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ORIGINAL ARTICLE

Evaluation of the chemical composition of *Eucalyptus sargentii* maiden leaf essential oil variations from Cyprus

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Abstract: 1,8-cineole-rich essential oils of *Eucalyptus* species have industrial importance. Eucalypts are heterophyllous, yet monthly chemical profiles of juvenile (J) and mature (M) leaves from the same individual have rarely been documented, and, to our knowledge, the oil composition of *E. sargentii*, a widely cultivated eucalypt in Cyprus, has not been previously reported from the island. This preliminary study describes monthly variation in essential oil yield and composition of J and M leaves collected from a single tree over one year to avoid confounding intraspecific genetic variation. Such data could be relevant to eucalypt essential oil quality and utilization. The oils were separately hydrodistilled and analyzed by GC-FID and GC/MS; hierarchical cluster analysis (HCA) and principal component analysis (PCA) were applied to visualize patterns. Oil yields were 0.9%–5.5% (M) and 1.9%–7.0% (J). Major constituents of M oils were 1,8-cineole (7.5%–68.9%), α-pinene (0.1%–25.2%), β-eudesmol (2.8%–32.3%), and *trans*-pinocarveol (2.2%–16.1%); J oils contained 1,8-cineole (7.4%–58.3%), α-pinene (7.3%–25.9%), *p*-cymene (0.4%–28.1%), and *trans*-pinocarveol (0.9%–13.2%). HCA yielded two clusters and two single-sample outliers (November-M, October-J), and PCA indicated that variation in the essential oil composition was driven by several constituents. The result set reveals that the essential oil's not only content, but also yield changes significantly between mature and juvenile leaves, and two harvesting periods might be suggested for optimal commercial viability.

Keywords: *Eucalyptus*, *Eucalyptus sargentii*, Cyprus, hierarchical cluster analysis (HCA), principal component analysis (PCA)

1 Introduction

The genus *Eucalyptus* (belonging to the Myrtaceae family) includes 800–900 species worldwide (Brooker, 2000). *Eucalyptus* spp. dominate the forests and woodlands of Australia, covering about 74% of their area, and are ecologically and economically important (Ghasemian et al., 2019; Sørensen et al., 2020). The essential oils of eucalypts, especially 1,8-cineole-rich essential oils, are commercially important. The essential oil of *E. globulus* is one of the most represented species in pharmacopoeias as a source of *Eucalyptus* oil (Boland et al., 1991; Barbosa et al., 2016). 1,8-Cineole, a characteristic constituent in many *Eucalyptus* oils, is a valuable medicinal source for many reasons, and many reports describe its various formulations and potential applications through clinical trials, as well as products for preventing and treating numerous diseases (Chandorkar et al., 2021). Pinene is another well-known compound with α- and β- isomers, and previous studies have summarized its pharmacological effects against a wide range of diseases (Salehi et al., 2019; Park et al., 2021). In addition to these medicinal effects, α-pinene repels detrimental pests such as the house fly (Haselton et al., 2015). The genus *Eucalyptus* is divided into 13 subgenera (Brooker, 2000). One of the subgenera, *Symphyomyrtus*, comprises a section *Bisectaria*, series *Erectae*, and subseries *Pedicellatae*, which includes *E. sargentii, together*

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with E. stowardii, E. occidentalis, E. aspratilis, and *E. astringens* (Brooker, 2000). The members of this subseries have typical morphological traits: leaf oil glands are numerous and discrete, not obscuring secondary veins; leaves are green or olive-green; the peduncle is long, flattened, or terete; buds are held loosely; and the staminophore is narrow (Brooker, 2000). In addition, *E. sargentii* (salt river gum) occurs in nature as a small tree (8–12 meters tall). The bark is rough and flaky, grey-brown on the lower trunk, then smooth and red-brown to grey above. *Eucalyptus* spp. has heterophyllous leaves: juvenile leaves are lanceolate, grey-green, concolorous, while mature leaves are narrowly lanceolate and acuminate. *E. sargentii*, which is cultivated in Cyprus, blooms from October through January; its flowers are white or cream-colored and arranged in upright, spreading, or pendulous clusters (Yıkıcı, 2015; Landrum, 1989).

A review summarizing the chemical composition of essential oils in this genus notes that the literature focuses mostly on *E. camaldulensis, E. cinerea, E. citriodora, E. globulus, E. grandis, E. saligna*, and *E. tereticornis* (Barbosa et al., 2016). On the other hand, there is no reports on the essential oil composition of *E. sargentii* from Cyprus, which is one of the most commonly cultivated species on the island, however, there are several reports in the literature from countries such as Australia, Tunisia, and Iran (Bignell et al., 1996; Sefidkon et al., 2006; Abravesh et al., 2007; Elaissi et al., 2010; Safaei-Ghomi and Batooli, 2010; Fathi and Sefidkon, 2012; Ameur et al., 2014; Ayed et al., 2023; Yıkıcı, 2015). To the best of our knowledge, these studies did not evaluate seasonal or monthly variation of the essential oil compositions. In these reports, the major compounds of *E. sargentii* essential oil were 1,8-cineole (48.6%–75.5%), α-pinene (4.9%–28.3%), *trans*-pinocarveol (1.9%–17.5%), and β-eudesmol (1.9%–6.0%) (Bignell et al., 1996; Sefidkon et al., 2006; Abravesh et al., 2007; Elaissi et al., 2010; Safaei-Ghomi and Batooli, 2010; Fathi and Sefidkon, 2012; Ameur et al., 2014; Ayed et al., 2023). Several reports on the essential oil compositions of other *Eucalyptus* spp. have recorded marked inconsistent 1,8-cineole fluctuations between seasons or even months. For instance, in a previous study done in Iran, 1,8-cineole content varied from 64.2% to 36.0% and back to 65.8% in *E. largiflorens* and it dropped from 41.1% to 19.3% and then rose to 75.4% in *E. microtheca* oil (Sefidkon et al., 2012), moreover, it ranged from 3.6% to 27.8% in mature and from 4.9% to 22.7% in juvenile leaves in *E. torquata* naturalized in Cyprus (Bulama Modu et al., 2025).

