stronger potency in case of older rockrose population. *In vitro* antioxidant capacity, evaluated by ORAC method, showed similar results for both populations, with its stronger potential between February and May.

1. Introduction

Owing to the increasing interest and demand of consumers and the industries for natural ingredients, alternative and still widely unexploited plant species are being investigated to be applied in the perfumery, cosmetics, pharmacological, agrochemical and food sectors (Verdeguer et al., 2012; Goyal and Jerold, 2023; González-Navarro et al., 2024). The use of these alternative species is crucial for the valorisation of endogenous resources, which are still widely underused (Ferraz et al., 2022). In Mediterranean flora, *Cistus ladanifer* L. represents such neglected plant species offering abundant biomass that can serve as a source of valuable compounds

* Corresponding author. Centre for the Development of Renewable Energies - Centre for Energy, Environmental and Technological Research CEDER-CIEMAT, Autovía de Navarra A-15, Salida 56, 42290, Lubia, Spain.

E-mail addresses: veronika.chaloupkova@ciemat.es (V. Chaloupková), irene.mediavilla@ciemat.es (I. Mediavilla), raquel.bados@ciemat.es (R. Bados), houdkovam@ftz.czu.cz (M. Houdková), rondevaldova@ftz.czu.cz (J. Rondevaldová), luis.esteban@ciemat.es (L.S. Esteban).

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(Raimundo et al., 2018; Alves-Ferreira et al., 2023).

Cistus ladanifer L. (hereafter referred as *C. ladanifer*), commonly known as rockrose, is a perennial fragrant plant from the Cistaceae family and *Cistus* genus, with its origin and primary habitat in the western Mediterranean region (Papaeftimiou et al., 2014). This pyrophyte plant usually forms a dense and continuous stands on acidic and siliceous soils (Guzmán and Vargas, 2009) and covers great areas of burnt degraded forests, abandoned pastures and poor uncultivated lands mainly in Spain, Portugal, south of France and north of Morocco (Frazao et al., 2018). The biggest extensions can be found in Spain, where it is distributed in more than 2 million hectares and is dominant in a little more than 460,000 ha (Montero et al., 2020). *C. ladanifer* exhibits strong climate adaptability and resilience, thriving in conditions of low water, high solar exposure and poor contaminated soils (Núñez-Olivera et al., 1996; Santos et al., 2016; Rossini-Oliva et al., 2016; Frazao et al., 2018). Furthermore, it has ability to inhibit a growth of several other plants through the release of allelochemicals (Herranz et al., 2005; Verdeguer et al., 2012). Its capacity to establish in disturbed sites also suggests rapid fuel accumulation and presents significant fire risks (Alves-Ferreira et al., 2019). Annual wildfires pose increasing threats to Mediterranean ecosystems due to ongoing climate changes (Vázquez et al., 2015). In response to this challenge and to mitigate risks, it is essential to adopt innovative forest and shrubland management strategies. While traditional methods like fuel reduction through brush clearing are costly and periodically required (Madrigal et al., 2017), a cost-efficient approach within a green economy involves developing high-value bioproducts, such as essential oils among others, alongside with fire prevention efforts (Verkerk et al., 2018).

To obtain bioproducts, various extraction and distillation techniques can be applied to the leaves and branches of *C. ladanifer*, yielding diverse types of extracts, including labdanum, essential oil (EO) and hydrolate (Frazao et al., 2018). These extracts have been used in traditional medicine since ancient times (Papaeftimiou et al., 2014) to treat various health issues like skin diseases, diarrhoea, dysentery, menstruation discomfort and catarrh (Barrajón-Catalán et al., 2010), as it has pharmacological potential (Adadi et al., 2022). Nowadays they have found numerous applications, particularly in the perfumery and cosmetics industry (Alves-Ferreira et al., 2023). Besides a labdanum resin, which is traditionally the main product obtained from the rockrose plants due to its well-known applications in the perfume industry for its aromatic and fixative properties (Barrajón-Catalán et al., 2016), more attention is paid to rockrose EOs (Verdeguer et al., 2012; Alves-Ferreira et al., 2019; Mediavilla et al., 2021a).

Over time various bioactive effects of *C. ladanifer* EO have been identified as antimicrobial (Greche et al., 2009; Mohammed et al., 2018), antifungal (El Karkouri et al., 2021; Pérez-Izquierdo et al., 2022), anti-inflammatory (Adadi et al., 2022) and analgesic (Youbi et al., 2015). It was reported that *C. ladanifer* is a source of natural antioxidants that can be exploited in the food or cosmetic industry given the high levels of existing flavonoids and phenolic compounds (Barrajón-Catalán et al., 2010; Guimarães et al., 2010; Amensour et al., 2010).

At present, the industrial-scale commercial exploitation of *C. ladanifer* is almost exclusively limited to the region of Andalusia in Southern Spain (Alves-Ferreira et al., 2019). Despite its presence in substantial areas across Spain (Montero et al., 2020), regions like Extremadura, Castilla La Mancha, and Castilla y León in Central Spain hold untapped potential (Mediavilla et al., 2021a). The harvesting of *C. ladanifer* is typically carried out manually during a short period of time, from June to November (Raimundo et al., 2018). It is presumed that there is a higher oil yield during the warmer months (Alves-Ferreira et al., 2023); although no comparative evidence is currently available. The yields of *C. ladanifer* EOs are usually low, with a market price exceeding 400 €/kg (Ruiz García et al., 2020). Several authors have reported EO yields ranging from 0.08 to 0.41% from dry plant material using laboratory Clevenger's apparatus (Mariotti et al., 1997; Gomes et al., 2005; Bechlaghem et al., 2019; Benali et al., 2020). On an industrial scale, steam distillation is the most commonly employed technique (Mediavilla et al., 2021a); however, limited information is available regarding EO yields from industrial plants. Experiments conducted in steam distillation pilot devices have also been underreported, with yield values ranging from 0.01% to 0.08% (Tavares et al., 2020; Mediavilla et al., 2021a).

Composition of *C. ladanifer* EO is highly complex, consisting of over 250 substances (>0.1%) belonging to various chemical classes. It comprises more than 400 components, including those present in trace amounts (Gomes et al., 2005). Generally, there are two compositional groups of *C. ladanifer* identified by authors (Gomes et al., 2005). First group is characterized by α -pinene as its principal component (Ferraz et al., 2022), while second group is distinguished by a higher quantity of viridiflorol and a low content of α -pinene (Greche et al., 2009; Zidane et al., 2013). First group is the most prevalent category, in which α -pinene, represents a dominant compound of monoterpenes hydrocarbons, bornyl acetate and *trans*-pinocarveol represents oxygenated monoterpenes, and in oxygenated sesquiterpene fraction mainly viridiflorol and ledol can be found (Verdeguer et al., 2012).

Yield as well as chemical composition of EOs vary. It may be caused, in general, by plant origin, developmental stage, environmental and climatic conditions, season of the collection and mentioned extraction method (Barra, 2009; Viuda-Martos et al., 2011; Barrajón-Catalán et al., 2016). Seasonal/annual changes in both the composition and concentration of EOs were documented for several aromatic plants, including *Salvia officinalis* L. (Santos-Gomes and Fernandes-Ferreira, 2001), *Rosmarinus officinalis* L./*Salvia rosmarinus* (Fumiere Lemos et al., 2015; Rathore et al., 2022), *Mentha spicata* (Kofidis et al., 2004) and *Eucalyptus* spp. (Emara and Shalaby, 2011), among others. To date, the report on the annual variation of yield and chemical composition, as well as antimicrobial and antioxidant activity of *C. ladanifer* EO, obtained by any extraction method is however missing. Thus, the objective of this paper is to provide knowledge on the annual evolution of EOs obtained via steam distillation from two differently aged rockrose (*Cistus ladanifer* L.) populations in central Spain, focusing on their EO yield, main composition, and antimicrobial and antioxidant activities.

2. Methodology

2.1. Characterisation of the study area

Cistus ladanifer L. (Cistaceae) was manually collected from its natural habitats in central Spain, from two sites – Bustares and Hiendelaencina in Guadalajara, Castilla-La Mancha, as illustrated in Fig. 1. The central point UTM coordinates (ETRS 89) of the

sampling areas were: Bustares (30N 495423 4552862) and Hiendelaencina (30 N 499691, 4546683). *C. ladanifer* represents the dominant species in these locations.

Populations in Bustares and Hiendelaencina were regenerated after wildfires happened in 2014 and 2009, respectively. Therefore, these two locations correspond to different rockrose shrub age. The vegetation in Bustares was approx. 7 years old, with a height of 1.03 m and a crown cover of 59%. The vegetation in Hiendelaencina was 12 years old, measuring 1.18 m in height and displaying a crown cover of 72%. Further in the text, the populations of Bustares and Hiendelaencina are named as '7-year-old' (or 'younger population') and '12-year-old' ('older population'), respectively.

The climatic conditions in the region exhibit typical Mediterranean characteristics, including a temperate climate with dry or temperate summers (Chazarra Bernabé et al., 2018). The temperatures and rainfalls including the one-year collection period (June 2021–July 2022), from the nearest meteorological station (Guadalajara), are depicted in Fig. 2. The annual mean temperature was 17 °C, with mean minimal temperature of 10 °C and maximum temperature of 23 °C. The soil in both locations is siliceous (Mateo Sanz, 2023); in Bustares it is characterized as loamy and slightly acid and in Hiendelaencina it is sandy loam with a neutral pH.

2.2. Plant material collection and preparation

Over the course of one year, from July 2021 to June 2022, samples of 80 kg of rockrose biomass were collected on a monthly basis (from February to July) or on a fortnightly basis during the period of highest essential oil production (from August to January); in total 18 harvests/collections were made. This sampling approach was adopted, based on previous observations (Mauri et al., 2020), to analyse the seasonal variation in both quantity and quality of the essential oil (EO) samples.

