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ORIGINAL PAPER

Comparative chemical profiling of *Laurus nobilis* L. leaf essential oils based on ontogenetic and locational variabilities: investigation of antioxidant and antimicrobial activities

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Abstract

Laurus nobilis L. (bay laurel) is among the most economically and pharmacologically significant aromatic plants cultivated in Türkiye; however, the extent to which its essential oil (EO) yield, chemical composition, and biological activities are shaped by both ontogenetic and locational factors within a single province remains poorly characterised. The present study addresses this gap by analysing 36 EO samples from six districts of Sinop province (Center, Ayancık, Erfelek, Türkeli, Dikmen, and Gerze) collected over six consecutive months (October-March 2023–2024). EOs were obtained by hydrodistillation and characterised by GC-MS/FID; antioxidant activity was assessed via DPPH radical-scavenging, and antimicrobial activity against nine microbial strains was evaluated by disk diffusion and microdilution. EO yields ranged from 0.5% to 1.5% (v/w). GC-MS analysis identified 10–22 components per sample (85.7–98.9% of total composition), with eucalyptol (43.9–65.5%), α-terpinyl acetate (7.7–15.4%), and sabinene (2.4–12.6%) as dominant constituents. Locational variation was statistically significant for six constituents including α-pinene ($p=0.002$), sabinene ($p<0.001$), and linalool ($p=0.026$). Temporal variation was limited to eucalyptol ($p<0.001$) and α-terpinyl acetate ($p=0.017$). DPPH assays yielded IC_{50} values of 119.8–426.8 µL/mL, with the strongest activity recorded for the Türkeli-November sample (IC_{50} = 119.8 µL/mL); neither locational nor overall temporal differences reached significance. Antimicrobial assays demonstrated broad-spectrum activity, with the lowest MIC values (320 µg/L) recorded against *Bacillus* spp. and *Klebsiella pneumoniae*; significant locational variation was detected only for *Enterococcus faecalis* ($p<0.01$). Overall, geographical location proved a more consistent source of compositional variability than the ontogenetic factor, with practical implications for harvest optimisation in pharmaceutical and food applications.

Keywords *Laurus nobilis* · Essential oil · Seasonal variation · Locational variation · Antioxidant activity · Antimicrobial activity · Sinop

Introduction

Laurel (*Laurus nobilis* L.) is a very important medicinal and aromatic plant, which belongs to the Lauraceae family [1]. Leaves of this plant produce a colorless to pale yellow, known for many therapeutic indications [2]. Turkey is the major producer of *L. nobilis* and exports it to 64 countries [3]. Turkey is one of the main producers and suppliers of bay leaves. The average amount of exported dried bay leaves from Turkey was 4 million kg per year during the 1999–2001 [4].

The EO of *L. nobilis* is a complex mixture of volatile secondary metabolites, and our study has focused primarily on the leaves, the most commercially important part of the

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plant. However, different botanical structures of *L. nobilis* including the fruits (berries), flowers, and bark also contain EOs with distinct chemical profiles. While leaf EOs are characterized by high levels of Eucalyptol, fruit oils (often referred to as 'laurel berry oil') contain significant amounts of fixed oils and different terpene compositions, leading to diverse biological activities and industrial applications. The different body parts of *L. nobilis* and its EO have been recognized to possess many interesting properties that have potential applications in many areas, including agriculture, medical, food, pharmaceutical industries, etc. The leaves are commonly used as a spicy, aromatic flavoring agent for soups, fish, meats, stews, puddings, vinegar, and beverages. The pharmaceutical properties of *L. nobilis* leaves and fruits have been known since Dioscorides time [5]. Due to its antimicrobial and insecticidal activities, bay is used in the food industry as a food preservative [6, 7]. The cosmetic industry also uses the *L. nobilis* EO in creams, perfumes, and soaps [8, 9]. EO exhibits beneficial functions such as antibacterial, antifungal, and antioxidant activities [10]. The seeds are reported to have antiulcer and antidiabetic effects [11]. Currently, the request for natural products for novel applications is increasing day by day; for example, bay laurel berries are used as a natural anthocyanin instead of synthetic dyes in the food, pharmaceutical, and cosmetic industries [12, 13].

Global demand for EOs is increasing at a significant rate (9.5% annually). This increasing demand is driven by the widespread use of EOs for their beneficial properties in various industrial applications such as medical treatment, food and beverages, cleaning and home, spa and relaxation, their use in aromatic products for their therapeutic and medicinal qualities, and also by the fact that health benefits and minimal side effects are key factors driving market growth [9, 14–22]. But, enormous quantities of plants are necessary for their production. For example, to produce around 500 g of EO of rose petals, lilac or lemons: 5000 kg, 130 kg, and 750 kg are required respectively [14]. These plants are very valuable and limited in quantity for living organisms.

Generally, the yield and composition of the oil vary depending on the origin, the collection period, and the growth stage of the plant [23]. Therefore, their use at the right time and collection from the most suitable locations based on yield are crucial for increasing productivity. While the phytochemical properties of *L. nobilis* have been widely studied across different regions, most research focuses either on isolated seasonal variations, broad geographical regions, or comparisons of different methods [8, 24–27]. Significant regional chemotypic variability was observed in the chemical composition of the EO of this species [13, 24]. Previous studies showed seasonal variation in the amount and/or composition of bay laurel EO [25, 26, 28–31]. According to some authors, the flowering stage is the best time for harvesting bay laurel leaves because this is when the plant contains the highest percentage of EO [32]. However, the results obtained from studies of seasonal variation in bay laurel EO were different. Some authors who studied the oil content during all four seasons found a higher amount of oil in the vegetative period [33] and others during the dormancy period [34]. Previous research also showed little or no variability between bay laurel oil from different geographic areas [25, 35–36].

This study addresses a significant research gap by simultaneously examining ontogenetic and locational differences across five districts and the center of Sinop province in Türkiye, unlike previous studies in the literature. By systematically analyzing 36 unique datasets (6 locations × 6 months), our research offers a novel perspective on how microclimatic and geographical factors within a single province influence the chemical consistency and biological activity (antioxidant and antimicrobial) of laurel EOs. This comprehensive approach provides vital data for optimizing harvest times and site selection for high-quality industrial production. To our knowledge, other studies in the literature have compared time or location. We have not come across any study that compares both, has such a rich dataset, and compares EO content and biological activity together. Furthermore, while seasonal comparisons are generally made in other studies, studies that conduct micro-level comparisons on a monthly basis are limited.

The aim of this study, which was conducted by considering existing literature, is to determine the optimal harvest time and location for *L. nobilis*, which has high medical and economic value. For this purpose, the yield, composition and biological activities of the EOs of samples taken from different locations and different periods were compared. Moreover, this study aims to emphasize the importance of variability in medicinal and aromatic plants, particularly how environmental and ontogenetic factors influence their chemical profiles.

Materials and methods

Plant material

Leaf samples of *L. nobilis* were collected by Dr. O. Elkiran from six distinct localities within Sinop province, Türkiye, spanning the Center district and five administrative districts (Ayancık, Erfelek, Türkeli, Dikmen, and Gerze), during the 2023–2024 vegetation period across six consecutive months (October, November, December, January, February, and March). The precise geographic coordinates of the collection sites were as follows: Sinop Center (42°1′10.38″N,



Fig. 1 Geographic locations of the six sampling sites (*L. nobilis* collection points) across the Sinop province, Türkiye (Center, Ayancık, Erfelek, Türkeli, Dikmen, and Gerze)

35°7′43.37″E), Ayancık (41°56′29.73″N, 34°34′2.76″E), Erfelek (41°52′47.47″N, 34°54′50.56″E), Türkeli (41°55′51.79″N, 34°19′15.05″E), Dikmen (41°38′52.05″N, 35°16′28.51″E), and Gerze (41°49′49.04″N, 35°8′50.68″E) (Fig. 1). Samples were coded based on their collection site and month (e.g., A-O for Ayancık in October) to allow for systematic comparison. A total of 36 samples were systematically harvested to evaluate the combined effects of ontogenetic (seasonal) and locational factors on the EO profiles. The plant material was identified by taxonomist Dr. O. Elkiran with reference to volume 7 of Flora of Turkey [37]. The average temperatures of the locations where plant samples were collected are given in Table 1.

Isolation of essential oil

The EOs were obtained from the dried leaves of *L. nobilis* (100 g for each sample) by hydrodistillation using a Clevenger-type apparatus for 3 h. The distillation was performed at a constant temperature of 100 °C. The extracted EOs were stored in amber-colored glass bottles at 4 °C until analysis. The volumes of the obtained EOs ranged from 0.5 mL to 1.5 mL, representing an extraction yield of 0.5%–1.5% (v/w).

Chromatographic analysis

The chemical composition of the EOs was analyzed using a Shimadzu GCMS-QP2010 system equipped with an RXI-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium (99.999% purity) was employed as the carrier gas at a constant flow rate of 1.0 mL/min. For the analysis, 1 µL of each EO sample (diluted 1:100 v/v in n-hexane) was injected in split mode (1:50 ratio) with the injector temperature maintained at 250 °C. The oven temperature program was initiated at 40 °C (held for 4 min), then increased to 240 °C at a rate of 3.8 °C/min, and finally

Table 1 Average monthly temperatures (°C) at the collection localities [38]

District / Month	October	November	December	January	February	March
Sinop (Center)	16.2	12.4	9.5	7	6.5	8.2
Ayancık	15.8	11.9	9	6.8	6.2	7.9
Gerze	16	12.2	9.3	7.1	6.6	8.1
Türkeli	15.7	11.7	8.8	6.5	6.1	7.7
Erfelek	14.5	10.2	7.1	4.8	4.5	6.8
Dikmen	14.8	10.5	7.4	5.1	4.9	7.1

held at this temperature for 53 min. The identification of the volatile components was performed by comparing their mass spectra with those stored in the Wiley and NIST electronic libraries. The relative percentages of the identified components were calculated based on GC-FID (Flame Ionization Detector) peak areas without the use of correction factors [39, 40].