Eucalypts are also heterophyllous, a single tree bears morphologically distinct leaves changes through leaf's age (Coppen, 2002). To the best of the authors' knowledge, monthly oil profiles of both leaf types from the same individual have not been documented for *E. sargentii*. As part of a research project ongoing on the possible monthly variations of the essential oils of several *Eucalyptus* spp. planted and naturalized in Cyprus (Bulama Modu et al., 2025) and the substantially varied compositions in the *E. sargentii* oils across the aforementioned countries lead the present study to elucidate possible variations in the chemical composition of the oils of *E. sargentii* grown in Cyprus. Sampling a single individual would be beneficial to avoid confounding intraspecific genetic variation, so that monthly yields and chemical changes in the oils can be examined within a single genotype.

The aim of this study is to investigate monthly variation in yield and chemical composition of essential oils obtained separately from juvenile and mature leaves of a same individual *E. sargentii* tree in Nicosia, which is one of the most commonly cultivated eucalypts in Cyprus, over twelve consecutive months (January-December 2025). In addition, multivariate statistical analysis, hierarchical cluster analysis (HCA) and principal component analysis (PCA), were applied to visualize patterns among the samples. To the best of our knowledge, this is the first report on the chemical composition of the oil of *E. sargentii* in Cyprus.

2 Materials and Methods

2.1 Plant Material

The juvenile and mature leaves, defined according to the morphological characteristics in Yıkıcı (Yıkıcı, 2015) and Landrum (Landrum, 1989), belonging to a same individual tree of *E. sargentii* were collected in Bakırdere, Lefkoşa, Turkish Republic of Northern Cyprus at the 10th of each month and approximately same time of the day for 12-months period (January-December 2025). Juvenile leaves were collected from relatively lower parts of the branch tips while mature leaves were collected from relatively upper-inner parts of the tree. The samples were shade-dried at room temperature for 15 days. The tree was identified by Prof. Dr. Dudu Özkum Yavuz, Assoc. Prof. Dr. Duygu Yiğit Hanoğlu and Assoc. Prof. Dr. Azmi Hanoğlu. The voucher specimens deposited at the Near East University herbarium (NEUN) with the number NEUN20010.

2.2 Isolation of the Essential Oil

The dried juvenile and mature leaves of the *E. sargentii* samples were gently crushed into small pieces and hydrodistilled separately for 3 h using a Clevenger-type apparatus. The resulting essential oils were collected in amber vials and stored at 4°C until the analysis. Essential oil yields were calculated on an air-dried weight as v/w. Table 1 tabulates sample codes, collection dates, and essential oil yields for all plant materials.

2.3 Gas Chromatography/Mass Spectrometry (GC/MS)

The GC and GC/MS analyses were performed using an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) set at 300°C, coupled to Agilent 5977B GC-MSD (Agilent Technologies, Santa Clara, CA, USA) system, respectively. Innowax FSC column (60 m × 0.25 mm, 0.25 µM film thickness) was used with helium as the carrier gas (0.8 mL/min). The GC oven temperature was held at 60°C for 10 min, ramped to 220°C at 4°C/min, held at 220°C for 10 min, then ramped to 240°C at 1°C/min. The split ratio was set to 40:1, and the injector temperature was set to 250°C. To obtain the same elution order in FID and MS detectors, simultaneous

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Table 1. The collection dates and essential oil yields of *E. sargentii* samples

Code	Collection date	Essential oil yields (%, v/w)	
		Mature leaves	Juvenile leaves
Jan	10.01.2025	1.7	6.7
Feb	10.02.2025	1.8	3.6
Mar	10.03.2025	1.4	1.9
Apr	10.04.2025	1.7	3.3
May	10.05.2025	1.4	6.7
Jun	10.06.2025	2.3	3.3
Jul	10.07.2025	5.0	4.3
Aug	10.08.2025	5.0	6.0
Sep	10.09.2025	5.5	6.0
Oct	10.10.2025	1.9	4.6
Nov	10.11.2025	0.9	6.7
Dec	10.12.2025	2.3	7.0
Mean ± SD		2.6 ± 1.6	5.0 ± 1.7
Median (min–max)		1.85 (0.9–5.5)	5.3 (1.9–7.0)

Note: Yields are expressed as % (v/w) based on air-dried leaf weight; each value derives from a single distillation. Mean ± SD and median were calculated from the twelve monthly values; SD therefore reflects month-to-month variability.

auto-injection was performed into the detectors through the splitter switch. The split ratio and synchronicity was maintained through the use of uncoated fused silica columns of differing lengths (1.44m for MS, 0.53m for FID). The relative percentage amounts of the separated compounds were calculated from the FID chromatograms. Mass spectra were recorded at 70 eV. The mass range was 35–450 m/z.

