



Comprehensive Analysis of Essential Oil Constituents in *Lathyrus laxiflorus* subsp. *laxiflorus* via SPME and GC-MS/FID and Their Antimicrobial Properties

Lathyrus laxiflorus subsp. *laxiflorus*'un Esansiyel Yağ Bileşenlerinin SPME ve GC-MS/FID ile Kapsamlı Analizi ve Antimikrobiyal Özellikleri

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Abstract

In this study, the essential oil contents of *Lathyrus laxiflorus* subsp. *laxiflorus* plant were investigated comparatively by GC-MS/FID and SPME-GC-MS/FID techniques. It is known that some of the essential oils used for medicinal purposes are plant origin. For this purpose, in this study, the content of the essential oil components obtained by steam distillation method was analyzed by comparing with SPME method and its content was elucidated more comprehensively. In addition, the antimicrobial activities of the essential oil obtained by steam distillation were investigated. The antimicrobial activity was tested against six bacterial strains (*Escherichia coli*, *Yersinia pseudotuberculosis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis*) and one fungal strain (*Candida albicans*). 27 volatile compounds were detected by GC-MS/FID and 30 volatile compounds were detected by SPME-GC-MS/FID method for the plant *L. laxiflorus*, and a total of 42 volatile compounds were identified. The identified constituents predominantly belonged to the monoterpene and sesquiterpene classes, while diterpenes were detected in trace levels. The essential oil exhibited notable antimicrobial activity particularly against *E. coli*, *Y. pseudotuberculosis*, *S. aureus*, and *C. albicans* and inhibition zones were measured in millimeters.

Keywords: Antimicrobial activity, essential oil, *Lathyrus laxiflorus* subsp. *laxiflorus*, SPME-GC-MS/FID, volatile compounds.

Öz

Bu çalışmada, *Lathyrus laxiflorus* subsp. *laxiflorus* bitkisinin uçucu yağ içerikleri GC-MS/FID ve SPME-GC-MS/FID teknikleri ile karşılaştırmalı olarak araştırılmıştır. Tıbbi amaçlarla kullanılan uçucu yağların bir kısmının bitkisel kaynaklı olduğu bilinmektedir. Bu amaçla, bu çalışmada buhar damıtma yöntemi ile elde edilen uçucu yağ bileşenlerinin içeriği, SPME yöntemi ile karşılaştırılarak analiz edilmiş ve içeriği daha kapsamlı olarak aydınlatılmıştır. Ayrıca, buhar damıtma ile elde edilen uçucu yağın antimikrobiyal aktiviteleri araştırılmıştır. Antimikrobiyal aktivite altı bakteri suşuna (*Escherichia coli*, *Yersinia pseudotuberculosis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis*) ve bir mantar suşuna (*Candida albicans*) karşı test edilmiştir. *L. laxiflorus* bitkisinde GC-MS/FID yöntemiyle 27, SPME-GC-MS/FID yöntemiyle ise 30 uçucu bileşik tespit edilmiş ve toplam 42 uçucu bileşik tanımlanmıştır. Tanımlanan bileşenlerin büyük çoğunluğu monoterpen ve seskiterpen sınıflarına aitken, diterpenler eser miktarda tespit edildi. Uçucu yağ, özellikle *E. coli*, *S. aureus* ve *C. albicans*'a karşı belirgin antimikrobiyal aktivite göstermiş ve inhibisyon alanları milimetre cinsinden ölçülmüştür.

Anahtar Kelimeler: Antimikrobiyal aktivite, esansiyel yağ, *Lathyrus laxiflorus* subsp. *laxiflorus*, SPME-GC-MS/FID, uçucu bileşikler.

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1. Introduction

Lathyrus laxiflorus subsp. *laxiflorus*, a perennial non-endemic plant, is widespread in the Aegean coast, Marmara Region, Black Sea and Eastern Anatolia regions of our country at an altitude of approximately 0-1900 m (TÜBİVES 2024). Its habitats include woodlands, shrubs and shady coasts. *L. laxiflorus* belongs to the legume family Fabaceae. The Fabaceae family, one of the largest angiosperm families, comprises approximately 770 genera and more than 19,500 species worldwide, represented in herbaceous, shrubby, and rarely tree forms. *L. laxiflorus* subsp. *laxiflorus*, belonging to this family, is distributed from Italy to the Caucasus and is characterized by leaves with lavender to violet crowns, flowering between April and July. (Kocaeli bitkileri 2024, POWO 2024, Cronquist 1968, Lewis et al. 2005). Members of the genus *L. laxiflorus* are represented by more than 160 species globally, with ca. 75 taxa recorded in Turkey, many of which are endemic (Davis 1970, Arslan et al. 2018). These species are of agronomic, ecological, and pharmacological importance due to their roles as forage crops, sources of bioactive compounds, and contributors to biodiversity conservation.