Samples were randomly taken from plants of a similar age, and the biomass of the different plants was mixed to obtain samples of approximately 80 kg of green material from each location during each collection period. Aerial parts, including leaves, stems, flowers, and fruits (depending on the plant's phenological stage), were manually cut by a two-handed pruning shears at an approximate height of 20 cm above the ground to simulate mechanized collection.

The sampling procedure at both locations followed this method: Initially, a central point was randomly selected to commence the sample collection, spanning a different area each day within the total sampling area (10 ha in Bustares and 3 ha in Hiendelaencina). The plants were bundled together in groups weighing 12–15 kg each to ease handling and transportation. Moving 20 m in a random direction from this point, the next sampling location was chosen, and rockrose plants were harvested to create another bundle. Subsequently, another collection point was established perpendicular to the previous direction, also 20 m away, and the process continued until a daily sample of 80 kg of rockrose was attained. Additionally, ten plants were selected each sampling day to measure dasometric parameters, including shrub crown cover, total height, and crown diameter, to characterize the scrubland area.

Prior to each distillation process, the biomass material was shredded using a shredder (18.5 kW, slow rotating single-shaft type, 70 rpm.) to achieve a size of 20 mm. Subsamples were then taken to determine the moisture content following the standard ISO 18134-2:2017 and to calculate biomass dry weight.

2.3. Essential oil extraction

The freshly shredded biomass was distilled each time in three batches of 15 kg during 1 h in a steam distillation pilot plant. The pilot plant comprised a 50 L stainless steel still, an electric boiler (ETE, Madrid, Spain) to generate steam (0.5 bar, 11.5 kg/h) and to extract the EO from the plant, a cooling system for steam condensation, and a glass Florentine flask to separate hydrolate (aqueous phase) and EO by density. The EO yield was calculated as a percentage, by measuring the weight of EO extracted per weight of dried plant material (% w/d.w.). Obtained EOs were dried using anhydrous sodium sulphate, weighted and stored at 4 °C until further analyses.

2.4. Essential oil analysis

To determine a volatile profile of EOs, the three samples of EOs obtained in each distillation were blended and analysed, in

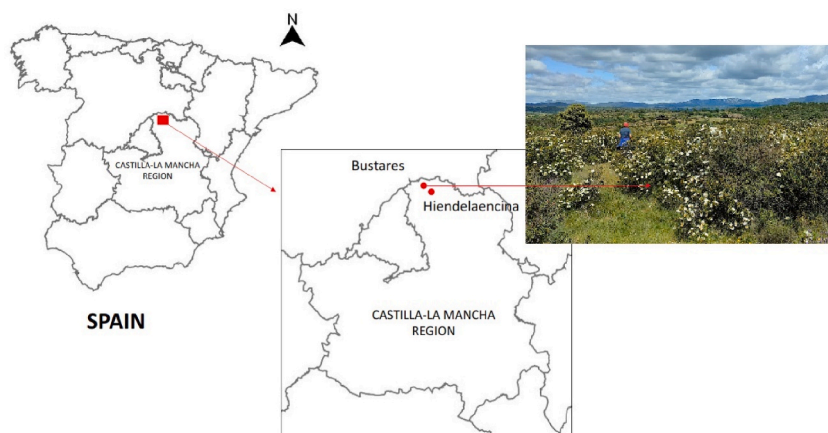


Fig. 1. Geographic locations of collection sites of *C. ladanifer*.

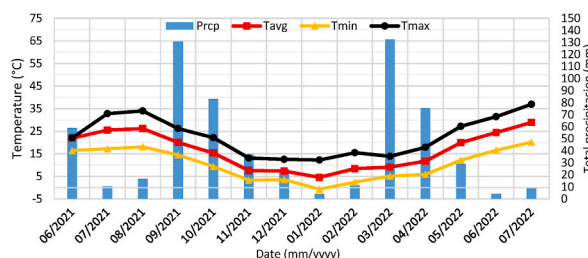


Fig. 2. Meteorological variables from Guadalajara in the period of biomass harvests. Note: Tmin – mean minimum temperature; Tmax – mean maximum temperature; Tavg – mean temperature; Prcp – total precipitation. (Source: meteostat.net).

triplicate, by gas chromatography GC (FID)-MS, using the same procedure as described in [Mediavilla et al. \(2021a\)](#). A GC-MS apparatus Agilent HP 8890/5977 (Agilent Technologies, Santa Clara, CA, USA) was used to identify the EOs components and Agilent HP 8890 (GC-FID) (Agilent Technologies, Santa Clara, CA, USA) was employed to quantify the identified components; both equipped with DB-WAX UI fused silica columns (60 m $\times$ 0.25 mm inner diameter, film thickness 0.5 μ m) and retention time locking. The column temperature program was 50 $^{\circ}$ C for 6 min, followed by an increase of 2 $^{\circ}$ C/min to reach 190 $^{\circ}$ C, then, a gradient of 4 $^{\circ}$ C/min to 220 $^{\circ}$ C was applied, which was maintained for 10 min. Finally, an increase of 4 $^{\circ}$ C/min to 250 $^{\circ}$ C was set, which was maintained for 10 min. The carrier gas was helium at a variable flow rate and a head pressure of 30.75 psi. The injection volume was 0.1 μ L and split mode injection (ratio 1:100) was employed. The temperatures of injector and detector were 240 $^{\circ}$ C. GC-MS was performed with the same capillary column, carrier gas and operating conditions described for GC analysis. Mass spectra were taken over the m/z range 33–350 with an ionisation voltage of 70 eV. The individual volatile components were identified by MS and their identity was confirmed by comparison of their Kovats retention index calculated using co-chromatographed standard hydrocarbons relative to C₆–C₃₀ n -alkanes and mass spectra with reference samples and those of the computer libraries (NIST 14, Wiley 10 and Chromessence library built through standards injection) and available literature ([Adams, 2007](#)). To characterize chemical profile of obtained EOs, 37 compounds were identified. To compare the annual variation in chemical composition, the most characteristic 16 compounds for *C. ladanifer* species were considered.

2.5. Bioactivity

To complement the analysis of the annual variation in rockrose EOs, we assessed the fluctuation in antimicrobial activity and antioxidant capacity.

2.5.1. Antibacterial activity

Gram-positive bacteria *Bacillus cereus* was selected for testing the variability of EOs' antimicrobial activity. Gram-positive bacteria are known to be more sensitive to natural extracts ([Kalemba and Kunicka, 2003](#)). Standard bacterial strain of the American Type Culture Collection (ATCC) 11778 was used (Oxoid, Basingstoke, United Kingdom). The cultivation and assay media (broth/agar) were Mueller-Hinton ((Oxoid)). The pH of broth was equilibrated to a final value of 7.6 using Trizma base (Sigma-Aldrich, Prague, Czech Republic). Bacterial strains from stock cultures were grown in a broth medium at 37 $^{\circ}$ C for 24 h prior to the testing. Turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standard using Densi-La-Meter II (Lachema, Brno, Czech Republic) to achieve a final concentration of 10^7 CFU/mL. The susceptibility of *B. cereus* to chloramphenicol was checked as a positive antibiotic control (conducted only in the liquid phase, as the antibiotic is not volatile).

The *in vitro* antibacterial potential of plant volatile compounds was assessed using newly developed broth-microdilution volatilization method by [Houdková et al. \(2017\)](#). This method determines antibacterial potential of plant volatiles simultaneously in the liquid and the vapour phase at various concentrations and addresses limitations of conventional antimicrobial susceptibility testing methods (broth dilution/agar diffusion) when testing volatile agents.

The experiments were performed in white standard 96-well immune plates (well volume = 400 μ L), covered by tight-fitting lids with flanges designed to reduce evaporation (SPL Life Sciences, Naechon-Myeon, Korea). Initially, 30 μ L of agar was pipetted into every flange on the lid (with exception of the outermost wells) and inoculated with 5 μ L of bacterial suspension after agar solidification. In the second part of the method, each sample of EO was dissolved in *dimethyl sulfoxide* (DMSO) at a maximum concentration of 1% and diluted in broth medium. Seven twofold serially diluted concentrations of samples were prepared, starting from 1024 μ g/mL. The final volume in each well was 100 μ L, with the exception of the outermost wells, which were left empty to prevent edge leakage effect. The plates were then inoculated with bacterial suspensions using a 96-pin multi-blot replicator (National Institute of Public Health, Prague, Czech Republic). Each plate also contained inoculated and non-inoculated broth, which served as growth and sterility controls, respectively. Chloramphenicol was used as a positive antibiotic control for verification of susceptibility of *B. cereus* strains in broth medium. Finally, the clamps (Lux Tool, Prague, Czech Republic) were used for fastening plate and lid together, with the handmade wooden pads (size 8.5 $\times$ 13 $\times$ 2 cm) for better fixing and the microtiter plates were incubated at 37 $^{\circ}$ C for 24 h in the incubator (Mettler GmbH & Co. KG, Buchenbach, Germany). The minimum inhibitory concentration (MIC) was evaluated by visual assessment of bacterial growth after colouring of metabolically active bacterial colony with thiazolyl blue tetrazolium bromide (MTT) (Sigma-Aldrich). 25 μ L of MTT at concentration 600 μ g/mL was pipetted in each well of microtiter plate and lid when the interface of

colour change from yellow and purple (relative to that of colours in control wells) was recorded in broth and agar. The MIC values were determined as the lowest concentrations that inhibited bacterial growth compared with the compound-free control and expressed in $\mu\text{g/mL}$. All experiments were conducted in triplicate across three independent experiments, and results were expressed as median/modal MICs values. In accordance with established practices in MIC testing, the mode and median were used for the final value calculation when triplicate endpoints were within the two- and three-dilution ranges, respectively.