2.4 Identification of the Constituents

The identification of the components was based on the comparison of the relative retention times of the essential oil constituents with those of authentic samples or by comparing their linear retention index (LRI) to a series of *n*-alkanes (C_8–C_{40}). Computer matching against commercial (Wiley GC/MS Library, NIST Chemistry WebBook) (Linstrom & Mallard, 2001; McLafferty & Stauffer, 1989) and in-house 'Başer Library of Essential Oil Constituents' built from genuine compounds and components of known essential oils, as well as MS literature data, was used for the identification (Babushok et al., 2011).

2.5 Statistical Analysis

All relevant data were imported into IBM SPSS Statistics v27.0 (International Business Machines (IBM) Corporation, USA). Principal Component Analysis (PCA) was performed using the Correlation Matrix method with sequential eigenvalues selected based on the introduction of "kinks" in the Scree Plot. The Varimax Rotation method was employed to examine associations of different chemical constituents and principal components. Correlation Matrices were employed to ascertain the effect of different constituents on chemical composition. Hierarchical Cluster Analysis (HCA) was performed using the Squared Euclidean Distance Between-Groups Linkage method with agglomeration schedules. The dendrograms were produced from HCA. We subjected the HCA-defined clades to Mann-Whitney U analysis to confirm chemical differences, using Levene's test to assess homogeneity of variances.

The phytochemicals absent in either juvenile or mature leaves, or present in half of the samples in either group in less than 0.1% of the total, were excluded from PCA and Wilcoxon Signed Rank analyses; some were detected only in either mature or juvenile leaves and not the other, therefore PCA and Wilcoxon Signed Rank analyses could not be conducted on these constituents.

3 Results and Discussion

Table 1 presents the codes, collection dates, and essential oil yields of mature and juvenile leaves of *E. sargentii* over a one-year period. Tables 2 and 3 present the major and important constituents (>3.0% in at least one month) of the mature and juvenile leaf essential oils of *E. sargentii* over a year. The full list of these analyses including with the LRI comparisons with relevant literature (Linstrom & Mallard, 2001, Babushok et al., 2011, Güleç et al., 2024, Demirci et al., 2025) were given in supplementary material (Tables S1 and S2).

Mature-leaf oil yields ranged from 0.9% to 5.5% (mean ± SD, 2.6 ± 1.6%); they were low (0.9%–2.3%) in most months but rose to 5.0%–5.5% from July to September and fell to 0.9% in November. Juvenile-leaf oil yields ranged from 1.9% to 7.0% (5.0 ± 1.7%) and fluctuated irregularly from month to month, with the lowest value in March (1.9%) and the highest in December (7.0%) (Table 1).

The major constituents of the mature leaves' essential oils were 1,8-cineole (7.5%–68.9%), α-pinene (0.1%–25.2%), β-eudesmol (2.8%–32.3%), and *trans*-pinocarveol (2.2%–16.1%). Other important compounds included pinocarvone (0.7%–4.2%), viridiflorol (1.1%–9.9%), and γ-eudesmol (0.3%–4.8%) (Table 2, See Supp. material, Table S1).

The main compounds in the essential oil of juvenile leaves were 1,8-cineole (7.4%–58.3%), α-pinene (7.3%–25.9%), *p*-cymene (0.4%–28.1%), and *trans*-pinocarveol (0.9%–13.2%). Other important compounds included β-eudesmol (0.3%–8.8%), viridiflorol (0.1%–6.9%), and pinocarvone (0.3%–4.9%) (Table 3).

The essential oil yields are mainly influenced by season, drying process, and plant part used, whereas both the yield and the essential oil composition are affected not only by environmental factors such as temperature, water availability, climate, and UV radiation, but also by edaphic factors such as soil minerals (Silva et al., 2011). Previous studies have reported the essential oil yields and compositions of the same species (Bignell et al., 1996; Sefidkon et al., 2006; Abravesh et al., 2007; Elaissi et al., 2010; Safaei-Ghomi & Batooli, 2010; Fathi & Sefidkon, 2012; Ameur et al., 2014; Ayed et al., 2023). In a previous Australian study, essential oil yield was reported as 1.55% (dry weight), obtained by vacuum distillation of dried, frozen mature leaves from a single tree (Bignell et al., 1996). In another study in Iran, the oil yield of fresh leaves collected during the flowering stage was reported as

Table 2. Major and important constituents (>3.0% in at least one month) of the essential oil of *E. sargentii* mature leaves over a year