Biological activity

Antioxidant activity

The antioxidant activities of EOs were determined by the method previously tested by Elkiran et al. [41]. The method is briefly as follows; the EO's antioxidant activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was assessed. The serial dilution procedure was used to extract the EO at various concentrations (500–31.25 µl/mL). Four milliliters of a 0.1 mM DPPH solution were combined with one milliliter of an ethanol solution of the EO at each concentration. These samples were left at room temperature in the dark for half an hour. At 517 nm, the absorbance was measured. The following formula was used to determine the DPPH radical's scavenging activity:

$$\% \text{ inhibition} = [(AB - AS) / AB] \times 100.$$

In this equation, AS represents the absorbance of the test substance and AB represents the absorbance of the control reaction. As a positive control, ascorbic acid (500–31.25 µg/mL) was used. As a blank, the chemical was used without a sample or standard. Median inhibitory concentration (IC_{50}), which is the extract concentration (µl/mL) needed to block 50% of the free radical scavenging activity, was used to express the scavenging activity. Microsoft Office Excel 2019 was used to create the graphic and Standard deviation (±).

Antimicrobial activity

Disk diffusion method

The antimicrobial activities of EOs obtained from *L. nobilis* samples were evaluated against four Gram-positive bacteria, three Gram-negative bacteria, and two fungal strains. The tested microorganisms included *Bacillus cereus* ATCC 10,987, *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25,923, and *Enterococcus faecalis* ATCC 29,212 (Gram-positive); *Escherichia coli* ATCC 25,922, *Klebsiella pneumoniae* ATCC 70,060, and *Pseudomonas aeruginosa* ATCC 27,853 (Gram-negative); as well as *Candida albicans* ATCC 10,231 and *Aspergillus niger* ATCC 16,404 (fungi). All reference strains were obtained from the American Type Culture Collection (ATCC).

The antimicrobial activity of EOs was determined using the disk diffusion method [41–43]. Overnight bacterial cultures were grown in Mueller–Hinton Broth (MHB), and fungal cultures were grown in Sabouraud Dextrose Broth (SDB). The turbidity of each culture was adjusted to match the 0.5 McFarland standard (~ 1.5×10^8 CFU/mL). Freshly prepared sterile Mueller–Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) plates were inoculated by spreading approximately 10^6 CFU of each microorganism across the surface.

Sterile paper disks (6 mm in diameter) were impregnated with 25 µL of the EO samples and left to dry completely under a laminar flow cabinet. The dried disks were then placed onto the inoculated agar surfaces. Bacterial plates were incubated at 37 °C for 24 h, whereas fungal plates were incubated at 28 °C for 48 h. Sterile distilled water was used as the negative control, while Ampicillin (AM10) and Gentamicin (CN10) were used as positive controls for bacteria, and Cycloheximide (Cyc) for fungi. After incubation, inhibition zone diameters were measured in millimeters.

Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MICs) of the EOs were determined using the micro dilution technique in 96-well micro plates, followed by agar incubation to confirm microbial growth inhibition. Stock solutions of EOs were serially diluted in Mueller–Hinton Broth (for bacterial assays) and Sabouraud Dextrose Broth (for fungal assays) to obtain a range of concentrations.

Each well received a defined concentration of the EO solution and a standardized microbial inoculum (approximately 10^6 CFU/mL). The micro plates were incubated at 37 °C for bacteria and 28 °C for fungi for the appropriate incubation periods.

After incubation, microbial growth was visually assessed by observing turbidity, and subcultures were performed on agar plates when necessary to confirm inhibition. The lowest concentration of EO that completely inhibited visible microbial growth was recorded as the MIC value.

Statistical Analysis

To assess whether ontogenetic and locational variations in EO yield, chemical composition, and biological activities of *L. nobilis* were statistically significant, one-way analysis of variance (ANOVA) or the Kruskal–Wallis H test was applied, depending on whether the underlying assumptions of parametric testing were met. Prior to all inferential analyses, the normality of the data distribution was evaluated using the Shapiro-Wilk test, which is considered

appropriate for small-to-moderate sample sizes. When the assumption of normality was satisfied, one-way ANOVA was performed, followed by Tukey's Honest Significant Difference (HSD) post hoc test to identify pairwise differences among groups. For variables that violated the normality assumption, the non-parametric Kruskal-Wallis H test was employed, with subsequent pairwise comparisons conducted using the Holm-Bonferroni correction to control the familywise error rate.

To elucidate the relationships between EO yield, chemical constituents, and biological activities across sampling periods and locations, Spearman's rank correlation coefficient (two-tailed) was calculated, given the non-normal distribution of several variables.

Principal component analysis (PCA) was performed to reduce data dimensionality and to clarify the interrelationships among mineral concentrations and selected ecological parameters. Components with eigenvalues greater than 1 were retained for further interpretation. All statistical analyses were carried out using OriginPro 2026 (64-bit).

Results and Discussion

In our study, a total of 36 samples, representing 6 distinct locations and collected over 6 months, were examined for EO, and their GC-MS analyses were carried out. The EO yields, extracted from 100 g of air-dried leaves per sample, ranged from 0.5% to 1.5% v/w, depending on location and harvest period. According to the results of GC-MS analysis, 10–22 chemical components greater than 0.5% were detected. Considering MS spectra and RI, 98.9–85.7% of the total oils were identified. Eucalyptol, α-Terpinyl acetate, and cis-Sabinene hydrate were detected in common in all the EOs. The main major components were Eucalyptol (43.9–65.5%), α-Terpinyl acetate (7.7–15.4%), Sabinene (2.4–12.6%) (Table 2).

Our study found that eucalyptol, the main component was higher in October and November than in other months. The highest eucalyptol value (65.5%) was found in November in the Erfelek district. Interestingly, the lowest level (43.9%) was found in a sample from the same district in December. In the literature review on bay laurel EO, it was found that the main component was eucalyptol [13, 32, 45–48]. When the average temperatures between locations are examined, Erfelek district has the lowest average temperature over the past 6 months (Table 1). The fact that eucalyptol levels are highest in the sample taken from Erfelek district in November indicates that temperature alone is not sufficient to determine the variation in EO components. However, the fact that eucalyptol levels are generally higher during periods of high average temperatures proves that temperature is a significant factor [49] (Fig. 1).

A study by Kremer et al. [25] also found that Eucalyptol levels were higher during warmer periods, as in our study. Similarly, the studies conducted in Türkiye found that laurel samples from the Mediterranean region contained higher levels of Eucalyptol than those from the Black Sea region [33, 50]. We see that this difference may be caused by temperature differences in the regions and that high average temperatures increase Eucalyptol yield in laurel.

The other main component. alpha-terpinyl acetate, was found at the highest concentration (15.4%) in a sample collected from the Erfelek district in January, and at the lowest concentration (7.7%) in a sample collected from the Dikmen district in November. Our third main component Sabinene, was found at the highest concentration (12.6%) in the Türkeli district in January and at the lowest concentration (2.7%) in the Gerze district in the same month.

Sabinene was the other major component. It was lowest in the Gerze district in January and highest in the Türkeli district. This result suggests that air temperature alone is not sufficient to determine EO content, but rather soil structure and other geographic conditions. Similar studies in the literature also list Sabinene as the major component [51].

The seasonal variations in the nine most abundant constituents of the identified oil obtained from the Sinop region are presented in Figs. 2 and 3. Other significant compounds identified in this study were α-Pinene (5.3–1.9%), Linalool (5.2–0.8%), Terpinen-4-ol (4.7–1.4%), β-Pinene (4.2–2.0%), α-Terpineol (4.0–0.5%), and cis-Sabinene hydrate (2.9–0.7%).

The comparison of EO compositions among stations revealed significant regional differences for several constituents (Fig. 2). According to one-way ANOVA results, α-pinene ($p=0.002$), sabinene ($p<0.001$), β-pinene ($p<0.001$), and α-terpinyl acetate ($p=0.036$) varied significantly among stations, whereas eucalyptol, cis-sabinene hydrate, and α-terpineol did not show statistically significant differences ($p>0.05$). Post-hoc comparisons indicated that sabinene content in Gerze (5.82 ± 1.77) was significantly lower than in Ayancık (8.48 ± 1.49), Erfelek (8.65 ± 0.95), Türkeli (10.46 ± 1.55), and the Center district (8.67 ± 0.57) ($p<0.05$).

Similarly, β-pinene content differed significantly between the Center (2.62 ± 0.40) and Erfelek (3.13 ± 0.59) stations, between Gerze (2.24 ± 0.19) and both Ayancık (3.47 ± 0.45) and Erfelek (3.13 ± 0.59), as well as between Dikmen (2.63 ± 0.38) and Ayancık (3.47 ± 0.45) ($p<0.05$).