2.5.2. Antioxidant activity

Antioxidant activity of EOs was determined with oxygen radical absorbance capacity (ORAC) method. Based on the method by [Ou et al. \(2001\)](#) and a slightly modified procedure from [Rondevaldová et al. \(2023\)](#), the essential oils (EOs) of *C. ladanifer* were tested to assess their ability to protect fluorescein against oxidative degradation by 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) (Sigma-Aldrich). The EOs and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma-Aldrich) were initially diluted with 1% methanol (Penta, Prague, Czech Republic). Further, stock solutions of AAPH radical (153 mM) and fluorescein (48 nM) (Sigma-Aldrich) were prepared in 75 mM phosphate buffer (pH 7.0). Two-fold serial dilutions of each sample were prepared in phosphate buffer in black absorbance 96-well microtiter plates using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, Switzerland). To secure better thermal mass stability, outer wells of each microtiter plate were filled with 200 μL of distilled water. Subsequently, 150 μL of fluorescein was added into each well and incubated for 10 min at 37 °C. The initiation of the reaction occurred by introducing 25 μL of AAPH, followed by a subsequent incubation of the plates at 37 °C for 90 min. To prevent the EOs' volatilization, the plates were sealed by silicone mats EVA capmat (Micronic, Aston, PA, USA). The tested samples covered a final concentration range of 1 and 256 $\mu\text{g/mL}$. Each microtiter plate included Blank A (fluorescein with AAPH in phosphate buffer) and blank B (fluorescein in phosphate buffer), with Trolox serving as a positive control. After the incubation, fluorescence changes were measured using the Multimode Reader Cytation 3 (BioTek Instruments, Winooski, VT, USA) with excitation and emission wave-lengths set at 485 and 528 nm, respectively. The experiments were conducted in triplicate as three independent tests. ORAC values were expressed as mean values of half-maximal inhibitory concentration $\text{IC}_{50} \pm \text{SD}$ in $\mu\text{g/mL}$.

2.6. Statistical analysis

Statistical analysis of the obtained data set was performed using IBM SPSS Statistics software package, version 29.0 (IBM, Armonk, NY, USA). Since the assumptions of normality of tested variables were violated (Shapiro-Wilk test, $P < 0.01$), non-parametric inference statistics (Mann-Whitney *U* test, Kruskal-Wallis test, post-hoc test) were used to compare the variables during a year. *P* value less than 0.05 was considered statistically significant.

To assess the relationship between selected variables (meteorological variables, EO yields, EO composition, ORAC values) Spearman's rank correlation was computed (at significance level of 0.01 or 0.05). The temperature variables (average temperature, minimum temperature, maximum temperature) were expressed as mean values and total precipitation variable as sum of everyday records of 15 days before biomass fortnight/monthly collection. The interpretation of the correlation degree (*r*) followed [Evans' guide \(1996\)](#), which categorizes the absolute value of *r* as follows: 0–0.19 (very weak), 0.20–0.39 (weak), 0.40–0.59 (moderate), 0.60–0.79 (strong), and 0.80–1 (very strong).

3. Results and discussion

3.1. Essential oil yield

Essential oil (EO) yields obtained over one year (July 2021–June 2022) from two locations with different age rockrose populations in Central Spain, expressed as % w/d.w. (weight/dry weight), are depicted in [Fig. 3](#).

The EO yields from 7-year-old and 12-year-old populations exhibited statistically significant variation ($P < 0.001$) throughout the year, ranging from 0.03 to 0.19% (± 0.05) and 0.001–0.12% (± 0.04), respectively. Notably, the EO yield was observed to be higher during the autumn and early winter months. As can be seen from [Fig. 3](#), the EO yield of both locations/plant populations showed very similar tendency. Starting from the end of September, the yield showed a consistent increase, peaking in the autumn and early winter month (December), until the beginning of February. The average EO yield for the younger population reached 0.14%, while for the older population, it was 0.10% during these months. However, towards the end of February, there was a significant decrease in EO

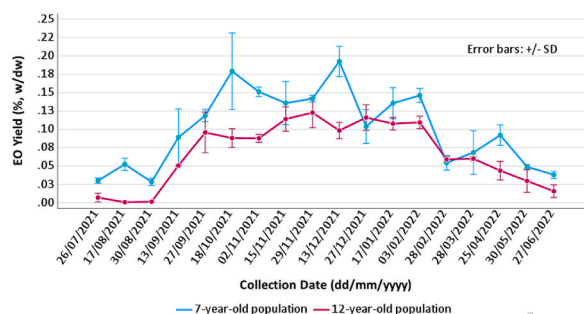


Fig. 3. Annual yield of *C. ladanifer* EOs from July 2021 to June 2022.

yield observed in both collection sites. The months from May until the end of August exhibited the lowest EO quantities, i.e. 0.04% and 0.01% for younger and older rockroses, respectively.

Considering ‘plant age’ factor, a statistically significant difference was confirmed between younger and older rockrose populations ($P < 0.001$). It was observed that the EO yield from Bustares, i.e. younger population, was higher than EO yield from Hiendelaencina and its older population ($U = 974$, $P < 0.001$). On average, the 7-year-old population yielded annually 0.10% (± 0.05), approximately 51% more than 12-year-old population ($0.07\% \pm 0.04$).

The obtained EO yield values are within the range reported in the literature for *C. ladanifer*, as can be seen in Table 1, where other relevant studies are discussed and presented.

In this study, steam distillation was conducted in a steam distillation pilot device, while the majority of the reported values for *C. ladanifer* were obtained using a Clevenger system in laboratory settings. In this context, it is observed that laboratory conditions often yield values exceeding 0.1% (dry weight), whereas industrial steam distillation typically results in yields close to 0.1% (Mediavilla et al., 2021a). While the scientific literature generally assumes a higher EO yield during warmer months (Alves-Ferreira et al., 2023), the results of this study revealed that the summer months exhibited lower quantity of EO. The evaluation of the relationship between weather variables for the studied period (July 2021–June 2022), obtained from the nearest meteorological station (Guadalajara), and EO yields is presented in Table 2.

We can observe a significant ($P < 0.001$) negative correlation between the mean temperature variables and EO yields for both the 7-year-old and 12-year-old populations, which means that with an increasing maximum/minimum/average temperature, EO yields tended to decrease. Very strong relationships between these variables were observed for the EO yields of the older population ($r = -0.84$, -0.86 and -0.87). No correlation ($P > 0.01$) was found between total precipitations and EO yield for both populations. Besides meteorological aspects, lower EO yields in summer months in studied region could also be influenced, among others, by seasonal changes of plant phenology (Llusià and Peñuelas, 2000). Starting in May, when the EO yield notably decreased, both plant populations were experiencing a flowering season and by the end of June and beginning of July the seeds were produced. Both seasonality and phenology can influence the EO yield as also leaves, where the rockrose EO is mainly present (Frazao et al., 2018), undergo morphological and metabolic modifications (Aronne and Micco, 2001; Amaral et al., 2015). Leaf characteristics of *C. ladanifer* are influenced by seasonal changes, as the adaptative response to the climatic conditions. In summer there is a decrease of chlorophyll content and leaf area (Núñez-Olivera et al., 1996). Altogether, this issue is more complex and requires further investigation involving additional meteorological, environmental and phenological indicators, which is planned as a continuation of this research.

To compare *C. ladanifer* with other Mediterranean tree and shrub species producing EOs, the following values have been reported for: *Eucalyptus globulus* Labill. – 1.59%, *Eucalyptus nitens* Maiden – 0.45%, *Juniperus communis* L. – 0.31%, *Salvia rosmarinus* Spenn. –

Table 1
EOs yield reported in previous papers for *C. ladanifer*.

Collection month	Origin	Plant condit.	Plant part	Distill. method	Distill. time h	Sample weight	Yield %w/d.w.	Yield %v/d.w.	Reference
March	Portugal	Fresh	Twigs	SD	1.5	100 kg	–	0.01	Tavares et al. (2020)
April	Morocco	Dry	Twigs	HD-Clev.	–	–	–	0.21	El Karkouri et al., 2021
May	Morocco	Fresh	Twigs	HD-Clev.	5	–	0.08	–	Bechlaghem et al. (2019)
May	France	Fresh	Twigs	SD	2	100 g	0.12	–	Robles et al. (2003)
May	Morocco	Dry	Leaves	HD-Clev.	5	–	0.14	–	Zidane et al. (2013)
July	Portugal	Fresh	Leaves	HD-Clev.	3	100 g	–	0.63*	Guimarães et al. (2010)
July	Portugal	Fresh	Twigs	SDE	1	15 g	0.15	–	Teixeira et al. (2007)
July–August	Portugal	Fresh/Dry	Twigs	HD-Clev.	2	20 g	0.2–0.3	–	Gomes et al. (2005)
August	Portugal	Fresh	Twigs	SD	1.5	100 kg	–	0.01–0.04	Tavares et al. (2020)
August	Portugal	Fresh	Twigs	HD-Clev.	3	270 g	–	0.15	
August	France-Corsica	Fresh	Twigs	HD	8	–	0.07	–	Mariotti et al. (1997)
August		Fresh	Twigs	HD-Clev.	8	–	0.16–0.41	–	
August	Morocco	Fresh	Twigs	HD-Clev.	4	100 g	–	0.3–0.4*	Greche et al. (2009)
September	Spain -	Fresh	Twigs	SD	0.5	5 kg	0.036	–	Mediavilla et al. (2021b)
October	Guadalajara	Fresh	Twigs	SD	–	–	0.037	–	
September	Spain - Extremadura	Dry	Twigs	HD-Clev.	3	200 g	–	0.35	Pérez-Izquierdo et al. (2022)
October	Spain - Madrid	Fresh	Leaves	HD-Clev.	3	–	0.34	–	Verdeguer et al. (2012)
October	Spain - Guadalajara	Fresh	Twigs	SD	2	20 kg	0.075	–	Mediavilla et al. (2021a)
-	Morocco	Dry	Twigs	HD-Clev.	4	100 g	0.26	–	Benali et al. (2020)
-	Portugal	Dry	Twigs	SD	–	50 g	0.33	–	Gonçalves et al. (2013)

Note: SD – steam distillation; HD-Clev. – hydro-distillation via Clevenger apparatus; SDE – simultaneous distillation-extraction; %w/d.w. – % of weight/dry weight; %v/d.w. – volume/dry weight; *%v/f.w. – weight/fresh weight.