LRI PS	Compound	Jan M	Feb M	Mar M	Apr M	May M	Jun M	Jul M	Aug M	Sep M	Oct M	Nov M	Dec M
983	α-pinene	4.8	1.2	2.9	2.5	2.0	9.3	21.8	25.2	23.1	3.4	0.1	4.0
1183	1,8-cineole	64.6	68.9	56.4	63.3	57.3	62.6	50.7	48.6	50.9	62.5	7.5	53.3
1545	Pinocarvone	3.3	4.1	4.1	3.8	4.2	2.9	0.8	0.7	0.8	3.8	1.6	3.5
1621	*trans*-pinocarveol	13.6	15.6	14.5	14.9	16.1	10.4	2.7	2.2	2.5	13.1	10.0	14.1
2047	Viridiflorol	1.9	1.1	2.7	1.9	2.6	2.7	1.4	1.4	1.6	1.9	9.9	2.6
2125	γ-eudesmol	0.6	0.3	1.3	0.7	1.1	0.5	1.6	1.5	0.8	0.7	4.8	1.1
2184	α-eudesmol	0.3	0.1	0.4	0.2	0.4	0.3	-	2.8	2.3	0.5	3.6	1.3
2195	β-eudesmol	4.1	3.6	9.2	6.3	7.9	2.8	5.4	5.2	5.5	6.6	32.3	9.2
Sum of listed compounds (%)		*93.2*	*94.9*	*91.5*	*93.6*	*91.6*	*91.5*	*84.4*	*87.6*	*87.5*	*92.5*	*69.8*	*89.1*
Total identified compounds (%)		99.6	99.7	99.3	99.5	99.4	99.4	95.6	99.7	99.4	99.6	93.5	98.7

Note: LRI PS: linear retention indices calculated against *n*-alkanes in the present study; % values calculated from FID data; -: not detected. Only compounds present at >3.0% in at least one month are listed; the complete list is given in the Supplementary Material (Table S1).

Table 3. Major constituents (>3.0% in at least one month) of the essential oil of *Eucalyptus sargentii* juvenile leaves (J) over a year

LRI PS	Compound	Jan J	Feb J	Mar J	Apr J	May J	Jun J	Jul J	Aug J	Sep J	Oct J	Nov J	Dec J
983	α-pinene	16.4	12.5	8.0	15.1	7.7	18.0	21.8	25.2	25.9	7.3	21.1	19.3
1183	1,8-cineole	58.0	57.7	58.3	54.4	51.2	56.5	50.8	50.0	48.2	7.4	50.3	52.2
1241	*p*-cymene	0.6	0.6	0.5	0.5	0.7	0.5	0.5	0.5	0.5	28.1	0.4	0.4
1545	Pinocarvone	0.9	2.7	3.5	1.8	3.4	1.6	0.9	0.6	0.3	4.9	0.5	0.5
1621	*trans*-pinocarveol	3.2	9.9	13.2	6.4	12.5	5.0	3.0	1.7	0.9	5.3	1.5	1.5
1964	Methyl eugenol	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	8.2	0.1	0.1
2047	Viridiflorol	1.9	1.7	2.0	1.9	0.1	3.1	1.7	1.5	1.6	6.9	1.7	1.8
2184	α-eudesmol	1.5	0.4	0.3	1.6	0.1	0.7	2.2	2.4	3.2	0.2	3.3	3.1
2195	β-eudesmol	5.5	4.7	5.4	6.2	8.8	1.8	5.2	4.8	5.1	0.3	6.7	6.3
Sum of listed compounds (%)		*88.1*	*90.3*	*91.3*	*88.0*	*84.6*	*87.4*	*86.2*	*86.8*	*85.8*	*68.6*	*85.6*	*85.2*
Total identified compounds (%)		99.1	99.7	99.5	99.6	98.6	99.7	99.4	99.5	99.7	97.4	99.6	99.7

Note: LRI PS: linear retention indices calculated against *n*-alkanes in the present study; % values calculated from FID data. Only compounds present at >3.0% in at least one month are listed; the complete list is given in the Supplementary Material (Table S2).

2.36% (Sefidkon et al., 2006). Essential oil yield from mature leaves collected in Tunisia in January was reported as 5.7% (Elaissi et al., 2010). A study investigated the effects of drying methods and distillation methods on the essential oil yields of the mature leaf samples collected in the middle of spring in Iran and reported the highest oil yield obtained from the shade-dried samples (2.30%–3.39%) and the hydrodistilled oils (1.35%–3.39%) (Fathi & Sefidkon, 2012). Our mature-leaf yields largely overlap with these findings, whereas juvenile-leaf yields, as far as the authors are aware no previous data exist for this species, reach higher values.

The literature review identified 1,8-cineole as dominant in *E. sargentii*, which agrees with our findings (Bignell et al., 1996; Sefidkon et al., 2006; Abravesh et al., 2007; Elaissi et al., 2010; Safaei-Ghomi & Batooli, 2010; Fathi & Sefidkon, 2012; Ameur et al., 2014; Ayed et al., 2023). While studies of Iranian origin reported 1,8-cineole proportions ranging from 55.48% to 75.50%, research from Tunisia and Australia reports levels between 48.56% and 55.93% (Bignell et al., 1996; Ayed et al., 2023). In eleven of the twelve months of the present study results, 1,8-cineole in the mature (48.6%–68.9%) and juvenile (48.2%–58.3%) leaf oils was within or close to this range.

Regarding *trans*-pinocarveol levels, the reported range of 1.90%–17.54% (Sefidkon et al., 2006; Ayed et al., 2023) aligns with our values (M: 2.2%–16.1%; J: 0.9%–13.2%). In both leaf types, *trans*-pinocarveol was lowest and α-pinene highest from July to September (*trans*-pinocarveol, M: 2.2%–2.7%; J: 0.9%–3.0%; α-pinene, M: 21.8%–25.2%; J: 21.8%–25.9%), and in juvenile leaves these shifts persisted into November–December. α-Pinene values reported for *E. sargentii* range from 4.9% to 28.3% (Abravesh et al., 2007; Safaei-Ghomi & Batooli, 2010; Fathi & Sefidkon, 2012); our monthly values (M: 0.1%–25.2%; J: 7.3%–25.9%) cover most of this range, suggesting that sampling time may account for part of the variation.