Numerous studies have addressed the agronomic characteristics of *Lathyrus* species, including plant height, pod and seed number, seed weight, and overall yield, providing valuable data for cultivation strategies in different agroecological zones (Acar and Basaran 2007). Parallel investigations into the nutritional composition—such as crude protein content determined via the Kjeldahl method and mineral composition assessed by Atomic Absorption Spectrometry (AAS)—highlight their potential as protein-rich crops for human and animal consumption (Basaran et al. 2008). Beyond agricultural traits, *Lathyrus* species have been evaluated for cytotoxic, antimicrobial, anti-inflammatory, and antioxidant properties (Yildirim et al. 2023). Cytotoxicity assays, typically employing the MTT method, have revealed species- and extract-specific variations in cell viability (Acar and Basaran 2007). Antimicrobial screening, using both disk diffusion and minimum inhibitory concentration (MIC) methods, has demonstrated selective activity against Gram-positive and Gram-negative bacteria as well as fungi, depending on the solvent polarity and plant part extracted (Heydari et al. 2019). Anti-inflammatory potential has been assessed through *in vitro* protein denaturation inhibition and 5-lipoxygenase enzyme inhibition assays, supporting the possible therapeutic relevance of these taxa (Heydari et al. 2019).

Phytochemical investigations employing spectrophotometric antioxidant assays—including DPPH, ABTS, FRAP, CUPRAC, metal chelation (MCA), and phosphomolybdenum (PDA) methods—have consistently reported measurable radical-scavenging capacity, often correlating with total phenolic content (TPC) and total flavonoid content (TFC) determined via Folin–Ciocalteu and AlCl_3 colorimetric methods (Heydari et al. 2015). More advanced chemical profiling by LC-MS/MS has identified diverse phenolic compounds, flavonoids, and other secondary metabolites, with some endemic taxa from Turkey exhibiting notable enzyme inhibitory activities against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α -amylase, and α -glucosidase, indicating potential applications in neuroprotection, dermatology, and metabolic health (Yıldırım et al. 2023). Complementing the chemical and bioactivity studies, morphological and micromorphological analyses have contributed significantly to the taxonomy and systematics of the genus. Morphological and cytogenetic studies have provided valuable information for the taxonomy of the genus. SEM analyses of seed and pollen micromorphology revealed diagnostic traits for species delimitation (Güneş and Aytuğ 2010, Güneş and Çırpıcı 2011) while karyotype analyses documented chromosome numbers and centromere positions, contributing to the understanding of evolutionary relationships (Güneş and Çırpıcı 2008). Analysis and comparison of the fatty acid components of *L. boissieri* sirj. and *L. laxiflorus* subsp. *laxiflorus* (Desf.) O. Kuntze, belonging to the genus *Lathyrus* L., which is a food source for domestic animals, were carried out. Gas chromatographic analysis of the fatty acid components revealed the following: myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and behenic (22:0). In same study it was also found that these plants contain more saturated fatty acids than unsaturated fatty acids. *L. boissieri* sirj. was found to have a high content of linolenic acid (20.16%), an essential fatty acid for animals (Dinç 2007).

Essential oils are highly valued natural products composed mainly of volatile terpenes and their derivatives, known for a wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, and insecticidal effects. Due to their efficacy, safety, and natural origin, they are extensively used in pharmaceuticals, cosmetics, aromatherapy, and food preservation, as well as in agriculture for pest control. (Pezantes-Orellana et al. 2024). Solid phase micro extraction (SPME) is widely preferred in volatile analysis due

to its solvent-free, sensitive and efficient sampling advantages (Şahin et al. 2024, Şener et al. 2024)

The genus *Lathyrus* (Fabaceae) comprises species of agronomic, nutritional, ornamental, and pharmacological interest, and has been investigated across complementary biological scales—from macromolecular markers to volatile/primary metabolite chemistry. Electrophoretic profiling of seed storage proteins has shown that *Lathyrus* and allied *Pisum* taxa display distinctive SDS-PAGE banding patterns, supporting their use as taxonomic markers and indicators of genetic diversity (Osman 2023). At the lipid level, comparative seed fatty-acid work demonstrates that linoleic, oleic, and palmitic acids predominate, yet their relative proportions vary among species—information that is valuable both for chemotaxonomy and for assessing nutritional/industrial potential (Bağcı and Şahin 2004).