For compounds that did not meet parametric assumptions, the Kruskal–Wallis test revealed significant regional differences for linalool ($p=0.026$) and terpinen-4-ol ($p=0.006$). Pairwise comparisons showed that linalool

Table 2 Essential oil composition of aerial parts of L. nobilis

No	RRI	RRI*	Compounds	Relative area (%) (peak area relative to the total peak area)															
				A-o	A-*n*	A-d	A-j	A-f	A-m	E-o	E-*n*	E-d	E-j	E-f	E-m	T-o	T-*n*	T-d	T-j	T-f	T-m
1	924	924–930	α-Thujene	-	-	-	0.5	-		-	-	-	-	0.5	-	*	*	*	*	*	*
2	932	932–939	α-Pinene	4	3.7	4.7	4	4.2	4.1	4	2.2	5.3	4.4	4.3	3.5	3.1	2.8	3.4	4.1	4.3	2.4
3	954	946–954	Camphene	-	-	0.6	-	0.5	0.5	-	-	1.2	-	0.5	0.6	-	-	0.9	0.5	0.5	-
4	**969**	**969–975**	**Sabinene**	**8.3**	**6.4**	**7.9**	**11**	**8.8**	**8.5**	**9.3**	**8.1**	**-**	**9.6**	**-**	**7.6**	**9.5**	**9.5**	**11.6**	**12.6**	**-**	**9.1**
5	974	974–979	β-Pinene	3.6	3.5	4.2	2.9	3.5	3.1	3.7	2.2	3.8	3.1	3.2	2.8	3.1	2.9	3	3.2	3.4	2.4
6	988	988–990	Myrcene	-	-	-	0.8	0.5		0.8	0.5	0.6	0.9	0.6	0.8		0.5	1.2	1.1	0.5	-
7	1002	1002	α-Phellandrene	*	*	*	*	*	*	-	-	-	-	0.8	-	*	*	*	*	*	*
8	1014	1014–1017	α-Terpinene	*	*	*	*	*	*				0.5			*	*	*	*	*	*
9	1020	1020–1024	p-Cymene	1.1	2.2	1	-	-		0.9	-	-			1.3	0.7	0.5	-	-	-	-
10	1025	1025–1029	β-Phellandrene	1.6	-	1.6	-	-		2	-	**9.3**		**7.2**		-	1.4	-	-	**12.5**	
11	**1026**	**1026–1031**	**Eucalyptol**	**58**	**53.9**	**51.8**	**49.6**	**49.2**	**49**	**50.8**	**65.5**	**43.9**	**45.9**	**46.4**	**51.9**	**60.2**	**62.5**	**52.5**	**48.3**	**50.1**	**59.6**
12	1054	1054–1059	γ-Terpinene	-	0.5	0.5	-	-	0.7	-	-	0.5	0.5	0.5		-	-	-	0.5	-	-
13	1070	1065–1070	cis- Sabinene hydrate	1.1	1.1	1.5	2.1	1.9	1.9	0.9	1.4	1.4	1.2	1.2	1.5	1.5	1.4	1.3	1.7	1.1	2.9
14	1088	1083–1089	Linalool	1.9	0.8	1	1.9	2	2	2.7	2.1	2.2	2.8			2.3	2.2	3.9	3.5	2.3	3.4
15	1098	1098	trans-Sabinene hydrate	*	*	*	*	*	*					0.8		*	*	*	*	*	*
16	1122	1122	Isophorone	-	-	-	-	0.5		*	*	*	*	*	*	-	-	-	-	-	0.5
17	1132	1128–1132	Allo-Ocimene	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	0.6	-
18	1135	1135–1139	trans-Pinocarveol	*	*	*	*	*	*					5.4	4.2	*	*	*	*	*	*
19	1164	1160–1164	Pinocarvone	*	*	*	*	*	*	-	0.8	-		0.5	0.6	-	-	0.5	0.5	-	0.6
20	1177	1174–1177	Terpinen-4-ol	2.1	3.5	2	2.2	3	3.3	1.9	1.8	3.5	3.5	3.6	3.4	1.7	1.4	1.7	1.7	1.7	2.2
21	1188	1186–1188	α-Terpineol	1.3	2.3	1.4	2.8	3.6	2.8	1.3	3.8	2.6	2	2.3	3.2	2.5	2.6	2.8	2.9	2.7	3.2
22	1239	1235–1239	Isobornyl formate	-	-	-	0.6	-		*	*	*	*	*	*	*	*	*	*	*	*
23	1285	1284–1285	Bornyl acetate	-	-	-	-	0.7	0.5	0.6	-	1.4		0.6		-	-	0.5	-	0.7	0.6
24	1316	1304–1324	Piperitenone	0.7	0.7	-	-	-	0.7	-	-	1	0.9			0.6	-	-	-	-	-
25	**1349**	**1346–1349**	**α-Terpinyl acetate**	**12**	**12.1**	**12.9**	**13.7**	**12.4**	**13.4**	**15**	**8.9**	**14**	**15.4**	**13.4**	**13.4**	**11.4**	**9.8**	**14.2**	**14.6**	**14.7**	**10.8**
26	1359	1356–1359	Eugenol	-	0.7	0.7	-	1.3	1.5	0.5		1.6		1.7		0.6	-	1.2	1.5	1.5	0.7
27	1369	1366–1369	Dihydroeugenol	-	-	-	1.4	-					0.8		0.9	*	*	*	*	*	*
28	1403	1403	Methyl eugenol	1.1	1.4	1.1	2.3	2.4	2.3	1.6	0.7	1.9	2	3.5	2	*	*	*	*	*	*
29	1577	1577–1578	Spathulenol	-	0.8	-	-	-	1.3				0.6	0.5		*	*	*	*	*	*
30	1582	1582–1583	Caryophyllene oxide	0.6	1	-	0.9	1.1	1.3	-	-	0.7	-	0.6		*	*	*	*	*	*
31	1650	1649–1650	β-Eudesmol	*	*	*	*	*	*	-	-	1.3	0.9	0.8		-	-	-	0.5	-	-
Total identified compounds (%)				**97**	**94.6**	**92.9**	**96.7**	**95.6**	**96.9**	**96**	**98**	**96.2**	**95**	**98.9**	**97.7**	**97.2**	**97.5**	**98.7**	**97.2**	**96.6**	**98.4**
No	RRI	RRI*	Compounds	Relative area (%)(peak area relative to the total peak area)																	
				C-o	C-*n*	C-d	C-j	C-f	C-m	G-o	G-*n*	G-d	G-j	G-f	G-m	D-o	D-*n*	D-d	D-j	D-f	D-m
1	932	932–939	α-Pinene	2.6	-	3.4	2.9	2.9	2.5	1.9	2.3	2.7	3.5	2.3	2.3	2.7	2	3.6	3.5	3.6	3.9
2	954	946–954	Camphene	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	-
3	**969**	**969–975**	**Sabinene**	**8.7**	**8.6**	**9.1**	**9.2**	**7.6**	**8.8**	**5.9**	**6.5**	**5.8**	2.4	**7**	**7.3**	**7.7**	**6.8**	**9.3**	**9.4**	**8.1**	**-**
4	974	974–979	β-Pinene	2.7	3.1	2.7	2.6	-	2	2.1	2.5	2.3	-	2.3	2	2.7	2.2	2.3	2.5	3.2	2.9
5	988	988–990	Myrcene	0.6	-	0.6	0.6	-	1	-	-	-	0.7	-	0.7	-	0.7	0.6	0.6	-	0.5

Table 2 (continued)

No	RRI	RRI*	Compounds	Relative area (%)(peak area relative to the total peak area)																
				C-o	C-*n*	C-d	C-j	C-f	C-m	G-o	G-*n*	G-d	G-j	G-f	G-m	D-o	D-*n*	D-d	D-j	D-f	D-m
6	1002	1002	α-Phellandrene	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-
7	1020	1020–1024	p-Cymene	-	0.6	-	-	-	-	0.8	0.7	-	-	-	-	0.6	-	-	-	-	-
8	1025	1025−1029	β-Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.1
9	**1026**	**1026–1031**	**Eucalyptol**	**62.1**	**59.8**	**52.8**	**51.9**	**56.8**	**56**	**64.8**	**62.6**	**62**	**50.7**	**57.1**	**57.9**	**62.5**	**64.7**	**49.1**	**52.2**	**54.6**	**47**
10	1054	1054−1059	γ-Terpinene	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	0.5	-	0.7
11	1070	1065–1070	cis- Sabinene hydrate	1.3	1.4	2.1	0.9	2.1	1.6	1.4	1.6	0.8	1.7	2	1	1.7	1.4	1.7	1.7	2	0.7
12	1088	1083–1089	Linalool	1.7	0.9	2.1	1.9	1.7	1.2	5.1	3.1	1	**3.7**	2.9	1.6	2.6	2.8	4	1.7	0.9	5.2
13	1135	1135–1139	trans-Pinocarveol	-	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-	0.6	-
14	1136	1135–1140	Nopinone	-	-	-	-	-	-	-	-	0.9	-	-	0.8	-	-	0.5	-	-	-
15	1164	1160–1164	Pinocarvone	-	-	0.7	-	-	-	-	-		-	0.6	-	0.6	0.5	2.3	0.6	0.6	-
16	1177	1174–1177	Terpinen-4-ol	1.7	2.1	-	2.7	3.4	2.1	2.6	2.4	4.7	2.8	3.4	3.7	1.9	1.7	2.8	3.5	2.8	3.9
17	1188	1186−1188	α-Terpineol	1.2	-	3.3	3.2	3.2	0.5	1.3	3.3	3.5	2.8	2	3.8	3.1	4	4	1.9	1.5	1.8
18	1285	1284–1285	Bornyl acetate	-	0.8	0.8	0.7	0.6	0.5	0.5	0.5	0.8	-	0.5	-	0.7	0.5	0.5	-	-	0.7
19	1300	1298–1308	Methyl geranate	-	-	0.7	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	1316	1304–1324	Piperitenone	0.7	-	-	-	0.6	-	0.5	0.5	-	-	0.8	0.7	0.5	-	1.1	1.1	-	1.2
21	**1349**	**1346–1349**	**α-Terpinyl acetate**	**12.6**	**11.1**	**10.2**	**12.2**	**11**	**10.9**	**8.5**	**8.7**	**10.9**	**14**	**10.8**	**11**	**8.2**	**7.7**	**10.6**	**12.4**	**10.3**	**12.8**
22	1359	1356–1359	Eugenol	0.9	1	1.3	2.9	-	-	0.5	-		-	0.8	-	1	0.6	3.8	1.1	1.5	1.5
23	1361	1359–1361	Neryl acetate	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-
24	1369	1366–1369	Dihydroeugenol	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-
25	1389	1387–1392	Dodecanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	-	-	-
26	1403	1403	Methyl eugenol	-	-		3.2	-	-	1.8	1.1	1.6	3.4	2.8	2.3	1	1.7	-	0.7	2.9	1.9
27	1443	1429–1446	Aromadendrene	-	-	-	-	-	1.1	-	-	0.5	-	-	-	-	-	-	-	0.5	-
28	1492	1491–1492	Methyl isoeugenol	-	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	1521	1521–1522	Eugenol acetate	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
30	1577	1577–1578	Spathulenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9
31	1582	1582–1583	Caryophyllene oxide	-	0.8	0.7	-	1.4	-	-	-	-	-	0.8	-	-	-	-	0.9	-	1.1
32	1650	1649–1650	β-Eudesmol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	0.5
Total identified compounds (%)				**90.2**	**92.2**	**95.7**	**93**	**89.7**	**97.7**	**95.8**	**97.5**	**85.7**	**97.2**	**96.3**	**97.5**	**97.3**	**97.6**	**94.9**	**93.9**	**94.3**	**90.2**