Two samples, NovM and OctJ, departed markedly from the general pattern and formed single-sample outliers. In the NovM sample, the concentrations of the major monoterpenoids (α-pinene 0.1%, 1,8-cineole 7.5%), dropped, while the concentrations of sesquiterpenoids underwent an increase (β-eudesmol 32.3%, viridiflorol 9.9%, γ-eudesmol 4.8%). A simultaneous decrease in the essential oil yield was observed, accompanied by the lowest oil yield across the entire sampling period (0.9%) (Tables 1–3, S1, S2). On the other hand, a simultaneous increase in *p*-cymene (28.1%), methyl eugenol (8.2%), γ-terpinene (2.5%), and carvacrol (1.2%) was recorded in OctJ sample together with a concentration collapse of 1,8-cineole (7.4%) and α-pinene (7.3%).

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The existing literature reveals information about essential oil production can be strongly affected by physiological factors, including responses to environmental (Ben Rabeh et al., 2025) or leaf type (Batish et al., 2006; Li et al., 1996). Heterophyllous leaves may respond differently to the same environmental stimuli due to different physiological and/or developmental profiles (Batish et al., 2006; Bulama Modu et al., 2025; Li et al., 1996).

Of the compounds that demonstrated an increase in the OctJ sample, *p*-cymene acts as a prominent precursor for biosynthesis (Poulose & Croteau, 1978). This might indicate that a metabolic shift occurs in the juvenile leaves in October, at the time of the onset of flowering. Eucalypts are known to have flowers developing from buds adjacent to the juvenile leaves, tying the metabolic profiles of the inflorescences and the juvenile leaves to an extensive degree, introducing a degree of metabolic interconnection and proximity effects that should have a greater effect on the juvenile leaves than on the mature leaves (Coppen, 2002). During this period, rapid inflorescence development and the chemoattraction integral to flower function require extensive modifications to the essential oil's chemical profile to maximize the chances of successful pollination while minimizing the risk of infestation by insects that might be detrimental to its well-being. Among the many chemical contributors, constituents such as 1,8-cineole produce potent chemoattractant effects on beneficial pollinators (Haselton et al., 2015; Schiestl & Roubik, 2003). In addition, methyl eugenol has been suggested to function in defence as a possible antifeedant, and pollinator attraction (Tan & Nishida, 2012). This period of inflorescence in *E. sargentii* begins in October and continues through January. Even though both samples (NovM and OctJ) were collected during the flowering period (October–January), the other samples from this period were not outliers, and entomological data was not recorded, so these explanations remain in hypotheses. Alternatively, the very high temperatures recorded in the Cypriot summer might manifest in the leaves as abiotic stress, contributing to the production of stress-response metabolites. Regardless of the potential explanations for these outliers, neither climatological nor entomological data was recorded at the site of sample collection, therefore, they remain as hypotheses, rather than concrete explanations, and shall remain outside the scope of the present study.

Although previous studies on this species' essential oil composition do not include seasonal or monthly samples for comparison, several reports marked transient drops in 1,8-cineole have also been reported in other eucalypts. For instance, in a previous study in Iran investigating the best harvest time for *E. largiflorens* and *E. microtheca* to improve essential oil quality, 1,8-cineole content showed severe and inconsistent fluctuations. It fell from 64.2% to 36.0% and returned to 65.8% the following season in *E. largiflorens*, while in *E. microtheca* oil, it dropped from 41.1% to 19.3% and then rose to 75.4% (Sefidkon et al., 2012). Likewise, in five *Eucalyptus* species studied in Morocco, 1,8-cineole concentrations varied randomly during the year, and the month of maximum content differed among species (Zrira & Benjilali, 1996). Moreover, a previous study on the essential oil composition of *E. torquata* naturalized in Cyprus reported that 1,8-cineole fluctuated during the year in mature samples (3.6%–27.8%) and in juvenile samples (4.9%–22.7%) (Bulama Modu et al., 2025). Such fluctuations are therefore not unique to *E. sargentii*.

The HCA of the 24 samples (12 mature and 12 juvenile) revealed the existence of a total of 4 divisions, A-D. Of these, A and B were true clusters, meeting the condition of at least 3 samples per cluster, whereas C and D, with only one sample each, were single sample outliers and were therefore excluded from further clustering analysis. Cluster A comprised 11 samples: eight juvenile and three mature. Cluster B comprised 11 samples: eight mature and three juvenile. The outliers were OctJ (C) and NovM (D), discussed above. The grouping thus reflected both leaf type and collection month: mature leaves collected from July to September clustered with the juvenile samples, whereas juvenile leaves collected in February, March, and May clustered with the mature samples (Figure 1). Cluster A was characterized by higher α-pinene and limonene and lower *trans*-pinocarveol and pinocarvone contents than cluster B (Table 4). The same July–September shift in α-pinene and *trans*-pinocarveol occurred in both independently analyzed leaf-type series.

After HCA divided the samples into clusters, the Mann-Whitney U test was used to determine whether individual constituents differed significantly between clusters. A single membered pseudoclusters C and D each contained one member; they were excluded from further comparative analyses.