Phytochemically, *Lathyrus* is rich in flavonoids. A genus-wide survey reports widespread flavonol aglycones—particularly kaempferol and quercetin—occurring in multiple glycosidic forms, with sectional patterns that aid systematics and distinguish *Lathyrus* from other Viciaeae genera (Ranabahu and Harborne 1993). Beyond targeted phenolics, untargeted GC-MS metabolite profiling has mapped sugars, organic acids, amino acids, and selected phenolics, and multivariate analyses (e.g., PCA) reveal species- and geography-linked separations in chemical space (İbrahim et al. 2021).

Compound-level structure elucidation further refines this picture: NMR studies on *L. odoratus* seeds identified lathyritol—a galactosyl-bornesitol derivative—as a seed reserve carbohydrate with plausible physiological roles (Obendorf et al. 2005, Ranabahu and Harborne 1993). The floral scent dimension of *L. odoratus* has likewise been characterized by SPME-GC-MS, highlighting limonene, linalool, benzyl alcohol, and related aromatics, with cultivar-dependent profiles relevant to breeding and fragrance optimization. (Porter et al. 1999). The floral volatiles of *Lathyrus odoratus* were profiled using HS-SPME-GC-MS, leading to the identification of 71 compounds with clear seasonal variation. Notably, enantioselective GC analysis revealed distinct chiral distributions of key terpenes, reported here for the first time in this species (Calva et al. 2024). Finally, work in a related Fabaceae member, *Cercis siliquastrum*, combining LC-MS/MS (non-volatiles) and SPME-GC-MS (volatiles), underscores family-level phytochemical diversity and provides a comparative framework for interpreting *Lathyrus* chemistry (Moghaddam et al. 2025). In a recent study, the phenolic profile of *L. laxiflorus* draws attention with both high anti-

oxidant activity and the presence of various phenolic/flavonoid compounds (Kıran Acemi 2020).

Collectively, findings from agronomy, cytogenetics, antioxidant chemistry, and antimicrobial studies emphasize that *Lathyrus* species are an important source of genetic diversity and bioactive compounds. The integration of morphological, phytochemical, and bioactivity data provides a comprehensive framework for the exploitation and conservation of *Lathyrus* taxa, particularly in biodiversity-rich regions such as Türkiye⁷, where endemic species hold both scientific and economic significance. No data on the analysis of the essential oil of *L. laxiflorus* has been found in the literature. This study attempted to elucidate the volatile profile using both Clevenger and SPME methods. The antimicrobial properties of the resulting essential oil were also evaluated.

2. Material and Methods

2.1. Chemicals and Instrumentation

Volatile oil analysis was carried out using a Shimadzu QP2010-4 Ultra gas chromatograph (Japan). The system was equipped with a Shimadzu 2010 Plus FID detector and a Shimadzu mass spectrometer detector, operated through Shimadzu Class-5000 Chromatography Workstation software. The SPME headspace volatiles were collected using a Supelco 57348 2 cm, 50/30 µm DVB/Carboxen/PDMS Stable-Flex fiber. HPLC grade n-Hexane, Wisetherm heating mantle used for steam distillation, Poly Science chiller used for heat control.

2.2. Plant Material and Identification

The plant *L. laxiflorus* was collected from the Kopuz Village (2900 m) area of Bayburt Province in the northeast of Turkey in July 2010 shown Figure 1. After grinding, 50 grams of the plant was put into a Clevenger flask in a shredded form. Approximately 400 mL of water was added to the flask. The plant was subjected to water vapor distillation in the Clevenger apparatus for 3 hours. It was collected on the water by cooling it with a cooling bath at -15 °C. The essential oils obtained from the plant were taken by dissolving it with 0.5 mL of n-hexane. 2 µL were analyzed in GC-MS/FID. Also, after the plant gringing into pieces of 0.2 cm and smaller, it was crushed in a mortar and 7 g of the powdered material was weighed and placed in a 10 mL glass flask and placed in the chamber. *L. laxiflorus* was heated for 30 minutes, then the fiber (DVB/Carboxene/PDMS Stable) was immersed in the empty part of the bottle to perform the adsorption process. The adsorbed compounds were then re-



Figure 1. Image of *Lathyrus laxiflorus* subsp. *laxiflorus* plant (Kurt, 2014).

heated at the injection port, desorbed, and introduced into the column and analyzed.