Table 2

Spearman's correlation between weather conditions and EO yields over the course of one year.

Population	Maximum temperature		Minimum temperature		Average temperature		Total precipitations	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
7-year-old	−0.63	0.005**	−0.64	0.004**	−0.67	0.003**	0.33	0.176*
12-year-old	−0.84	<0.001**	−0.86	<0.001**	−0.87	<0.001**	0.31	0.212*

Note: * no significance; ** statistically significant at 0.01; *r* – correlation coefficient.

0.50%, *Pinus sylvestris* L. – 0.15% (Mediavilla et al., 2021b), *Eucalyptus patellaris* – 0.25% (Khedhri et al., 2022), *Eucalyptus occidentalis* – 1.62% (Khammassi et al., 2022).

3.2. Essential oil composition

GC (FID)-MS analyses of the extracted rockrose EOs resulted in the identification of 37 constituents for each collection date, representing in average 73.40–91.50% of their respective total content across all investigated samples. The Tables containing those data are placed in the end of the article in the Appendix A - Supplementary data. The studied EOs are composed mainly by high volatility monoterpenes hydrocarbons (α -pinene, camphene, limonene, *p*-cymene, sabinene, verbenene), medium volatility compounds of sesquiterpene hydrocarbons (*allo*-aromadendrene, γ -cadinene, α -copaene, ledene) and oxygenated monoterpenes (pino-carvone, bornyl acetate, terpinen-4-ol, *trans*-pinocarveol, borneol, α -campholenal, 2,2,6-trimethylcyclohexanone, myrtenol), and low volatility oxygenated sesquiterpens (viridiflorol, ledol). Monoterpene hydrocarbons and oxygenated monoterpenes were leading chemical classes across all EO samples.

Comparing with other studies, Xavier et al. (2021) also identified α -pinene as a principal constituent ($42.50 \pm 0.96\%$) of *C. ladanifer* EO from south-west Spain, other important compounds included: ledol (6.94 ± 0.36 and $4.06 \pm 0.17\%$), bornyl acetate (5.01 ± 0.02 and $4.16 \pm 0.10\%$) and camphene (6.66 ± 0.01 and $2.15 \pm 0.07\%$). Mediavilla et al. (2021b) reported the main components of *C. ladanifer* EOs in September and October harvests as follows: α -pinene (51.93 and 39.11%), followed by viridiflorol (6.40 and 10.21%), bornyl acetate (2.89 and 3.16%), camphene (2.42 and 3.13%), *trans*-pinocarveol (2.15 and 2.22%), and ledol (1.84 and 2.94%). Our findings also align with the results of Mariotti et al. (1997), who evaluated *C. ladanifer* of Spanish origin harvested in Corsica, wherein α -pinene (35%), viridiflorol (11.8%), ledol (3.3%), and bornyl acetate (3.1%) were also identified as the major constituents. On the other hand, Verdeguer et al. (2012) reported chemical profile of *C. ladanifer* from central Spain containing *trans*-pinocarveol (20.00%), bornyl acetate (7.03%) and terpinen-4-ol (6.37%) as the main monoterpene compounds. Viridiflorol (13.59%) and ledol (4.36%) were the main constituents of the oxygenated sesquiterpene fraction. However, α -pinene in that study was represented only by 4.70%. Similar result was obtained by Greche et al. (2009), who determined 5.0% of α -pinene in *C. ladanifer* from Morocco and the major constituents were viridiflorol (19.4%), bornyl acetate (16.5%), camphene (12.1%), 2,2,6-trimethylcyclohexanone (3.6%) and terpinen-4-ol (4.1%). Zidane et al. (2013) identified camphene (15.5%) as a major component of *C. ladanifer* EO, from eastern Morocco, followed by borneol (11.1%), cyclohexanol-2, 2, 6 tremethyl (7.3%), terpineol-4 (6.3 %) and α -pinene (4.2 %). The composition of EO appears to exhibit significant variability when comparing these aforementioned studies conducted in different regions. Variations in the EO composition are commonly attributed to geographic, climatic, physiological and seasonal factors (Jordán et al., 2006; Herranz et al., 2005), as extensively documented in other plant species.

To compare the annual variability, the main EOs' compositions of both populations (represented by 16 compounds) are presented in Fig. 4–9. The constituents are depicted in the graphs based on their percentage representation, from the mayor components to the minor ones. Figs. 4 and 5 depict α -pinene, the principal constituent with higher values than the rest, on a different scale in the right axis of the figures.

α -Pinene was the principal constituent of rockrose EOs in all collection dates, found in the largest quantities from the late September to the end of December. This trend coincided with periods of elevated EO yields; a very strong positive correlation for the 7-year-old population ($r = 0.83$, $P < 0.01$) was found between the EO yields and α -pinene contents throughout the year. For the following

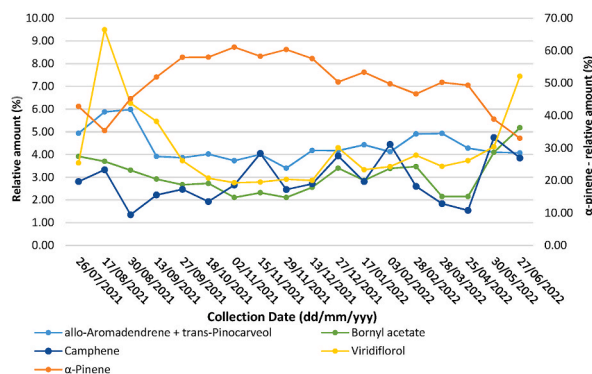


Fig. 4. Chemical composition of EO of 7-year-old rockrose population – main compounds (mean values 2.00–50%).

components, strong negative correlations with EO yield were observed: *allo*-aromadendrene + *trans*-pinocarveol ($r = -0.62$, $P = 0.007$), viridiflorol ($r = -0.63$, $P = 0.005$), bornyl acetate ($r = -0.62$, $P = 0.006$). This indicates a tendency for these components to decrease as EO yield increases. Camphene content showed no correlation ($P > 0.05$) with EO yields. Similarly, the 12-year-old population displayed higher amounts of α -pinene in autumn and winter months, aligning with periods of increased EO yields. A very strong positive correlation ($r = 0.88$, $P < 0.01$) between the EO yield and α -pinene content was observed. For the following components, a strong negative correlation was observed: *allo*-aromadendrene + *trans*-pinocarveol ($r = -0.79$, $P < 0.001$), viridiflorol ($r = -0.71$, $P = 0.001$), bornyl acetate ($r = -0.77$, $P < 0.001$). Additionally, a moderate positive correlation ($r = 0.50$, $P = 0.034$) was determined for camphene.

It is apparent from the graphs that an increase in α -pinene corresponds to a decrease in less volatile compounds. The evaluation of relationship between the increase or decrease of α -pinene and other compounds is presented in Table 3.

Very strong negative correlations ($r \geq -80$, $P < 0.001$) were found between composition of α -pinene and *allo*-aromadendrene + *trans*-pinocarveol, α -copaene, bornyl acetate, viridiflorol and myrtenol, in case of older population. For younger population, there was a strong correlation of α -pinene with borneol and bornyl acetate.

Additionally, the annual variability of pinocarvone, limonene, *p*-cymene, terpinen-4-ol and γ -terpinene is presented in Figs. 6 and 7, for younger and older population, respectively.

It is evident, that the level of *p*-cymene, terpinen-4-ol and γ -terpinene content in 7-year-old rockrose EOs were more stable compared to EOs from 12-year-old rockroses, where fluctuations throughout the year occurred. *p*-Cymene content of both rockrose populations reached during June and July the highest levels. It can be noted that the γ -terpinene content of the younger population reached its highest values in August, while the older population exhibited the lowest contents during the same period.

In the last Figs. 8 and 9, the variability of minor compounds with mean percentage area below 1 (verbenene, myrtenal, borneol, α -copaene, myrtenol and ledene) is presented.

All studied compounds from Figs. 8 and 9 exhibited higher contents during the late spring and summer months and lower contents in autumn and winter months. Both rockrose population had the highest content of ledene and α -copaene by mid-August. Borneol and verbenene reached maximum levels at the end of May. Myrtenal content of both populations reached minimum values by mid-September.

Regarding the variation among collection dates, from September to February, there was registered an increase of α -pinene and a decrease in the rest of the main compounds (*allo*-aromadendrene + *trans*-pinocarveol, bornyl acetate and viridiflorol) in these months. The similar findings were reported by Tavares et al. (2020), who compared two harvests – in March and August, for following compounds: α -pinene (27.9 and 24.7%), bornyl acetate (3.7 and 6.3%), viridiflorol (1.7 and 6.2%), *p*-cymene (2.3 and 2.9%) and borneol (0.7 and 1.3%). On the contrary, limonene (6.6 and 1.0%) and myrtenal (0.1 and 0.4%) showed opposite results than this study. The study of Llusà and Peñuelas (2000) demonstrated that *Cistus albidus* stores large amounts of terpenes with maximum value in autumn and with the lowest in summer-spring season. In summer, the most volatile monoterpenes, such as α -pinene, were emitted to the environment in greater quantities (and thus stored in less amounts in plants) due to the increase in temperature (Llusà and Peñuelas, 2000). In our case, evaluation of relationship between temperature variables and composition of α -pinene showed moderate negative correlation (for younger population: Tmax $r = -0.50$ ($P > 0.05$); Tmin $r = -0.55$ ($P > 0.05$) and Taver $r = -0.52$ ($P > 0.05$) and for older population: Tmax $r = -0.55$ ($P > 0.05$); Tmin $r = -0.61$ ($P > 0.01$) and Taver $r = -0.58$ ($P > 0.05$)). On the other hand, larger emission rates of the least volatile terpenes such as limonene were reported in spring (Llusà and Peñuelas, 2000), which means that plant stored less limonene in spring season. This result also coincides with our results, both plant populations exhibited less amount of limonene in spring months. However, no correlations ($P > 0.05$) were found between limonene content of both rockrose populations and temperature variables.