10 constituents, chosen for their relevance and importance in *Eucalyptus*, between types and between clusters. The initial distribution analysis revealed that approximately a third of the chemical constituents did not conform to a Gaussian (Normal) distribution, and the homogeneity of variances was not ensured; therefore, non-parametric analysis was selected over parametric analyses to determine inter-cluster and mature-juvenile comparisons to compare the compositions between types (mature vs. juvenile) and between clusters (Cluster A vs. Cluster B). Of these 10 compounds, β-eudesmol, γ-eudesmol, *p*-cymene, and viridiflorol showed no significant variation between clusters or sample types, indicating that their concentrations depend on neither leaf type nor clustering.

Figure 2 shows the PCA results for the first three components. The PCA results also revealed a complex profile, with the 4 components with eigenvalues greater than or equal to 1 (PC1 = 6.128, PC2 = 5.475, PC3 = 5.254, PC4 = 1.291) accounting for 95.513% of the total variance. That 4 principal components are able to explain over 95% of the total variance indicates that the number of variation sources that contribute to the development of essential oil clusters is relatively low, favoring an explanation where a small number of chemicals contribute to the development of essential oil variances, over an explanation that employs a large number of chemicals.

Tables 4 and 5 tabulate the primary intercluster differences and correlations among the major and important essential oil constituents, respectively.

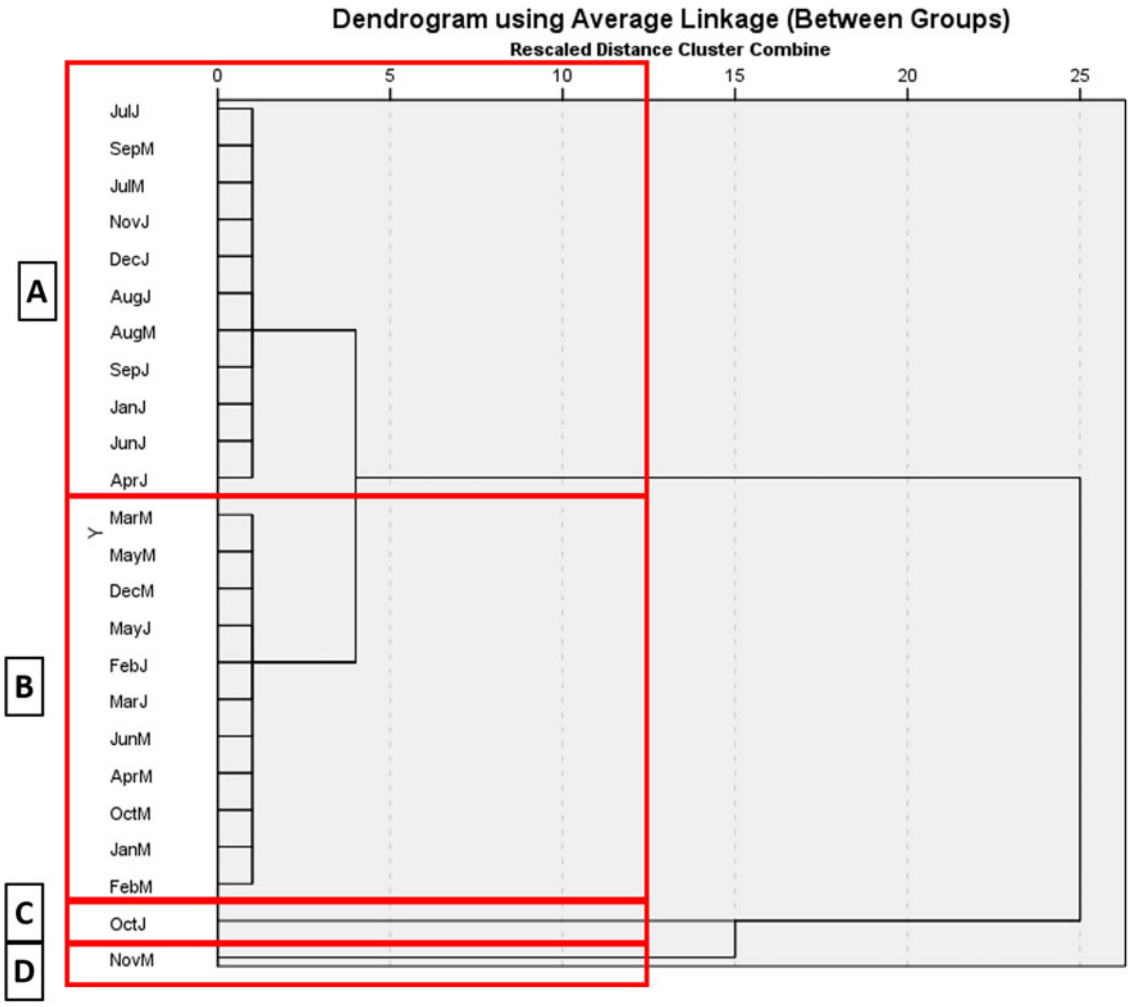

Figure 1. The HCA dendrogram of both juvenile and mature essential oils of *E. sargentii*

Table 4. Summary of the statistical significance of important essential oil constituents in both *E. sargentii* oils