A: Ayancık, D: Dikmen, E: Erfelek, G: Gerze, C: Center, T: Türkeli, o: October, n: November, d: December, j: January, f: February, m: March (A-o: Ayancık- October, E-d: Erfelek- December, etc.; RRI: Relative retention indices; RRI*: [44]; (-): Not detected / Trace amount (below 0.01%).

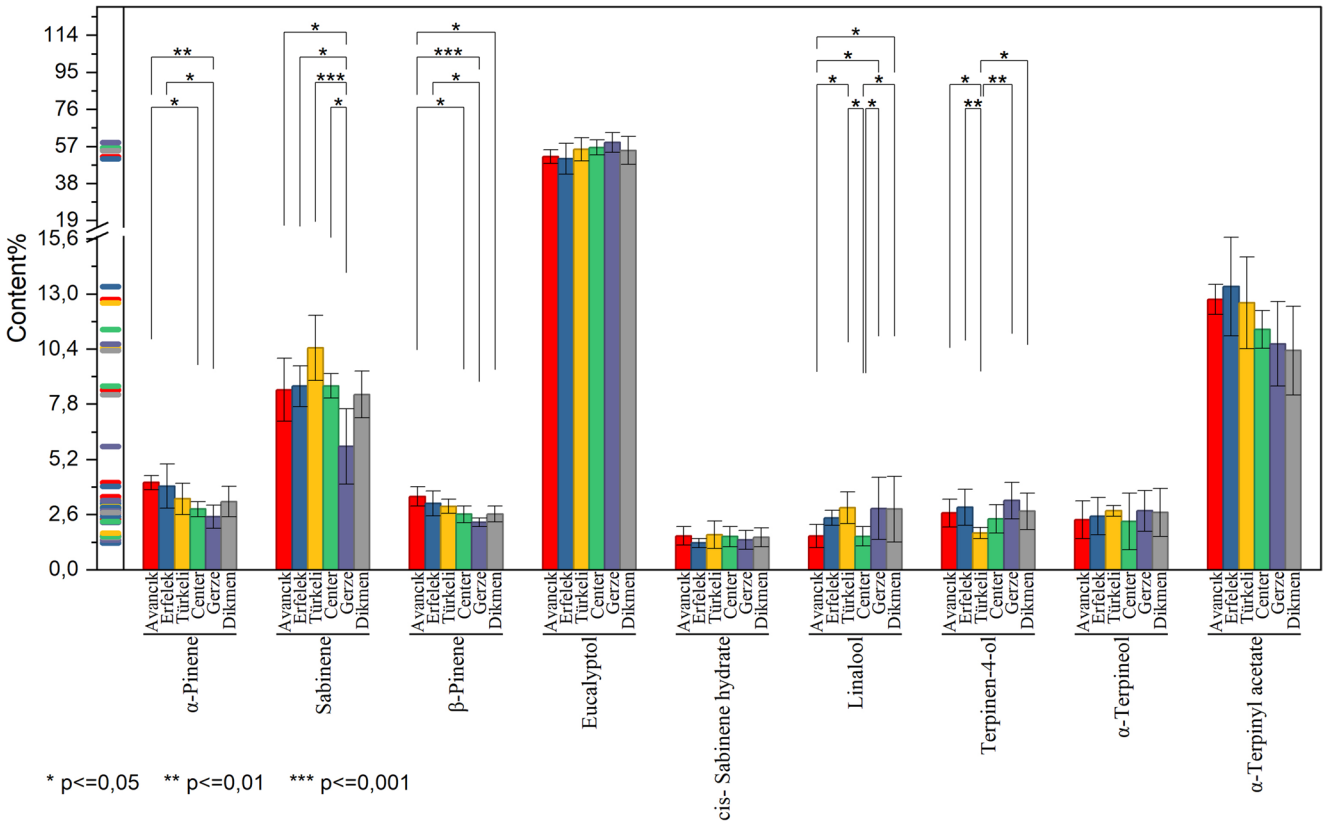


Fig. 2 Variation (%) in the nine most abundant essential oil components in bay leaves across different stations (regions) (Mean ± SE)

content differed significantly between Center (1.58 ± 0.45) and Türkeli (2.93 ± 0.75), Türkeli (2.93 ± 0.75) and Ayancık (1.60 ± 0.55), Gerze (2.90 ± 1.47) and Center (1.58 ± 0.45), Gerze (2.90 ± 1.47) and Ayancık (1.60 ± 0.55), Dikmen (2.87 ± 1.55) and Center (1.58 ± 0.45), and Dikmen (2.87 ± 1.55) and Ayancık (1.60 ± 0.55) ($p < 0.05$).

In addition, terpinen-4-ol content was significantly higher in Gerze (3.27 ± 0.86) and Dikmen (2.77 ± 0.86) compared with Türkeli (1.73 ± 0.26), while significant differences were also observed between Türkeli (1.73 ± 0.26) and Erfelek (2.95 ± 0.85), as well as between Gerze (3.27 ± 0.86) and Ayancık (2.68 ± 0.66) ($p < 0.05$).

The locational analysis of EO composition revealed that several monoterpene hydrocarbons and oxygenated monoterpenes varied significantly across the six sampling districts. These patterns indicate that the chemical profile of *L. nobilis* EO is not uniform across the Sinop province; rather, district-level differences in the concentrations of specific constituents are statistically demonstrable, suggesting that geographical location within the same province constitutes a meaningful source of compositional variability.

The analysis of monthly variation revealed that most constituents did not show statistically significant differences across the sampling period (Fig. 2). According to one-way ANOVA, significant temporal variation was observed only for eucalyptol ($p < 0.001$) and α-terpinyl acetate ($p = 0.017$), whereas α-pinene, sabinene, β-pinene, cis-sabinene hydrate, and α-terpineol did not differ significantly among months ($p > 0.05$). Non-parametric Kruskal–Wallis analysis also indicated no significant monthly variation for linalool ($p = 0.921$) and terpinen-4-ol ($p = 0.093$).

Post-hoc comparisons showed that eucalyptol content differed significantly between January (49.77 ± 2.39) and October (59.73 ± 4.94) ($p < 0.01$), January (49.77 ± 2.39) and November (61.50 ± 4.22) ($p < 0.01$), and February (52.37 ± 4.42) and November (61.50 ± 4.22) ($p = 0.020$). In addition, α-terpinyl acetate showed a significant difference between January (13.72 ± 1.24) and November (9.72 ± 1.64) ($p < 0.01$). Although the overall tests for terpinen-4-ol were not statistically significant, exploratory pairwise comparisons suggested differences between March (3.10 ± 0.77) and October (1.98 ± 0.34) ($p = 0.017$), February (2.98 ± 0.69) and October (1.98 ± 0.34) ($p = 0.032$), March (3.10 ± 0.77) and November (2.15 ± 0.74) ($p = 0.041$), and December (2.94 ± 1.21) and October (1.98 ± 0.34) ($p = 0.049$).