When considering the ‘plant age’ element, a statistical significance difference ($P < 0.05$) was found only in the content of *allo*-aromadendrene + *trans*-pinocarveol between younger and older population. In the rest of the compounds, no difference ($P > 0.05$) was revealed. Several studies evaluated the impact of the endogenous factor ‘plant age’ on the chemical composition of EOs of different aromatic plants (Pagoula et al., 2000; Hudaib et al., 2002; Afolajan and Asekun, 2008). However, in the case of *C. ladanifer*, these seasonal variations are not reported. The only relevant information was found in the study of Torcato (2015), where α -pinene was the

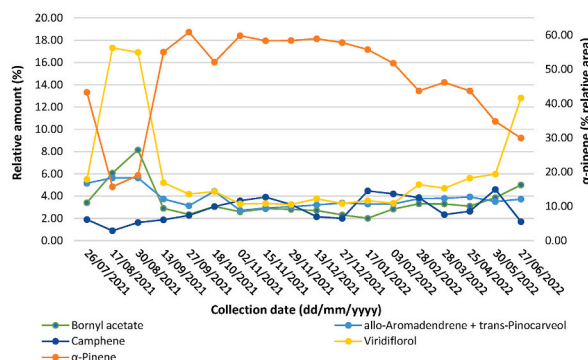


Fig. 5. Chemical composition of 12-year-old rockrose population – main compounds (mean values 3.00–50.00%).

Table 3
Spearman's correlation between α-pinene and other EO's constituents.

Main EO constituents	7-year-old popul.		12-year-old popul.	
	r	P value	r	P value
allo-Aromadendrene + trans-pinocarveol	−0.53**	0.023	−0.80**	<0.001
α-Copaene	−0.63***	0.005	−0.85***	<0.001
Borneol	−0.84***	<0.001	−0.58**	0.011
Bornyl acetate	−0.87***	<0.001	−0.91***	<0.001
Camphene	−0.32*	0.189	0.42*	0.085
γ-Terpinene	−0.05*	0.852	0.61***	0.007
Ledene	−0.50*	0.037	−0.71***	0.001
Limonene	0.49*	0.038	0.28*	0.266
Myrtenal	−0.41*	0.088	−0.35*	0.155
Myrtenol	−0.77***	<0.001	−0.83***	<0.001
p-Cymene	−0.59**	0.010	−0.76***	<0.001
Pinocarpone	0.24*	0.342	0.39*	0.111
Terpinen-4-ol	0.27*	0.285	0.61***	0.007
Verbenene	−0.70***	0.001	−0.07*	0.776
Viridiflorol	−0.79***	<0.001	−0.93***	<0.001

Note: *no significance; **Significant difference at $P < 0.05$; *** Significant difference at $P < 0.01$.

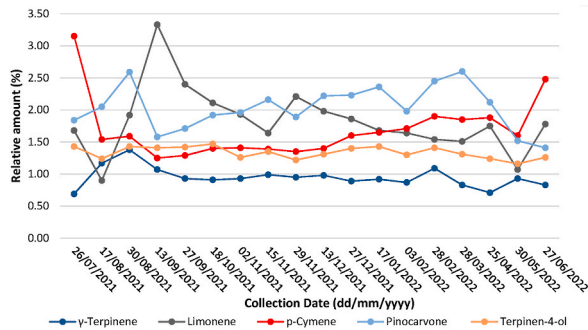


Fig. 6. Chemical composition of EO of 7-year-old rockrose population – main compounds (mean values 1.00–2.00%).

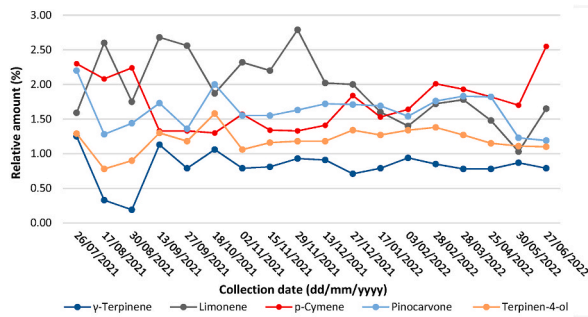


Fig. 7. Chemical composition of EO of 12-year-old rockrose population – main compounds (mean values 0.50–3.50%).

dominant component in the EO of young leaves, accounting for 40–44%, its relative amount decreased significantly in mature leaves, ranging from 9 to 22%. There was also difference in viridiflorol content, ranging from 1 to 2% in young leaves and 5–22% in mature leaves. Our results revealed that the average annual viridiflorol content was 4.27% (± 1.76) for the younger population and 6.20% (± 4.40) for the older population.

3.3. Bioactive properties

Finally, to complement the study, variability in antibacterial activity and antioxidant capacity was introduced, and the results are presented in the following subchapters.

3.3.1. Antibacterial activity

B. cereus, a foodborne pathogen, was selected due to the traditional use of *C. ladanifer* extracts against diarrhoea and

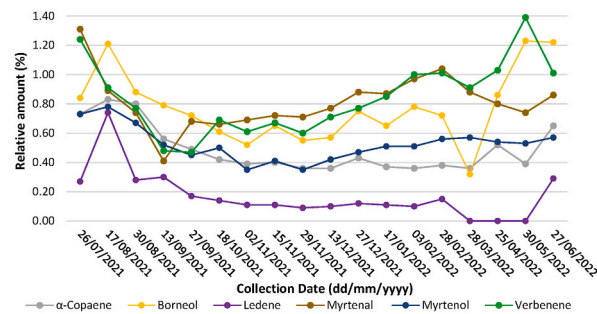


Fig. 8. Chemical composition of EO of 7-year-old rockrose population – main compounds (mean values 0.20–1.00%).

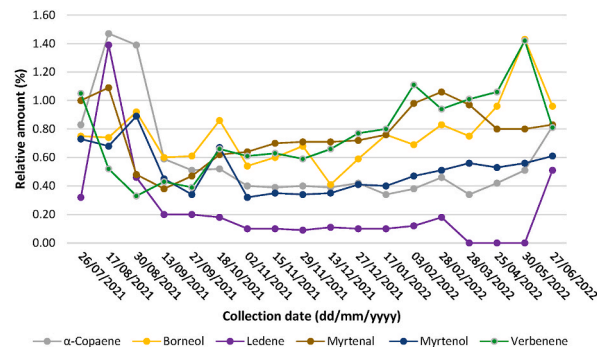


Fig. 9. Chemical composition of EO of 12-year-old rockrose population – main compounds (mean values 0.20–1.00%).

gastrointestinal illness, among others (Barrajón-Catalán et al., 2010). In addition, it is reported that gram-positive bacteria are more sensible to natural extracts (Kalemba and Kunicka, 2003; Xavier et al., 2021). The used method determined antibacterial potential of plant volatiles of *C. ladanifer* EOs simultaneously in the liquid and the vapour phase at various concentrations and eliminated limitations of conventional antimicrobial susceptibility testing methods like broth dilution and agar diffusion when testing volatile agents, as in case of EO (Houdková et al., 2017).

Table 4 presents the results of the *in vitro* antibacterial capacity of EOs in liquid and vapour phase against *B. cereus* strain, expressed

Table 4
Minimum inhibitory concentrations (MICs) of tested rockrose EOs against *B. cereus*.

Collection Date (dd/mm/yyyy)	7-year-old		12-year-old	
	MIC broth µg/mL	MIC agar µg/mL	MIC broth µg/mL	MIC agar µg/mL
26/07/2021	1024	>1024	512	1024
17/08/2021	1024	1024	nt	nt
30/08/2021	1024	>1024	nt	nt
13/09/2021	1024	>1024	512	>1024
27/09/2021	1024	>1024	512	1024
18/10/2021	>1024	>1024	1024	>1024
02/11/2021	1024	>1024	1024	>1024
15/11/2021	>1024	>1024	1024	>1024
29/11/2021	>1024	>1024	1024	>1024
13/12/2021	>1024	>1024	512	1024
27/12/2021	1024	1024	512	>1024
17/01/2022	>1024	>1024	1024	>1024
03/02/2022	1024	>1024	1024	>1024
28/02/2022	1024	1024	512	1024
28/03/2022	>1024	>1024	1024	>1024
25/04/2022	>1024	>1024	1024	>1024
30/05/2022	>1024	>1024	1024	>1024
27/06/2022	1024	>1024	512	1024
Positive antibiotic control				
Chloramphenicol	2	nd	2	nd

Note: nt – not tested (due to the unavailability of EOs' quantity); nd – not determined.

as minimum inhibitory concentration (MIC) values in $\mu\text{g/mL}$. A lower MIC value indicates higher antibacterial activity.

From the results of Table 4, we can see that tested EOs from 12-year-old rockroses produced a moderate antibacterial level of *in vitro* growth-inhibitory activity against the *B. cereus* in liquid phase, with MICs ranging from 512 to 1024 $\mu\text{g/mL}$. The older rockrose population exhibited stronger potency than the younger one, as the latter demonstrated a weak action with MICs of 1024 $\mu\text{g/mL}$ or higher. Statistically, the difference between MIC values of liquid phase is significant ($P < 0.01$). The results of vapour phases, in Table 4 referred to as agar (lid), showed weaker antibacterial activity compared to liquid phase (broth); none of the EO samples achieved values below 1024 $\mu\text{g/mL}$. A comparable pattern of lower potency of vapour phase compared to liquid phase has been observed in prior studies focused on EOs isolated from medicinal plants (Houdková et al., 2018a, 2018b). No correlation ($P > 0.05$) was found between MIC values and previously studied variables (EO yield, EO composition and meteorological variables).