Compound	Range M (%)	Range J (%)	Main PC contribution	M vs J (p)[a]	Cluster A vs B (p)[b]
1,8-cineole	7.5–68.9	7.4–58.3	PC2 (moderate)	0.178	<0.001
α-Pinene	0.1–25.2	7.3–25.9	PC1 (Strong)	0.020	<0.001
Limonene	tr-2.4	0.3–2.8	PC1 (Strong)	0.014	<0.001
trans-Pinocarveol	2.2–16.1	0.9–13.2	PC1 (Strongest)	0.012	<0.001
β-Eudesmol	2.8–32.3	0.3–8.8	PC3 (Strong)	0.291	0.401
γ-Eudesmol	0.3–4.8	0.1–1.2	PC3 (Strongest)	0.160	0.133
α-Terpineol	0.1–1.9	0.4–2.7	PC1 (strong)	0.014	<0.001
Pinocarvone	0.7–4.2	0.3–4.9	PC1 (strong)	0.089	<0.001
p-cymene	0.1–0.6	0.4–28.1	PC2 (Strongest)	0.242	0.076
Viridiflorol	1.1–9.9	0.1–6.9	PC2 and PC3 (Moderate)	0.551	0.116

Note: M = mature leaf oil, J = juvenile leaf oil, PC = principal component. [a]Wilcoxon Signed-Rank Test, [b]Mann-Whitney U Test.

One major compound, 1,8-cineole (M: 7.5%-68.9%, J: 7.4%–58.3%), contributed moderately to PC2, and mature and juvenile leaf oils did not differ significantly (p = 0.178). However, this compound differed significantly between clusters A and B (p < 0.001). 1,8-Cineole was negatively correlated with α-pinene (p = 0.014), γ-eudesmol (p = 0.031), limonene (p = 0.001), and α-terpineol (p = 0.001). In contrast, this compound positively correlated with pinocarvone (p = 0.011) and *trans*-pinocarveol (p < 0.001) (Tables 4, 5).

The other major compound, α-pinene (M: 0.1%–25.2%, J: 7.3%–25.9%), made a strong contribution to PC1, and there was a significant difference between the oils of mature and juvenile leaves (p = 0.020) and a significant difference between clusters A and B for this compound (p < 0.001).

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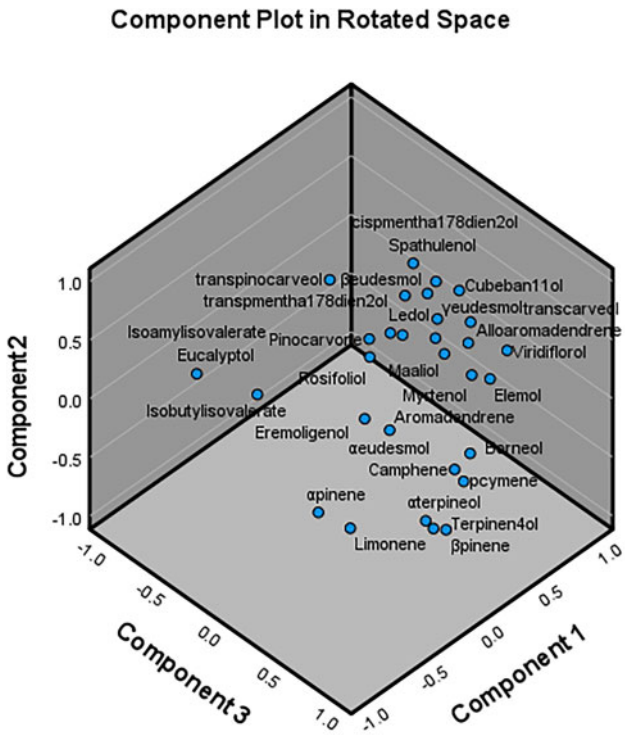

Figure 2. The PCA biplot of the first 3 principal components

Table 5. Pairwise correlations between important constituents of both *E. sargentii* oils

Compound 1	Compound 2	Correlation direction	*p*-value
1,8-Cineole	α-Pinene	Negative	0.014
	γ-Eudesmol	Negative	0.031
	Limonene	Negative	0.001
	α-Terpineol	Negative	0.001
	Pinocarvone	Positive	0.011
	trans-Pinocarveol	Positive	<0.001
α-Pinene	Pinocarvone	Negative	<0.001
	Limonene	Positive	<0.001
	trans-Pinocarveol	Negative	<0.001
	α-Terpineol	Positive	<0.001
	Viridiflorol	Negative	0.012
Limonene	Pinocarvone	Negative	<0.001
	trans-Pinocarveol	Negative	<0.001
	α-Terpineol	Positive	<0.001
trans-Pinocarveol	α-Terpineol	Negative	<0.001
	Pinocarvone	Positive	<0.001
β-Eudesmol	γ-Eudesmol	Positive	0.039
Pinocarvone	α-Terpineol	Negative	<0.001
	Viridiflorol	Positive	0.023
	p-cymene	Positive	0.019
γ-Eudesmol	*p*-cymene	Negative	0.041

There were negative correlations between α-pinene and the following: pinocarvone ($p < 0.001$) and *trans*-pinocarveol ($p < 0.001$) (Tables 4, 5).

Based on either the percentage amounts in the oils or the PCA results, other important compounds included *trans*-pinocarveol, γ-eudesmol, *p*-cymene, and viridiflorol.