Monthly variation analysis indicated that temporal effects on EO composition were largely limited to eucalyptol and α-terpinyl acetate, which exhibited opposing seasonal trajectories: eucalyptol peaked in early autumn and declined toward mid-winter, while α-terpinyl acetate accumulated

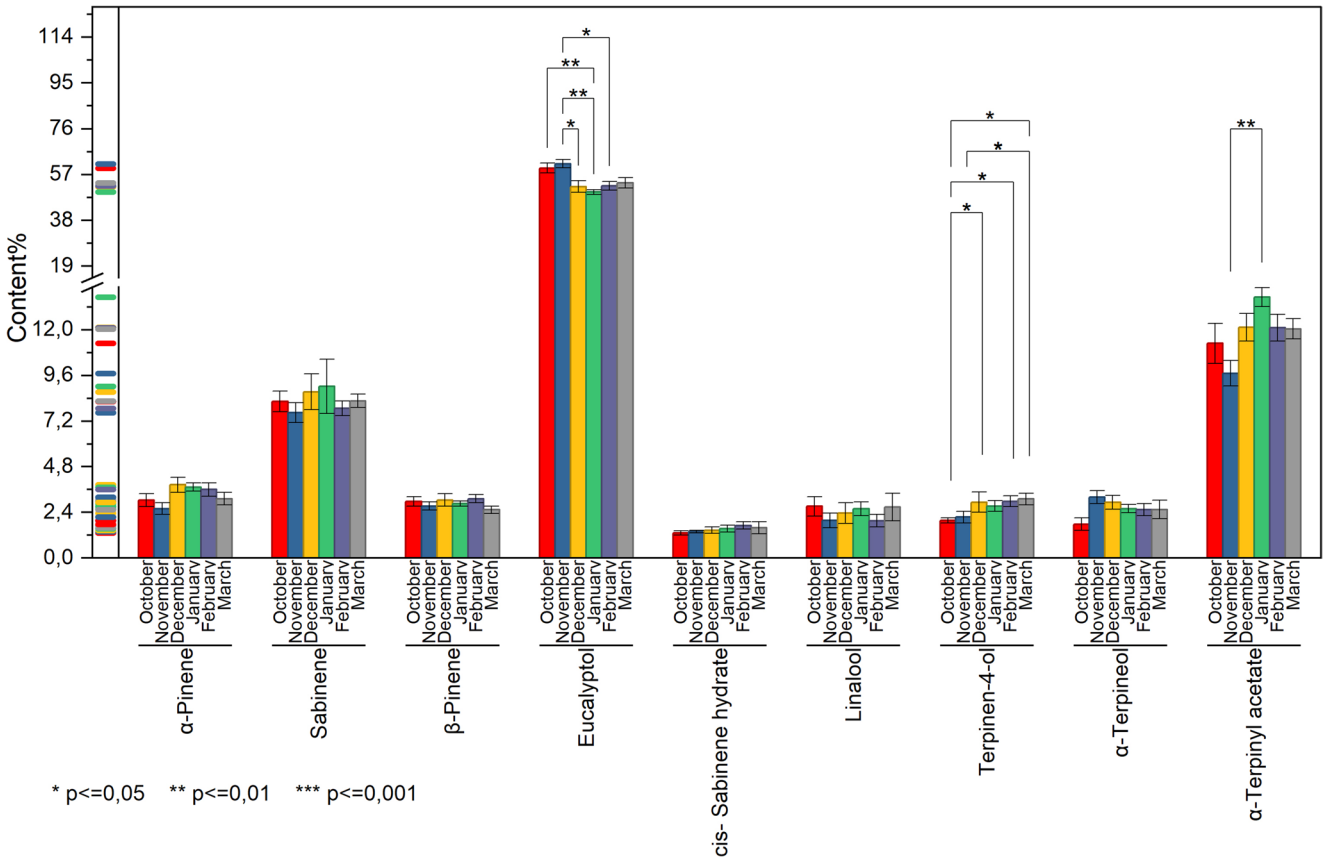


Fig. 3 Monthly seasonal variation (%) of the nine most abundant essential oil components in bay leaves (Mean ± SE)

more prominently during colder months. The remaining constituents showed no statistically significant monthly variation, indicating relative temporal stability across the sampling period.

Moreover, the presence and proportions of monoterpenes such as α-pinene, β-pinene and linalool are similar to previous studies [51, 52]. Overall, the highest eucalyptol concentration was observed in Erfelek district in November (65.5%), while the highest average concentration over the 6-month period was seen in Gerze district (59.1%). Camphene, Myrcene, γ-Terpinene, Terpinen-4-ol, and α-Terpineol were the compounds found at the lowest levels in our study (0.5-4%). Similar results were obtained in studies conducted in Türkiye and other countries [13].

Compared with the positive control, ascorbic acid (IC_{50} = 65.2 µL/mL), the IC_{50} values of the samples were approximately twofold or higher, ranging from 119.8 to 426.8 µL/mL. Among the tested samples, those collected from Gerze in December (125.3 µL/mL), Türkeli in November (119.8 µL/mL), Dikmen in January (125.6 µL/mL), Gerze in January (133.6 µL/mL), Gerze in February (130.8 µL/mL), and the Center in February (137.3 µL/mL) exhibited antioxidant activities relatively closer to that of ascorbic acid compared with the other samples.

When the regions (Ayancık, Erfelek, Türkeli, Center, Gerze, and Dikmen) were compared, samples collected in December generally demonstrated higher antioxidant activity (IC_{50} values between 125.3 and 199.8 µL/mL) than those collected in the other months. A more detailed analysis also revealed considerable variability in antioxidant activity among samples obtained in the same month. For instance, in February, the IC_{50} value of the Ayancık sample was 352.8 µL/mL, whereas the Gerze sample exhibited a markedly lower IC_{50} value of 130.8 µL/mL. (Table 3)

The comparison of the evaluated parameter among the sampling districts using the Kruskal–Wallis test did not reveal any statistically significant differences ($p = 0.524$). Consistent with this result, subsequent pairwise comparisons also showed no significant differences between any district pairs ($p > 0.05$). These findings indicate that the examined variable remained relatively consistent across all sampling locations (Ayancık 240.71 ± 83.66; Erfelek 197.17 ± 56.41; Türkeli 166.50 ± 26.63; Center 231.86 ± 86.33; Gerze 208.44 ± 83.65; Dikmen 326.04 ± 79.39) (Fig. 4a).

Furthermore, IC_{50} values (µL/mL) did not differ statistically significantly across months overall ($p = 0.051$). However, exploratory pairwise comparisons suggested that some pairs of months differed significantly. Specifically,

Table 3 IC_{50} values (µL/mL) (±SD) of *L. nobilis* essential oils collected from different locations and at different time intervals

	October	November	December	January	February	March
Ayancık	182.8	242.5	176.3	305.7	352.8	210
Erfelek	198.3	190	180.9	324.7	216.6	321.5
Türkeli	381.4	119.8	199.8	223.1	245.2	426.8
Center	217.2	179.5	170.8	278.5	137.3	275.2
Gerze	300.7	171.3	125.3	133.6	130.8	323.9
Dikmen	163.9	279.8	146	125.6	167.9	398.8

significant differences were observed between March (213.67±105.42) and December (266.00±115.92) ($p<0.001$), March (213.67±105.42) and November (238.69±66.45) ($p<0.01$), March (213.67±105.42) and February (197.59±90.63) ($p<0.01$), and March (213.67±105.42) and January (209.76±57.87) ($p=0.032$). No significant differences were found among the remaining pairs of months ($p>0.05$) (Fig. 4b).

Antioxidant activity did not vary significantly across sampling districts, indicating that geographical location within Sinop province had no meaningful effect on the radical scavenging capacity of the EOs. Regarding monthly variation, although the overall Kruskal–Wallis test did not reach significance ($p=0.051$), exploratory pairwise comparisons revealed that March consistently yielded lower IC_{50} values - and thus higher antioxidant activity - compared with the preceding winter months. These pairwise patterns should nonetheless be interpreted cautiously given the absence of overall test significance.

Many studies have been conducted in various regions on the antioxidant activities of *L. nobilis* EOs. Although direct comparison may not be logical considering ecological differences, it is anticipated that it will be informative at least in terms of evaluating its biological activity. When examined from this perspective, the antioxidant activities of different parts of this plant (seeds. roots. stems. leaves. etc.) have been investigated in many studies using different extraction techniques, and the presence of antioxidant capacity has been reported. Here are some of the studies published in parallel with our study, in which we found that antioxidant activity was present, although lower than that of the positive control, Ascorbic acid. Saab et al. [53] studied the antioxidant activity of seed and leaf EOs of *L. nobilis* using the DPPH technique and reported effective results compared to Ascorbic acid. When comparing our results with this study, we can state that we detected significantly higher activity in the context of ascorbic acid, which served as a positive control.

Beyene et al. [35] reported that methanol and fatty acid extracts of *L. nobilis* leaves collected from Ethiopia have significant antioxidant capacity. In their study, Dobroslavić et al. [36] aimed to obtain extracts from *L. nobilis* leaves using microwave, ultrasound, and heat-reflux-assisted green and conventional methods and to examine their antioxidant capacities. They reported that the plant extract had high antioxidant capacity, which was best achieved using the heat-reflux-assisted method. Mssillou et al. [36] reported that the EOs of *L. nobilis* flowers collected from Morocco had high antioxidant activity. Considering the DPPH method used by the researchers and the positive control they compared, we can state that our results contain a higher antioxidant capacity.

Dias et al. [54] studied the antioxidant capacity and nutritional content of wild and cultivated *L. nobilis* leaves and reported that wild varieties have high nutritional content, while cultivated types have high antioxidant capacity. Altın et al. [15] stated that the *L. nobilis* leaves they collected from Mersin province have effective antioxidant capacity,

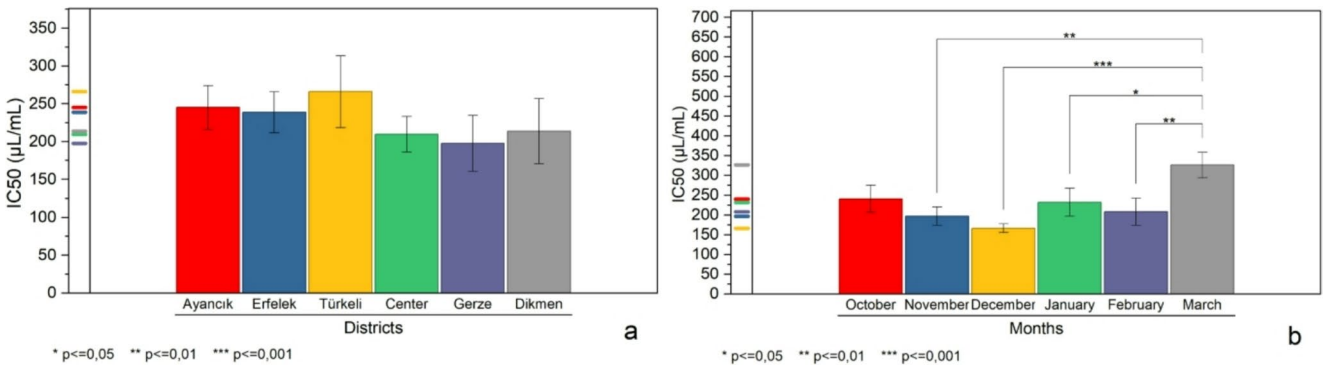


Fig. 4 Comparison of IC_{50} values (µL/mL) of *L. nobilis* essential oils collected from different locations (**a**) and at different time intervals (**b**), with Ascorbic Acid (65.2±8.4 µL/mL) as a positive control (Mean±SE)

which is due to their high phenolic content. Nabiha et al. [55] studied the antioxidant capacity of *L. nobilis* flower bud extracts collected from Tunisia and reported that they were more effective than butylated hydroxytoluene, which served as a positive control. They emphasized that this is also due to the high levels of eugenol, elemicin, and methyl eugenol it contains. Ramos et al. [56] studied the biological properties of the EO, ethanol extract, and hot/cold water extract of the *L. nobilis* plant collected from Portugal and reported that the EO showed the lowest activity in terms of antioxidant capacity.