To compare with other studies, Boy et al. (2023) evaluated *C. ladanifer* from Extremadura as a promising agent against several microorganisms (yeasts, molds and bacteria), including *B. cereus*, following different procedure (agar disk-diffusion method). Guinoiseau et al. (2011) determined that *C. ladanifer* from Corsica exhibited similar antibacterial activity against *B. cereus* at concentration of 0.8 mg/mL. Xavier et al. (2021) evaluated antibacterial activity of Spanish *C. ladanifer* from two locations against *Escherichia coli* (0.6 mg/mL), *Listeria monocytogenes* (0.3–0.6 mg/mL), MRSA (0.3–0.6 mg/mL) and *Enterococcus faecalis* (0.6–1.25) mg/mL. Stronger antibacterial potency in this study was attributed to rockrose EO with lower α -pinene content and higher share of viridiflorol. This result is consistent with the general theory that α -pinene, the primary component of the studied EOs' samples, has lower antibacterial activity (Santoyo et al., 2005). Thus, it is essential to consider the potential contribution of less abundant constituents to the antibacterial effect. Guinoiseau et al. (2011) separated EO of *C. ladanifer* from Corsica into several fractions by column chromatography and identified the main EO' constituents involved in the antibacterial activity-monoterpene alcohols (including *trans*-pinocarveol and borneol), and sesquiterpene alcohols (as viridiflorol and ledol); this fraction strongly inhibited the growth of *B. cereus* at concentration of 0.1 mg/mL. Unfortunately, the EO samples from older population containing in higher amounts above mentioned compounds (17/08/2021 and 30/08/2021) were not tested due to insufficient remaining quantity for analysis. To date, this study is the first evaluation of *C. ladanifer* by newly developed broth microdilution volatilization method in the liquid and the vapour phase, thus further investigation of other bacteria is needed.

To compare *C. ladanifer* with other Mediterranean tree and shrub species producing EOs, the following values have been reported. The EOs of *Eucalyptus* spp (*E. occidentalis*, *E. striatocalyx* and *E. stricklandii*) exhibit strong antibacterial potential, inhibiting the growth of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* at concentrations of 25–45 $\mu\text{L/mL}$ (Khammassi et al., 2022). Less antibacterial activity than *C. ladanifer* was found for *Juniperus communis* L. with MIC values of 1.25 mg/mL (*Escherichia coli*) and >2.5 mg/mL (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Morganella morganii*, MRSA and others) (Xavier et al., 2021).

3.3.2. Antioxidant capacity

In recent years, EOs as herbal antioxidants gained scientific interest, especially in food science and medicine (Guimarães et al., 2010), due to concerns over the potential harm of synthetic antioxidants to human health (Amorati et al., 2013). The variation in antioxidant activity of *C. ladanifer* EOs throughout the year, determined by oxygen radical absorbance capacity (ORAC) method and expressed as $\text{IC}_{50} \pm \text{SD}$ in $\mu\text{g/mL}$, is presented in Table 5. A lower inhibition value indicates higher antioxidant activity.

Over the course of one year, the antioxidant capacity of 7-year-old population varied from $117.47 \pm 15.45 \mu\text{g/mL}$ to $>256.00 \mu\text{g/mL}$, with annual mean value of $213.81 \pm 42.95 \mu\text{g/mL}$. ORAC values of 12-year-old population ranged from $147.47 \pm 15.19 \mu\text{g/mL}$ to $>256.00 \mu\text{g/mL}$, with annual mean value of $213.90 \pm 79.79 \mu\text{g/mL}$. As can be seen, the antioxidant activity of both populations was highly similar and followed the same trend during all harvest dates. Comparing both populations, there was not statistically significant difference between them ($U = 1219$, $P > 0.05$). Comparing the collection dates, from February to May, the both EOs showed a stronger antioxidative action compared to the rest of the year, on average 7-year-old population at $165.69 \pm 32.40 \mu\text{g/mL}$ and 12-year-old population at $165.35 \pm 29.76 \mu\text{g/mL}$. In November, there was also a slight increase in antioxidant action in both populations, reaching levels of $190.82 \pm 14.65 \mu\text{g/mL}$ and $204.78 \pm 12.86 \mu\text{g/mL}$ for younger and older population, respectively, in the middle of the month. During the summer period, from July to October, ORAC values remained relatively constant ($\geq 243.50 \mu\text{g/mL}$).

Considering the factor of harvest period, Mediavilla et al. (2021b) found that the antioxidant activity of *C. ladanifer*, as determined by the ORAC assay (however in different units), was $187.93 \mu\text{mol Trolox/g EO}$ for the September harvest and $209.36 \mu\text{mol Trolox/g EO}$ for the October harvest. As in our case, the antioxidant capacity of October harvest was slightly stronger. Tavares et al. (2020) did not detect much difference in the antioxidant activity between the samples of *C. ladanifer* harvested in different periods of the year; ABTS method showed a moderate antioxidant capacity of $68.1 \pm 0.1\%$ for March and $69.7 \pm 1.2\%$ for August, and by xanthine oxidase inhibiting activity method determined a strong inhibition of $96.9 \pm 0.2\%$ for March and 98.5 ± 0.2 for August.

The relationship between antioxidant activity values and other variables, such as EO yield, EO composition and meteorological variables throughout the year, was also evaluated. Correlation analyses revealed no interaction ($P > 0.05$) with EO yield and meteorological variables. Regarding interactions between ORAC values and EO composition, significant correlations in EOs of 7-year-old population were found for contents of verbenene ($P = 0.006$), which demonstrated a strong negative correlation ($r = -0.62$) and ledene, that showed a moderate positive correlation ($r = 0.53$, $P = 0.025$). In EOs of older population, correlations were found for contents of verbenene ($r = -0.73$, $P = 0.001$), myrtenal ($r = -0.63$, $P = 0.01$), limonene ($r = 0.56$, $P = 0.024$), borneol ($r = -0.56$, $P = 0.025$), and α -pinene ($r = 0.50$, $P = 0.048$). Negative correlations indicate that an increased content of mentioned compounds resulted in stronger antioxidant capacity across the EO year's evolution. Contrarily, positive correlations suggest that as the content of these compounds increased, the antioxidant potential decreased. Ruberto and Baratte's analysis (2000) of individual compounds determined similar results for α -pinene and limonene, but opposite findings for borneol. Their study also identified γ -terpinene as a potent

Table 5Antioxidant capacity of *C. ladanifer* EOs evaluated by ORAC method.

Collection date dd/mm/yyyy	7-year-old		12-year-old	
	Mean µg/mL	SD	Mean µg/mL	SD
26/07/2021	243.51	9.32	>256.00	0
17/08/2021	>256.00	0	nd	nd
30/08/2021	>256.00	0	nd	nd
13/09/2021	>256.00	0	>256.00	0
27/09/2021	>256.00	0	>256.00	0
18/10/2021	235.19	4.31	247.66	2.95
02/11/2021	228.26	2.82	251.20	1.64
15/11/2021	190.82	14.65	204.78	12.86
29/11/2021	205.36	10.80	215.70	14.27
13/12/2021	>256.00	0	>256.00	0
27/12/2021	227.03	7.04	235.65	4.88
17/01/2022	244.32	9.99	251.28	0.76
03/02/2022	158.99	7.71	151.30	3.54
28/02/2022	168.51	15.28	147.47	15.19
28/03/2022	192.64	5.48	163.68	11.08
25/04/2022	147.67	6.30	156.33	4.94
30/05/2022	117.47	15.45	149.33	15.33
27/06/2022	208.88	7.23	224.02	11.93
Control Trolox	16.55	1.35		

Note: nd - EO from 17/08/2021 and 30/08/2021 collection dates not tested for older population, due to the unavailable quantity of mentioned EOs; SD – standard deviation.

antioxidative constituent; however, in the present study, no relationship was found between its content and ORAC value throughout the year. Altogether, the antioxidant activity of EOs may be attributed not only to two or three predominant compounds within their chemical profile but also to the synergistic interactions with their minor components (Araújo Couto et al., 2019).

In general, we can consider all obtained values as indicating moderate-weak antioxidative action. For comparison, a very strong antioxidant action (via ORAC method) presents a Trolox, synthetic analog of a well-known antioxidant vitamin E (IC₅₀ 16.6 µg/mL) or *Citrus lumia* EO with IC₅₀ of 46 µg/mL (Smeriglio et al., 2018). On the other hand, other evaluation methods, including the DPPH, β-carotene bleaching, and ABTS assays, revealed significant antioxidant capacity in rockrose EO (Zidane et al., 2013; Upadhyay et al., 2018; Luís et al., 2020). Xavier et al. (2021) reported *C. ladanifer* from south-west Spain that inhibited about 83% of oxidation (tested by Reducing Power assay) and exhibited a GI₅₀ of 336 ± 8 µg/mL. Guimarães et al. (2010) determined DPPH scavenging activity of *C. ladanifer*, harvested in Portugal (in July) as 36.28 ± 0.36 mg/mL.