Among the compounds with relatively high percentages in the oils, *trans*-pinocarveol (M: 2.2%–16.1%, J: 0.9%–13.2%) contributed most to PC1 variance. Mature and juvenile leaf oils differed ($p = 0.012$), and clusters A and B differed significantly for this compound ($p < 0.001$). α-terpineol was negatively correlated ($p < 0.001$), and pinocarvone was positively correlated ($p < 0.001$) (Tables 4, 5).

Another important compound was γ-eudesmol (M: 0.3%–4.8%, J: 0.1%–1.2%), which contributed most to PC3, as indicated by its strong coupling coefficient to the PC3 component of variance. Mature and juvenile leaf oils did not differ ($p = 0.160$), and clusters A and B did not differ for this compound ($p = 0.133$). As mentioned above, this compound showed a negative correlation with 1,8-cineole ($p = 0.031$) and a positive correlation with β-eudesmol ($p = 0.039$) (Tables 4, 5).

Another important compound was *p*-cymene (M: 0.1%–0.6%, J: 0.4%–28.1%), which contributed most to PC2 due to its high concentration, especially in juvenile leaf oil. Mature and juvenile leaf oils did not differ significantly (p = 0.242), and also clusters A and B did not differ significantly ($p = 0.076$) (Tables 4, 5).

Finally, viridiflorol contributed moderately to PC2 and PC3 and had relatively high concentrations in both essential oil types (M: 1.1%–9.9%, J: 0.1%–6.9%). Mature and juvenile leaf oils did not differ ($p = 0.551$), and clusters A and B did not differ for this compound ($p = 0.116$). As mentioned above, this compound showed a negative correlation with α-pinene ($p = 0.012$) (Tables 4, 5).

Overall, 1,8-cineole was the dominant constituent in both leaf types in eleven of the twelve months (M: 48.6%–68.9%; J: 48.2%–58.3%), and mature and juvenile oils did not differ significantly in 1,8-cineole content (Table 4). Both leaf types showed a similar shift toward higher α-pinene and lower *trans*-pinocarveol contents from July to September, and the cluster analysis grouped samples according to both leaf type and collection month.

The present study represents the first reporting of *E. sargentii* in Cyprus, a species with a widespread cultivation profile across the island. The present work represents the first of its kind in the essential oil of *E. sargentii*, where essential oils samples were obtained from heterophyllous leaves over an annual period, excluding the differences that might arise due to genotypic and/or phenotypic divergences between different individual trees. It will assist in the generation of baseline data on the chemical composition and temporal variations in the essential oil content of *E. sargentii* naturalized in Cyprus.

4 Conclusion

The present study, by identifying the profile of the essential oil throughout the cycle of one entire year, may provide valuable insight into the essential oil producing behavior of *E. sargentii*. The dataset will be helpful to elucidate certain

characteristics important for the utilization of this plant as a medicinal product, in commercial or industrial capacity. Two different windows of opportunity to harvest the leaves of *E. sargentii* for the purposes of obtaining its essential oils were identified: The maximal time of harvest was identified as summer (Jul & Aug) for mature leaves, whereas winter (Nov-Jan) was identified as the ideal time to maximize the yield of essential oils from juvenile leaves. In the mature leaves, the peak essential oil volume coincides with the maximum α-pinene content in the oil (~21 to 25%) while the 1,8-cineole constitutes ~50% of the oil content, thus is important if these constituents are the target of economic manufacture. In the juvenile leaf oils, however, the peak oil period, α-pinene content remains relatively meager (~16 to 20%), however, the 1,8-cineole content remains over 50% over the same time period. Therefore, both essential oils are composed of a majority of two major compounds constituting approximately three thirds of the total oil content, indicating that both peak periods have the potential to serve as commercially relevant sources of α-pinene and 1,8-cineole. The identification of this dual-maximum behavior is therefore of essential import in economic respects.

The most important limiting factor in the veracity of the present paper is that the entire sampling volume was obtained from a single tree. Therefore, the extrapolation of the present dataset to the entire species must be made carefully, in full understanding of the fact that a single tree may not necessarily represent its entire species, might have been affected by biotic or abiotic factors, or might have been affected by some other unknown factors. In the future, it would be well-advised to repeat the present study using multiple originator trees, and over longer periods of time, perhaps up to or exceeding 3 complete years, to provide a much stronger statistical basis for drawing conclusions. *E. sargentii* is in general underrepresented in the literature, with only a limited number of studies available to draw comparisons. While the present study itself is of pioneering nature, it will also provide a comparative baseline upon which future studies will not only compare, but also improve upon, providing essential literature data for this underrepresented species.

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Author Contributions

Conceptualization, DÖY, FBA, AH, DYH, KHCB; Methodology, MA, FBA, KHCB, AH, DYH, MBM; Software, FBA, KHCB, AH, DYH; Writing—Original Draft Preparation, MA, FBA, AH, DYH; Writing—Review & Editing, KHCB, DÖY, DYH, FBA, AH; Supervision, AH, DÖY, DYH, KHCB; Project Administration, DYH, DÖY, AH, KHCB, FBA.

Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Artificial Intelligence (AI) Statement

The authors affirm that no Artificial Intelligence (AI) tools were employed in the preparation of this manuscript, which includes language editing, graphical abstract preparation, figure generation, or image editing.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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