In another study, Belasli et al. [57] investigated the antioxidant activity of EOs from *L. nobilis* plants collected in Algeria and found that their results were parallel to ours when compared to the positive control.

In this study, the antimicrobial effects of EOs obtained from the leaves of *L. nobilis* were evaluated by the disk diffusion method against four Gram-positive bacteria, three Gram-negative bacteria, and two fungi. When the findings were compared with the existing literature, both notable similarities and differences were observed. The results presented in Table 4 indicate that bay leaf EO possesses a broad antimicrobial spectrum; however, its effectiveness varies depending on the type of microorganism and the time at which the samples were collected.

The antimicrobial activities of EOs obtained from different districts were evaluated using the Kruskal-Wallis test. No statistically significant differences among districts were detected for *B. cereus* ($p=0.722$), *B. subtilis* ($p=0.835$), *S. aureus* ($p=0.271$), *E. coli* ($p=0.512$), *K. pneumoniae* ($p=0.747$), or *P. aeruginosa* ($p=0.833$). In contrast, a significant regional variation was observed for *E. faecalis* ($p<0.01$). Pairwise comparisons revealed that inhibition zone values from Dikmen (15.17 ± 9.77) differed significantly from those recorded for Türkeli (10.83 ± 2.56) ($p<0.01$), Ayancık (10.00 ± 1.10) ($p=0.046$), and Erfelek (10.00 ± 0.63) ($p=0.046$), whereas no other pairwise differences were detected ($p>0.05$) (Fig. 5b).

Regarding seasonal variation, a statistically significant difference among months was observed for *E. coli* ($p=0.006$). Pairwise comparisons indicated significant differences between December (8.67 ± 0.82) and October (12.67 ± 1.97) ($p<0.001$), January (9.17 ± 0.98) and October (12.67 ± 1.97) ($p<0.001$), February (9.50 ± 1.52) and October (12.67 ± 1.97) ($p<0.01$), March (11.00 ± 2.19) and December (8.67 ± 0.82) ($p=0.014$), and November (10.50 ± 1.38) and October (12.67 ± 1.97) ($p=0.022$). For *K. pneumoniae*, the overall Kruskal–Wallis test did not reveal a statistically significant difference among months ($p=0.071$). However, exploratory pairwise comparisons suggested potential differences between January (24.00 ± 14.75) and October (12.00 ± 0.00) ($p<0.01$), January (24.00 ± 14.75) and December (15.00 ± 4.69) ($p=0.044$), and January (24.00 ± 14.75) and November (15.00 ± 2.45) ($p=0.044$) (Fig. 5a).

Antimicrobial activity against most tested organisms did not vary significantly across districts or months, suggesting a generally consistent inhibitory profile across the sampling matrix. The notable exception was *E. faecalis*, for which Dikmen yielded significantly higher inhibition zones than Türkeli, Ayancık, and Erfelek, indicating a district-specific enhancement in activity against this organism. Regarding seasonal variation, *E. coli* inhibition was significantly stronger in October relative to the winter months, pointing to a temporal peak in activity during early autumn. For *K. pneumoniae*, although the overall test was non-significant, exploratory comparisons suggested elevated inhibition in January; this pattern warrants cautious interpretation given the absence of confirmed overall significance.

In general, EOs exhibit stronger antimicrobial activity against Gram-positive bacteria and lower activity against Gram-negative bacteria. This difference is mainly attributed to the presence of the lipopolysaccharide (LPS) layer in the outer membrane of Gram-negative bacteria, which acts as a natural barrier limiting the penetration of hydrophobic EO components [58]. Consistent with this mechanism, large inhibition zones observed in Gram-positive species such as *S. aureus* and *B. cereus* (15–24 mm) indicate that EO components can more easily penetrate their cell walls and exert antimicrobial effects.

The significantly higher activity observed among certain samples suggests variations in EO chemical composition. Factors such as harvesting time, climatic conditions, soil characteristics, plant age, and distillation methods may influence the proportions of volatile compounds. Even samples sharing the same code showed differences in activity, indicating that the metabolite profile of *L. nobilis* is highly sensitive to environmental conditions.

Consistent with these observations, the EOs obtained from *L. nobilis* leaves in this study demonstrated higher activity against Gram-positive bacteria (*B. cereus*, *B. subtilis*, and *S. aureus*), whereas Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) and certain fungi showed lower sensitivity (Table 5).

When comparing our findings with previous studies, Fidan et al. [51] reported that *L. nobilis* EOs obtained from leaves, twigs, and fruits in Bulgaria showed organ-dependent variations in chemical composition and antimicrobial activity. The leaf EO (3.25% yield) had the highest yield with eucalyptol and α-terpinyl acetate as the main components and was effective against Gram-positive bacteria, whereas the twig EO was active only against *S. aureus*, and Gram-negative bacteria (*P. aeruginosa*, *E. coli*) were more resistant. Similarly, Yilmaz et al. [59] reported that leaf EO

Table 4 Antimicrobial activity of essential oils obtained from the leaves of *L. nobilis* determined by the disk diffusion method (mm)

	A-o	A-*n*	A-d	A-j	A-f	A-m	E-o	E-*n*	E-d	E-j	E-f	E-m
B. cereus	24	12	8	10	8	8	7	16	-	10	20	8
B. subtilis	20	16	10	10	8	8	7	14	-	10	20	7
S. aureus	15	12	10	10	8	8	10	10	9	9	10	10
E. faecalis	10	10	12	10	9	9	10	11	10	9	10	10
E. coli	15	10	8	8	8	8	12	10	9	9	10	11
K. pneumoniae	12	16	16	50	14	10	12	16	20	15	20	30
P. aeruginosa	15	18	10	10	10	8	10	9	9	8	12	11
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	20	-	-	-	-
	T-o	**T-n**	**T-d**	**T-j**	**T-f**	**T-m**	**C-o**	**C-n**	**C-d**	**C-j**	**C-f**	**C-m**
B. cereus	24	12	9	10	20	14	8	18	-	-	12	8
B. subtilis	20	10	10	20	10	15	10	18	-	10	20	7
S. aureus	10	12	12	9	10	10	10	9	8	10	12	12
E. faecalis	14	14	10	10	9	8	10	12	12	10	10	14
E. coli	15	13	8	9	10	14	12	11	10	9	9	10
K. pneumoniae	12	16	16	20	14	12	12	16	16	-	-	-
P. aeruginosa	9	10	10	10	11	10	10	8	12	9	10	12
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	14	-	16	-	-	-	14	8	6	-	-	-
	G-o	**G-n**	**G-d**	**G-j**	**G-f**	**G-m**	**D-o**	**D-n**	**D-d**	**D-j**	**D-f**	**D-m**
B. cereus	8	12	6	-	10	14	7	12	-	15	-	14
B. subtilis	10	8	8	-	15	15	7	14	-	10	-	15
S. aureus	10	12	10	12	12	12	10	9	8	8	10	12
E. faecalis	8	-	8	8	8	7	12	12	10	10	12	35
E. coli	10	9	8	11	12	13	12	10	9	9	8	10
K. pneumoniae	12	10	16	15	14	20	12	16	6	20	14	-
P. aeruginosa	8	10	10	12	14	14	10	12	12	9	9	10
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	14	8	7	-	-	-	20	-	8	-	-	-
	AM10	**CN10**	**Cyc**									
B. cereus	40	28	*									
B. subtilis	40	24	*									
S. aureus	28	40	*									
E. faecalis	25	15	*									
E. coli	15	20	*									
K. pneumoniae	40	30	*									
P. aeruginosa	25	15	*									
A. niger	*	*	15									
C. albicans	*	*	40									

A: Ayancık, D: Dikmen, E: Erfelek, G: Gerze, C: Center, T: Türkeli, o: October, n: November, d: December, j: January, f: February, m: March, Ampicillin (AM10), Gentamicin (CN10), Cycloheximide (Cyc)