4. Conclusions

The variability in yield, composition, antibacterial and antioxidant activity of the essential oil (EO) extracted from rockrose (*Cistus ladanifer* L.) sourced from two distinct locations with different plant age in central Spain was analysed and compared over a twelve-month period. It was confirmed that there are statistically significant differences in essential oil yield of *C. ladanifer* throughout the year. Strong correlations were found between EO yields and mean temperature variables in the studied region. However, further investigation is needed to fully understand the real influence of mean temperature and phenological factors on EO yields. The maximum essential oil yield of *C. ladanifer* was obtained between September and February, registering an increase of α-pinene and a decrease in the rest of the main compounds (*allo*-aromadendrene + *trans*-pinocarveol, bornyl acetate and viridiflorol) in these months. *C. ladanifer* EOs were rich in α-pinene, *allo*-aromadendrene + *trans*-pinocarveol, viridiflorol, bornyl acetate and camphene. On the other hand, the share of less volatile compounds increases from March and continues until the end of August. This period coincides with the highest antioxidant capacity, which is highest from February to May. The EOs obtained from both populations exhibited similar moderate-weak antioxidant capacity, with their strongest potential observed between February and May. Antimicrobial activity against the foodborne bacteria *Bacillus cereus* remained weak throughout the year, with stronger potency observed in case of the older rockrose population.

CRedit authorship contribution statement

Veronika Chaloupková: Writing – original draft, Investigation, Formal analysis. **Irene Mediavilla:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Raquel Bados:** Investigation, Data curation. **Markéta Houdková:** Resources, Methodology. **Johana Rondevaldová:** Resources, Methodology. **Luis Saúl Esteban:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2024.103279>.

Appendix A. Supplementary data

Table 1

Chemical profile of 7-year-old population of *C. ladanifer* EO

Compound	26/ 07/ 2021	17/ 08/ 2021	30/ 08/ 2021	13/ 09/ 2021	27/ 09/ 2021	18/ 10/ 2021	02/ 11/ 2021	15/ 11/ 2021	29/ 11/ 2021	13/ 12/ 2021	27/ 12/ 2021	17/ 01/ 2022	03/ 02/ 2022	28/ 02/ 2022	28/ 03/ 2022	25/ 04/ 2022	30/ 05/ 2022	27/ 06/ 2022
MONOTERPENE HYDROCARBONS																		
α -Pinene	42.83	35.37	45.22	51.87	57.96	57.98	61.07	58.27	60.35	57.56	50.34	53.35	49.76	46.69	50.23	49.34	38.89	33.02
α -p-Dimethylstyrene	0.44	0.27	0.18	0.12	0.11	0.14	0.10	0.10	0.08	0.10	0.13	0.14	0.14	0.17	0.21	0.20	0.31	0.36
β -Pinene	0.52	0.42	0.53	0.66	0.65	0.58	0.56	0.52	0.58	0.57	0.53	0.48	0.48	0.44	0.41	0.45	0.39	0.44
Camphene	2.81	3.33	1.35	2.22	2.47	1.93	2.66	4.05	2.46	2.71	3.94	2.81	4.45	2.60	1.84	1.54	4.75	3.85
Limonene	1.68	0.90	1.92	3.33	2.40	2.11	1.93	1.64	2.21	1.98	1.86	1.68	1.64	1.54	1.51	1.75	1.07	1.78
p-Cymene	3.15	1.54	1.59	1.25	1.29	1.40	1.41	1.39	1.35	1.40	1.60	1.65	1.71	1.90	1.85	1.88	1.60	2.48
Pinocarvone	1.84	2.05	2.59	1.58	1.71	1.92	1.96	2.16	1.89	2.22	2.23	2.36	1.98	2.45	2.60	2.12	1.52	1.41
Sabinene	0.70	0.39	1.14	1.47	1.40	1.13	1.19	1.07	1.40	1.01	0.85	0.83	0.83	0.74	0.80	0.87	0.75	1.18
Tricyclene	0.39	0.53	0.14	0.28	0.32	0.23	0.35	0.58	0.31	0.35	0.55	0.67	0.67	0.33	0.21	0.14	0.76	0.58
Verbenone	0.30	0.24	0.17	0.13	0.15	0.22	0.21	0.24	0.23	0.31	0.37	0.56	0.56	0.52	0.43	0.41	0.51	0.32
α -Terpinene	0.30	0.57	0.68	0.53	0.43	0.43	0.42	0.47	0.46	0.48	0.36	0.35	0.35	0.47	0.41	0.33	0.47	0.33
γ -Terpinene	0.69	1.17	1.38	1.07	0.93	0.91	0.93	0.99	0.95	0.98	0.89	0.92	0.87	1.09	0.83	0.71	0.93	0.83
Verbenene	1.24	0.91	0.77	0.48	0.47	0.69	0.61	0.67	0.60	0.71	0.77	0.85	1.00	1.01	0.91	1.03	1.39	1.01
Sum	56.89	47.69	57.66	64.99	70.29	69.67	73.40	72.15	72.87	70.38	64.42	66.65	64.44	59.95	62.24	60.77	53.34	47.59
OXYGENATED MONOTERPENES																		
Bornyl acetate	3.92	3.70	3.31	2.92	2.67	2.73	2.11	2.32	2.11	2.56	3.40	2.86	3.39	3.47	2.15	2.15	4.10	5.18
α -Campholenal	1.33	1.34	1.15	0.66	0.74	1.12	1.61	1.62	1.62	1.64	1.90	2.06	2.06	2.33	2.41	2.50	2.31	2.11
Borneol	0.84	1.21	0.88	0.79	0.72	0.61	0.52	0.65	0.55	0.57	0.75	0.65	0.78	0.72	0.32	0.86	1.23	1.22
Campholenal Isomer	0.33	0.29	0.27	0.14	0.15	0.20	0.27	0.28	0.27	0.28	0.33	0.35	0.35	0.39	0.37	0.35	0.33	0.29
Camphor	0.44	0.41	0.29	0.18	0.18	0.18	0.18	0.21	0.17	0.25	0.22	0.32	0.32	0.34	–	–	0.77	0.57
Isopinocamphe	1.11	1.03	1.09	0.77	0.66	0.85	0.67	0.80	0.64	0.74	0.71	0.72	0.72	1.25	1.24	1.54	3.91	1.45
Myrtenal	1.31	0.89	0.74	0.41	0.68	0.66	0.69	0.72	0.71	0.77	0.88	0.87	0.97	1.04	0.88	0.80	0.74	0.86
Myrtenol	0.73	0.78	0.67	0.52	0.45	0.50	0.35	0.41	0.35	0.42	0.47	0.51	0.51	0.56	0.57	0.54	0.53	0.57
Myrtenyl Acetate	0.36	0.42	0.44	0.33	0.28	0.30	0.25	0.30	0.27	0.30	0.35	0.33	0.33	0.38	0.68	0.28	0.25	0.36
p-Cymen-8-ol	0.31	0.24	0.20	0.20	0.22	0.26	0.10	0.11	0.08	0.11	0.16	0.19	0.19	0.35	0.14	0.17	0.19	0.25
p-Mentha-1,5-dien-8-ol	0.26	0.31	0.27	0.28	0.21	0.27	0.26	0.34	0.32	0.42	0.39	0.47	0.47	0.61	0.50	0.54	0.59	0.48
Terpinen-4-ol	1.43	1.24	1.43	1.41	1.42	1.47	1.26	1.35	1.22	1.31	1.40	1.43	1.30	1.41	1.31	1.24	1.16	1.26
allo-Aromadendrene + trans-Pinocarveol*	4.94	5.88	5.98	3.92	3.86	4.02	3.73	4.01	3.40	4.18	4.17	4.12	4.12	4.91	4.93	4.28	4.10	4.07
trans-Verbenol	0.61	0.67	0.65	0.51	0.58	0.71	0.70	0.77	0.85	0.99	1.07	1.41	1.41	1.44	1.35	1.61	1.25	0.85
Sum	17.26	17.59	16.51	12.31	12.11	13.07	11.97	13.24	12.02	13.95	15.49	15.73	16.36	18.56	16.30	16.25	20.75	18.67
SESQUITERPENE HYDROCARBONS																		
α -Murolene	0.35	0.51	0.39	0.39	0.37	0.31	0.17	0.16	0.17	0.13	0.19	0.11	0.11	0.27	0.06	0.06	0.11	0.24
Ledene	0.27	0.74	0.28	0.30	0.17	0.14	0.11	0.11	0.09	0.10	0.12	0.10	0.10	0.15	–	–	–	0.29
trans-Calamenene	0.06	0.04	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.02	0.05	0.02	0.02	0.04	0.03	0.03	0.03	0.06
α -Copaene	0.73	0.83	0.80	0.56	0.49	0.42	0.39	0.40	0.36	0.36	0.43	0.36	0.36	0.38	0.36	0.52	0.39	0.65
γ -Cadinene	0.77	1.34	1.03	1.04	0.65	0.76	0.31	0.30	0.36	0.25	0.40	0.14	0.14	0.34	0.02	0.02	0.31	0.85
Sum	2.84	4.28	3.39	3.05	2.43	2.48	1.74	1.65	1.55	1.45	1.90	1.29	1.29	1.82	1.02	1.24	1.55	2.66
OXYGENATED SESQUITERPENES																		
Viridiflorol	3.63	9.49	6.26	5.46	3.73	2.96	2.76	2.79	2.91	2.86	4.30	3.47	3.47	3.97	3.48	3.73	4.34	7.44
Ledol	1.08	2.73	1.82	1.63	1.09	0.79	0.79	0.75	0.78	0.75	1.19	0.98	0.98	1.05	0.90	0.99	1.25	2.16
Sum	4.71	12.22	8.08	7.09	4.82	3.75	3.55	3.54	3.69	3.61	5.49	4.45	4.45	5.02	4.38	4.72	5.59	9.60
OTHERS																		
2,2,6-trimethylcyclohexanone	2.77	1.73	1.45	0.81	0.73	0.75	0.64	0.57	0.76	0.83	1.04	1.33	1.33	1.42	1.52	1.69	2.16	2.73
1,2,4,4-Tetramethylcyclopentene	0.41	0.28	0.23	0.14	0.15	0.18	0.20	0.15	0.22	0.23	0.23	0.33	0.33	0.29	0.31	0.37	0.43	0.32
Sum	3.18	2.01	1.68	0.95	0.88	0.93	0.84	0.72	0.98	1.06	1.27	1.66	1.66	1.71	1.83	2.06	2.59	3.05
Total identified	84.88	83.79	87.32	88.39	90.53	89.90	91.50	91.30	91.11	90.45	88.57	89.78	88.20	87.06	85.77	85.04	83.82	81.57

Note: * - relative amounts (%) of *allo*-aromadendrene + *trans*-pinocarveol were determined together due to their coelution, in this context they are classified as oxygenated monoterpenes, since the proportion of *trans*-pinocarveol was higher, nevertheless the *allo*-aromadendrene belongs to sesquiterpene hydrocarbons.