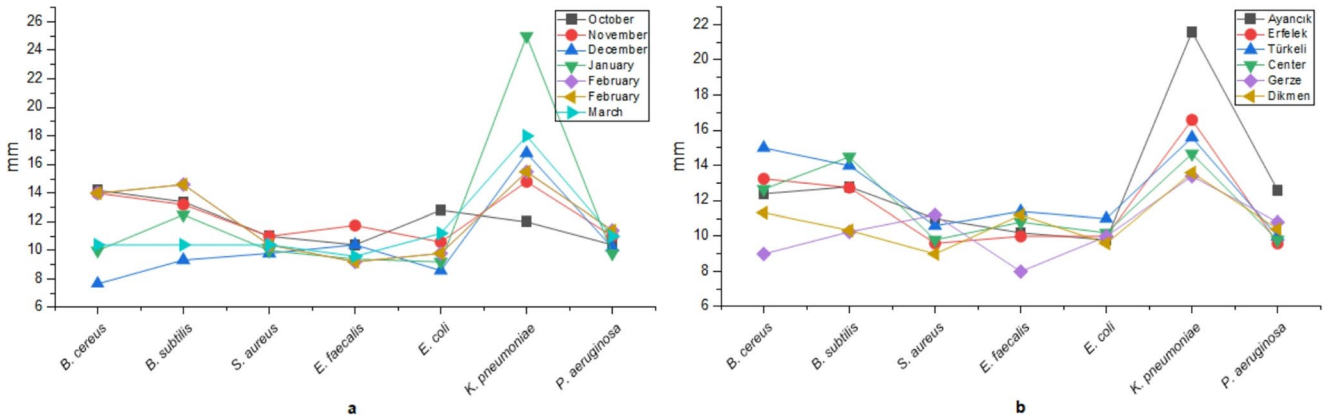


Fig. 5 Antimicrobial activity (mm) of essential oils obtained from *L. nobilis* leaves collected from different locations (a) and at different time intervals (b) (Mean ± SE)

from Antakya-Hatay contained 51.8% eucalyptol, 11.2% α-terpinyl acetate, and 10.1% Sabinene, exhibiting strong antibacterial, antifungal, and antioxidant activity. Ouibrahim et al. [60] also highlighted the role of eucalyptol and synergistic interactions among terpenes in EO antimicrobial activity. Our results are consistent with these studies: the high eucalyptol and linalool content in our leaf EOs likely explains the observed inhibition against both Gram-positive and Gram-negative bacteria. Despite minor compositional variations among samples due to environmental and regional factors, the findings align with previous reports, supporting the consistent antibacterial and antifungal potential of *L. nobilis* leaf EO.

Overall, these results emphasize that the antimicrobial potential of *L. nobilis* leaf EO is largely determined by its chemical composition and provide a data-driven interpretation that integrates our findings with previous literature.

Correlation analysis

Spearman correlation analysis was conducted to examine the relationships among temperature, major EO constituents, antioxidant activity (IC_{50}), and antimicrobial activities (Fig. 6).

Among the chemical constituents, several statistically significant correlations were identified. α-Pinene demonstrated a strong positive correlation with β-pinene ($r=0.86$, $p<0.001$) and a moderate positive correlation with sabinene ($r=0.43$, $p<0.05$), whereas it exhibited a strong negative correlation with eucalyptol ($r=-0.86$, $p<0.001$). Similarly, β-pinene was significantly negatively correlated with eucalyptol ($r=-0.55$, $p<0.001$) and positively correlated with α-terpinyl acetate ($r=0.63$, $p<0.001$). Sabinene was negatively correlated with eucalyptol ($r=-0.44$, $p<0.05$), and terpinen-4-ol also showed a significant negative relationship with eucalyptol ($r=-0.40$, $p<0.05$).

Temperature was significantly associated with several variables. A positive correlation was observed between temperature and eucalyptol ($r=0.49$, $p<0.01$), whereas a significant negative correlation was found between temperature and terpinen-4-ol ($r=-0.54$, $p<0.001$). Additionally, temperature exhibited significant positive correlations with *E. faecalis* ($r=0.38$, $p<0.05$) and *E. coli* ($r=0.41$, $p<0.05$).

With respect to antimicrobial activity, a strong positive correlation was detected between *B. cereus* and *B. subtilis* ($r=0.74$, $p<0.001$), indicating comparable antimicrobial response patterns for these two species. *S. aureus* showed significant positive correlations with *E. coli* ($r=0.37$, $p<0.05$) and *P. aeruginosa* ($r=0.43$, $p<0.01$), and a moderate positive correlation was also observed between *E. coli* and *P. aeruginosa* ($r=0.33$, $p<0.05$).

In contrast, IC_{50} values did not yield statistically significant correlations with most chemical constituents or antimicrobial variables, suggesting that antioxidant activity varied independently of the majority of the parameters investigated.

Taken together, the correlation analysis revealed two principal patterns: (i) pronounced interrelationships among several monoterpene compounds, particularly α-pinene, β-pinene, sabinene, and eucalyptol, and (ii) positive associations among multiple antimicrobial responses. Temperature further emerged as a variable influencing both chemical composition and certain antimicrobial activity patterns.

The correlation analysis revealed that eucalyptol concentrations were inversely associated with those of the co-occurring monoterpene hydrocarbons α-pinene, β-pinene, and sabinene, suggesting a competitive biosynthetic relationship among these constituents. Temperature emerged as a structuring variable, positively associated with eucalyptol and negatively with terpinen-4-ol, corroborating the seasonal patterns observed in the monthly variation analysis. Antimicrobial responses against phylogenetically related

Table 5 MIC Values (µg/L) of the leaves *L. nobilis* essential oils

	A-o	A-*n*	A-d	A-j	A-f	A-m	E-o	E-*n*	E-d	E-j	E-f	E-m
B. cereus	640	1280	--		-	-	-	640	-	-	320	-
B. subtilis	640	1280	-	-	-	-	-	1280	-	-	320	-
S. aureus	1280	-	-	-	-	-	-	-	-	-	-	-
E. faecalis	-	-	-	-	-	-	-	1280	-	-	-	-
E. coli	1280	-	-	-	-	-	1280	-	-	-	-	-
K. pneumoniae	1280	1280	-	320	640	-	1280	640	640	1280	640	320
P. aeruginosa	1280	1280	-	-	-	-	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	640	-	-	-	-
	T-o	**T-n**	**T-d**	**T-j**	**T-f**	**T-m**	**C-o**	**C-n**	**C-d**	**C-j**	**C-f**	**C-m**
B. cereus	320	1280	-	-	320	1280	-	640	-	-	1280	-
B. subtilis	320	1280	-	-	320	1280	-	320	-	-	320	-
S. aureus	-	1280	1280	-	-	-	-	-	-	-	1280	1280
E. faecalis	1280	1280	1280	1280	-	-	-	1280	1280	-	-	1280
E. coli	640	640	-	-	-	1280	1280	1280	-	-	-	-
K. pneumoniae	1280	640	640	320	640	1280	1280	640	640	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	1280	-	-	1280
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	1280	-	1280	-	-	-	1280	-	-	-	-	-
	G-o	**G-n**	**G-d**	**G-j**	**G-f**	**G-m**	**D-o**	**D-n**	**D-d**	**D-j**	**D-f**	**D-m**
B. cereus	-	1280	-	-	-	1280	-	1280	-	640	-	1280
B. subtilis	-	-	-	-	1280	1280	-	1280	-	-	-	1280
S. aureus	1280	1280	1280	1280	1280	1280	-	-	-	-	-	1280
E. faecalis	-	-	-	-	-	-	1280	1280	-	-	1280	160
E. coli	1280	-	-	1280	1280	1280	1280	-	-	-	-	-
K. pneumoniae	1280	1280	640	640	1280	320	1280	640	-	320	1280	-
P. aeruginosa	-	-	-	1280	1280	1280	-	1280	1280	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	1280	-	-	-	-	-	320	-	-	-	-	-

A: Ayancık, D: Dikmen, E: Erfelek, G: Gerze, C: Center, T: Türkeli, o: October, n: November, d: December, j: January, f: February, m: March, Ampicillin (AM10), Gentamicin (CN10), Cycloheximide (Cyc)