Table 2

Chemical profile of 12-year-old population of *C. ladanifer* EO

Compound	26/ 07/ 2021	17/ 08/ 2021	30/ 08/ 2021	13/ 09/ 2021	27/ 09/ 2021	18/ 10/ 2021	02/ 11/ 2021	15/ 11/ 2021	29/ 11/ 2021	13/ 12/ 2021	27/ 12/ 2021	17/ 01/ 2022	03/ 02/ 2022	28/ 02/ 2022	28/ 03/ 2022	25/ 04/ 2022	30/ 05/ 2022	27/ 06/ 2022
MONOTERPENE HYDROCARBONS																		
α-Pinene	43.29	15.73	19.04	55.03	60.87	52.13	59.83	58.34	58.44	58.93	57.81	55.78	51.78	43.73	46.20	43.72	34.8	26.96
α-p-Dimethylstyrene	0.29	0.35	0.19	0.10	0.09	0.13	0.11	0.10	0.09	0.10	0.15	0.11	0.14	0.18	0.22	0.23	0.36	0.36
β-Pinene	0.50	0.22	0.34	0.65	0.68	0.57	0.66	0.64	0.75	0.51	0.52	0.48	0.45	0.43	0.41	0.40	0.37	0.35
Camphene	1.90	0.89	1.63	1.87	2.27	3.06	3.59	3.91	3.24	2.15	2.00	4.47	4.20	3.87	2.34	2.64	4.60	1.71
Limonene	1.59	2.60	1.75	2.68	2.56	1.87	2.32	2.2	2.79	2.02	2.00	1.60	1.40	1.72	1.78	1.48	1.03	1.65
p-Cymene	2.30	2.08	2.24	1.33	1.33	1.30	1.57	1.34	1.33	1.41	1.84	1.53	1.64	2.01	1.93	1.82	1.70	2.55
Pinocarvone	2.20	1.28	1.44	1.73	1.36	2.00	1.55	1.55	1.63	1.72	1.71	1.69	1.54	1.76	1.83	1.82	1.23	1.19
Sabinene	0.63	0.23	0.77	1.57	1.46	1.01	1.32	1.32	1.26	1.17	0.98	0.92	0.75	0.73	0.84	0.69	0.63	1.03
Tricyclene	0.22	0.11	0.20	0.21	0.28	0.43	0.50	0.55	0.42	0.25	0.23	0.66	0.64	0.54	0.29	0.34	0.77	0.20
Verbenone	0.20	0.12	0.16	0.09	0.11	0.20	0.15	0.24	0.22	0.27	0.33	0.37	0.56	0.61	0.59	0.54	0.55	0.31
α-Terpinene	0.54	0.07	0.03	0.55	0.34	0.54	0.30	0.34	0.45	0.42	0.23	0.34	0.41	0.33	0.37	0.39	0.41	0.28
γ-Terpinene	1.25	0.33	0.19	1.13	0.79	1.06	0.79	0.81	0.93	0.91	0.71	0.79	0.94	0.85	0.78	0.78	0.87	0.79
Verbenene	1.05	0.52	0.33	0.43	0.39	0.66	0.61	0.63	0.59	0.66	0.77	0.80	1.11	0.94	1.01	1.06	1.42	0.81
Sum	55.96	24.53	28.31	67.37	72.53	64.96	73.30	71.97	72.14	70.52	69.28	69.54	65.56	57.70	58.59	55.91	48.74	38.19
OXYGENATED MONOTERPENES																		
Bornyl acetate	3.41	6.07	8.15	2.90	2.35	3.07	2.59	2.87	2.81	2.71	2.31	2.01	2.84	3.31	3.30	3.10	3.86	5.00
α-Campholenal	1.38	0.62	0.49	0.67	0.65	1.12	1.54	1.61	1.65	1.66	1.74	1.82	2.19	2.50	2.76	2.46	2.49	2.13
Borneol	0.75	0.74	0.92	0.60	0.61	0.86	0.54	0.60	0.68	0.41	0.59	0.76	0.69	0.83	0.75	0.96	1.43	0.96
Campholenal Isomer	0.31	0.20	0.14	0.13	0.12	0.21	0.26	0.27	0.27	0.27	0.30	0.27	0.34	0.39	0.40	0.36	0.35	0.27
Camphor	0.36	0.26	0.25	0.17	0.15	0.19	0.17	0.18	0.19	0.14	0.21	0.24	0.28	0.36	–	–	0.61	0.41
Isopinocamphe	0.98	0.71	0.78	0.80	0.62	0.97	0.67	0.58	0.68	0.68	0.80	0.78	0.81	0.94	0.94	1.52	2.29	0.95
Myrtenal	1.00	1.09	0.48	0.38	0.47	0.62	0.64	0.70	0.71	0.71	0.72	0.76	0.98	1.06	0.97	0.80	0.80	0.83
Myrtenol	0.73	0.68	0.89	0.45	0.34	0.67	0.32	0.35	0.34	0.35	0.41	0.40	0.47	0.51	0.56	0.53	0.56	0.61
Myrtenyl Acetate	0.33	0.63	0.73	0.30	0.23	0.29	0.27	0.30	0.25	0.35	0.27	0.24	0.27	0.31	0.33	0.33	0.28	0.43
p-Cymen-8-ol	0.24	0.60	0.56	0.17	0.16	0.20	0.10	0.11	0.07	0.10	0.19	0.14	0.18	0.35	0.17	0.15	0.24	0.32
p-Mentha-1,5-dien-8-ol	0.27	0.39	0.05	0.14	0.10	0.29	0.12	0.24	0.29	0.34	0.20	0.38	0.56	0.52	0.50	0.46	0.47	0.31
Terpinen-4-ol	1.29	0.78	0.90	1.30	1.18	1.58	1.06	1.16	1.18	1.18	1.34	1.27	1.34	1.38	1.27	1.15	1.11	1.10
allo-Aromadendrene + trans-Pinocarveol*	5.15	5.64	5.64	3.74	3.14	4.44	2.92	2.93	2.93	3.21	3.39	3.29	3.30	3.77	3.80	3.93	3.51	3.73
trans-Verbenol	0.54	0.49	0.50	0.43	0.43	0.72	0.59	0.78	0.76	0.96	0.91	1.14	1.35	1.54	1.56	1.34	1.30	0.95
Sum	15.73	17.64	19.17	11.68	9.97	14.43	11.09	12.05	12.28	12.42	12.74	12.98	15.06	17.13	16.75	16.31	18.69	18.37
SESQUITERPENE HYDROCARBONS																		
α-Murolene	0.38	0.95	0.79	0.34	0.31	0.40	0.14	0.20	0.15	0.17	0.20	0.16	0.17	0.22	0.06	0.08	0.12	0.31
Ledene	0.32	1.39	0.46	0.20	0.20	0.18	0.10	0.10	0.09	0.11	0.10	0.10	0.12	0.18	–	–	–	0.51
trans-Calamenene	0.03	0.07	0.06	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.04	0.02	0.06	0.04	0.03	0.03	0.03	0.05
α-Copaene	0.83	1.47	1.39	0.59	0.51	0.52	0.40	0.39	0.40	0.39	0.42	0.34	0.38	0.46	0.34	0.42	0.51	0.82
γ-Cadinene	0.96	2.08	2.39	0.98	0.66	0.76	0.35	0.35	0.34	0.26	0.27	0.22	0.38	0.37	0.02	0.03	0.56	1.33
Sum	3.53	7.22	6.40	2.64	2.29	2.69	1.73	1.70	1.66	1.60	1.67	1.36	1.65	1.91	1.01	1.34	1.83	4.03
OXYGENATED SESQUITERPENES																		
Viridiflorol	5.50	17.31	16.91	5.21	4.18	4.41	3.30	3.34	3.25	3.76	3.34	3.59	3.36	5.03	4.71	5.61	5.98	12.82
Ledol	1.57	4.86	5.04	1.49	1.19	1.23	0.94	0.88	0.93	1.05	0.89	0.95	0.87	1.32	1.26	1.52	1.57	3.58
Sum	7.07	22.17	21.95	6.70	5.37	5.64	4.24	4.22	4.18	4.81	4.23	4.54	4.23	6.35	5.97	7.13	7.55	16.40
OTHERS																		
2,2,6-trimethylcyclohexanone	2.38	1.67	1.26	0.88	0.63	0.85	0.8	0.79	0.73	0.78	0.91	0.96	1.38	1.85	1.76	1.88	3.30	2.56
1,2,4,4-Tetramethylcyclopentene	0.41	0.17	0.09	0.17	0.15	0.17	0.24	0.22	0.21	0.21	0.24	0.25	0.34	0.34	0.34	0.40	0.59	0.25
Sum	2.79	1.84	1.35	1.05	0.78	1.02	1.04	1.01	0.94	0.99	1.15	1.21	1.72	2.19	2.10	2.28	3.89	2.81
Total identified	85.08	73.40	77.18	89.44	90.94	88.74	91.40	90.95	91.20	90.34	89.07	89.63	88.22	85.28	84.42	82.97	80.70	79.80

Note: * - relative amounts (%) of *allo*-aromadendrene + *trans*-pinocarveol were determined together due to their coelution, in this context they are classified as oxygenated monoterpenes, since the proportion of *trans*-pinocarveol was higher, nevertheless the *allo*-aromadendrene belongs to sesquiterpene hydrocarbons.

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