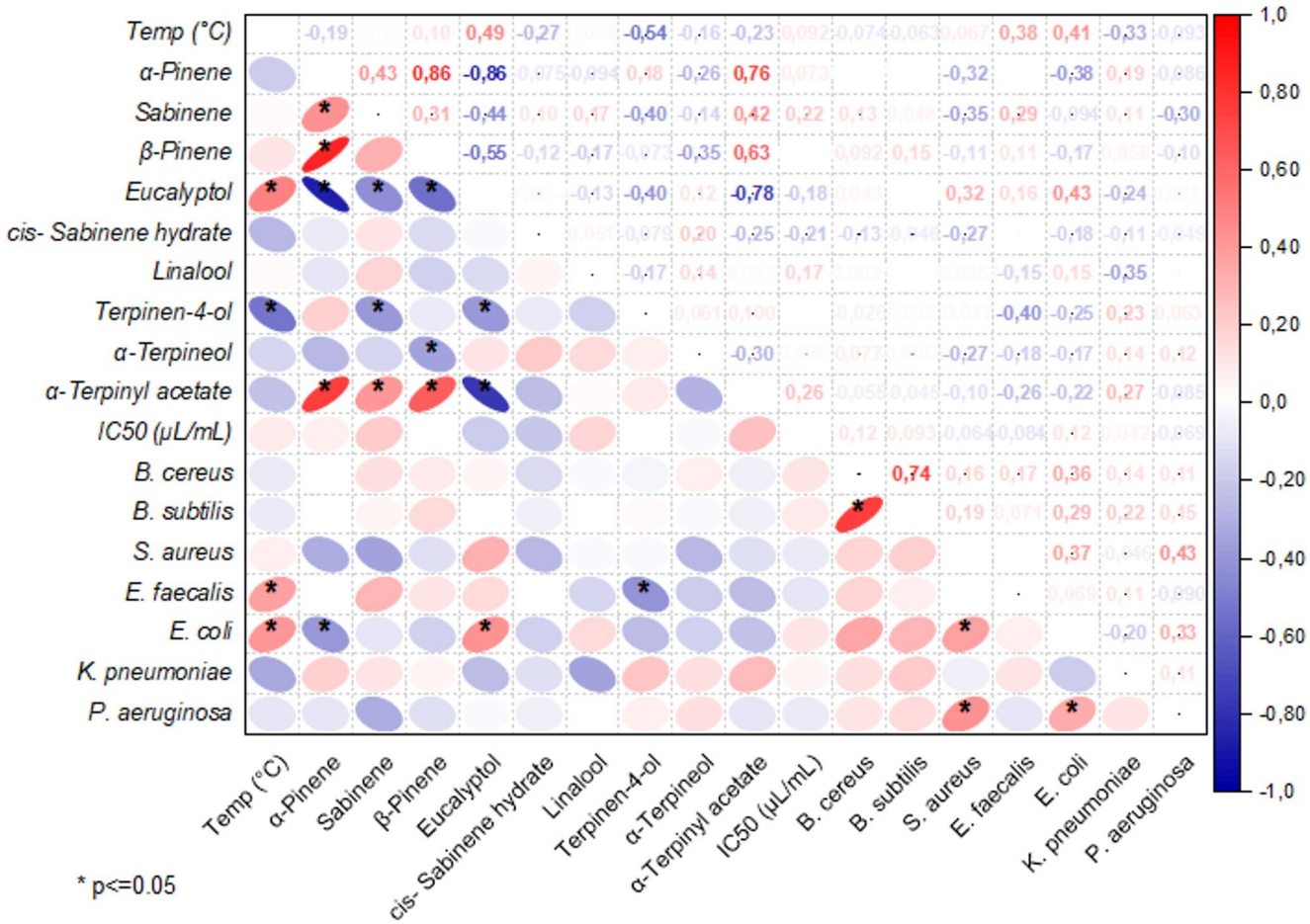


Fig. 6 Spearman correlation heatmap of essential oil components and biological activities

organisms tended to co-vary positively, whereas IC_{50} values showed no meaningful association with either chemical composition or antimicrobial activity, indicating that antioxidant capacity operates independently of the other measured parameters.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was applied to examine the multivariate relationships among temperature, major EO constituents, antioxidant activity (IC_{50}), and antimicrobial activities. The first five principal components (PCs) collectively accounted for 80.23% of the total variance (PC1: 27.90%, PC2: 20.43%, PC3: 13.39%, PC4: 11.49%, PC4: 7.03%), indicating that the dataset was adequately represented by these components. The biplot of PC1 and PC2, which together explained 48.33% of the total variance, provided a visual representation of the relationships among variables (Fig. 7).

PC1 (27.90%) was primarily characterized by an opposition between eucalyptol and the monoterpene hydrocarbons α-pinene and α-terpinyl acetate on one side, and temperature, *E. coli*, and *B. cereus* on the other. This pattern suggests that samples with higher eucalyptol content were associated with elevated temperature and stronger activity against certain Gram-negative bacteria, while samples rich in α-pinene and α-terpinyl acetate tended to occur under cooler conditions. The long, rightward arrow of eucalyptol in the biplot, pointing nearly along the positive PC1 axis, visually confirms its dominant role in structuring this dimension.

PC2 (20.43%) was dominated by the pinene-type monoterpenes: β-pinene showed the highest positive loading, followed by α-pinene and sabinene, whereas terpinen-4-ol and α-terpineol loaded negatively. In the biplot, the upward trajectory of β-pinene, α-pinene, and sabinene vectors, all pointing toward the upper-left quadrant, reflects their co-occurrence in samples, consistent with the strong positive correlations among these compounds reported in the Spearman analysis. Conversely, terpinen-4-ol and α-terpineol projected downward, indicating an inverse relationship with the pinene-type fraction along this component.

Temperature and the majority of antimicrobial variables (particularly *B. cereus*, *B. subtilis*, *E. faecalis*, *E. coli*, *S. aureus*, and *P. aeruginosa*) clustered in the upper-right

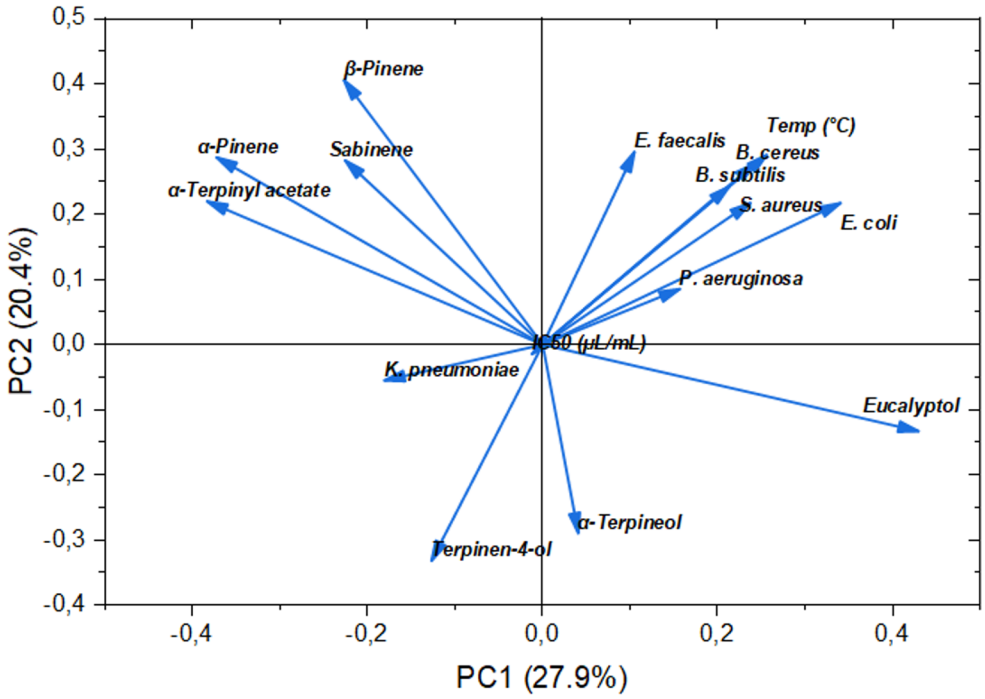


Fig. 7 PCA biplot of essential oil constituents and biological activities

quadrant of the biplot, where their vectors pointed broadly in the same direction as temperature (Fig. 6). This locational grouping implies a shared positive association between higher temperatures and the antimicrobial response patterns of multiple bacterial strains, corroborating the temperature antimicrobial correlations identified in the Spearman analysis. *K. pneumoniae* was a notable exception, with its vector oriented toward the lower portion of the plot, suggesting a divergent response pattern relative to the other test organisms.

IC_{50} values were represented by a notably short vector positioned near the origin of the biplot, indicating weak contributions to both PC1 and PC2 and confirming that antioxidant activity did not align consistently with any dominant grouping of chemical or biological variables. This finding is in accordance with the absence of significant Spearman correlations between IC_{50} and most other parameters.

Taken together, the PCA results revealed two primary axes of variation in the dataset: the first axis contrasted eucalyptol-dominant samples with pinene-dominant and α-terpinyl acetate-rich samples, mediated in part by temperature; the second axis reflected the internal differentiation within the monoterpene fraction, particularly between the β-pinene/sabinene group and the oxygenated terpene subgroup (terpinen-4-ol, α-terpineol). These patterns collectively underscore the influence of seasonal temperature variation on both the chemical composition and the bioactivity of *L. nobilis* EOs.

PCA confirmed the two dominant axes of variation identified in the Spearman analysis: PC1 separated eucalyptol-rich samples associated with warmer temperatures from α-pinene- and α-terpinyl acetate-dominant samples characteristic of cooler conditions, while PC2 differentiated the pinene-type hydrocarbon fraction from the oxygenated monoterpene subgroup. Antioxidant activity (IC_{50}) contributed minimally to both axes, reinforcing its independence from the remaining variables. *K. pneumoniae* diverged from the general antimicrobial clustering pattern, suggesting a distinct response profile relative to the other tested organisms.

Conclusion

This study provides an integrated, district-level characterisation of *L. nobilis* leaf EOs across six sampling locations in Sinop province over a six-month period, simultaneously examining ontogenetic and locational sources of variability a design that, to our knowledge, has not been previously reported for this species. The principal finding is that geographical location constitutes a more statistically consistent driver of compositional variation than the harvest month, with significant inter-district differences confirmed for six major constituents including sabinene, α-pinene, β-pinene, linalool, and terpinen-4-ol. Temporal effects were confined to eucalyptol and α-terpinyl acetate, which followed opposing seasonal trajectories across the study period. On the basis of the integrated analysis of EO yield, chemical composition, and biological activity, October and November harvests from the Erfelek and Gerze districts emerge

as the most promising combination for applications requiring high eucalyptol content (up to 65.5%) and relatively strong radical-scavenging capacity (IC_{50} as low as 119.8 µL/mL, Türkeli-November). For applications in which antimicrobial potency against Gram-positive bacteria is prioritised as evidenced by MIC values as low as 320 µg/L for *Bacillus* spp. and *Klebsiella pneumoniae* autumn collections from coastal districts similarly appear advantageous. These observations carry direct practical relevance for the optimisation of harvest scheduling and site selection in bay laurel cultivation destined for natural preservative, pharmaceutical, or cosmetic formulations. Taken together, the findings provide a scientific basis for the development of standardised EO-based preparations of *L. nobilis* with reproducible and targeted bioactivity profiles. This study is nonetheless subject to certain limitations. The dataset spans a single growing season, and multi-year replication would be necessary to confirm whether the observed locational and temporal patterns are stable across years or responsive to inter-annual climatic fluctuations. In addition, broader correlation analyses between minor constituent concentrations and specific bioactivity endpoints remain to be conducted. Future research should also explore the synergistic contributions of minor terpenoids to overall antimicrobial and antioxidant efficacy, and consider extending the sampling framework to additional provinces to assess the generalisability of these intra-regional findings.

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Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests The authors declare that there are no competing interests.

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