2.3. Chromatographic Studies

Gas chromatographic analysis was carried out using a RTX 5M capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness; Restek, USA). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The temperature program for the GC oven began at 60°C, held for 2 minutes, then increased to 240°C at a rate of 5°C/min, with a final 5-minute hold at 240°C. The injector and detector temperatures were set at 250°C and 230°C, respectively. Mass spectrometry was performed with an ionization voltage of 70 eV. Compound identification was achieved by comparing the obtained mass spectra with reference spectra of known standards. For SPME studies, the fiber was exposed to heat in the headspace of the bottle for 30 min. This time was determined to achieve the highest aroma compound concentration while maintaining a stable qualitative composition of the volatile profile. Following the extraction period, the SPME fiber was placed in the GC injector. Analysis was performed with a total run time of 62 min (Ulaş Çolak et al. 2018).

2.4. Antimicrobial Studies

All microorganisms used for antimicrobial activity testing were obtained from Refik Saydam Hıfzısıhha Institute. These microorganisms are; Gram Negative Bacteria: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, Gram Positive Bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* RSKK709, (Mycobacteria): *Mycobacterium smegmatis*, ATCC 607, Fungi (Yeast): *Candida albicans* ATCC 60193. The antimicrobial activities of the isolated essential oils were determined using the agar-gel diffusion method. After each

microorganism was kept in Mueller Hinton (MH) (Difco, Detroit, MI) broth for a while, it was diluted to form approximately 106 colonies per milliliter. Then, it was inoculated onto Mueller Hinton Agar (MHA) 24 and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) and dried. Only SDA was used for *Candida tropicalis* microorganism. 5 mm wells were cut from the agars using a sterile cork drill and 50 µl of sample was placed in these wells. The plates were incubated at 35 °C for 18 hours. Antimicrobial activity was determined by measuring the inhibition areas against the test organisms. Ampicillin and Fluconazole (10 µg) was used as positive control for bacteria, was used as a positive control for fungi and dimethylsulfoxide as the solvent (Kolaylı et al. 2016, Ulaş Çolak et al. 2025).

3. Results

3.1. GC-MS/FID Analysis Results

The percentage yields of the oils from *L. laxiflorus* calculated on a moisture-free basis were 0.22%. The number of volatile components was 27 for *L. laxiflorus* shown in Table 1. When evaluated in terms of main components, of *L. laxiflorus* consists of 11 monoterpenes (27.03%), 14 monoterpenoids (69.49%), 1 sesquiterpene (0.14%), 1 sesquiterpenoid (0.20%) compounds. It was observed that the main components belong to the monoterpenoid and monoterpene classes. As a result of this study, 96.86% of the components were elucidated with the GC-MS/FID method. With the GC-MS/FID Camphor (%44.60) is found most abundant compound in *L. laxiflorus*. The chemical class distribution of compounds obtained from the GC-MS/FID analysis for *L. laxiflorus* is shown in Table 2. MS and FID chromatograms are shown in Figure 2.

3.2. SPME-GC-MS/FID Analysis Results

The identity, retention index, and the percentage composition of SPME-GC-MS/FID analysis of *L. laxiflorus* are presented in Table 1. SPME (Solid-Phase Microextraction) enabled the analysis of the entire plant material under uniform conditions, despite its complex composition something that other extraction methods might not achieve consistently. By utilizing a nonpolar poly(dimethylsiloxane) (PDMS) phase, a range of compounds including terpenoid hydrocarbons, alcohols, cyclic ethers, and esters were effectively extracted. The retention indices of the components were verified by comparing with literature data. As a result of the analysis of the *L. laxiflorus* obtained by the method, 30 volatile compounds were detected. When evaluated in

Table 1. Compounds found as a result of GC-MS/FID and SPME-GC-MS/FID analyses.

No	Compounds	GC-MS/FID		SPME-GC-MS/FID		RI	KI	Class
		%S	%Area	%S	%Area			
1	α -thujene	98	5.99			938	931	Monoterpene
2	α -pinene	97	1.71	97	2.60	945	939	Monoterpene
3	2,4-thujadiene	96	3.83			955	953	Monoterpene
4	Camphene	97	9.64	97	3.16	957	946	Monoterpene
5	Sabinene	97	0.55	97	2.76	973	969	Monoterpene
6	β -pinene	97	0.5	97	11.04	978	974	Monoterpene
7	Myrcene			96	12.85	997	991	Monoterpene
8	α -phellandrene	96	0.15	95	0.27	1009	1002	Monoterpene
9	Δ -3-carene			97	1.09	1020	1008	Monoterpene
10	α -terpinene	98	0.57			1020	1014	Monoterpene
11	p-cymene	95	2.89	85	0.61	1028	1020	Monoterpene
12	Thujol	88	2.75			1036		Monoterpenoid
13	β -phellandrene			95	16.28	1038	1031	Monoterpene
14	<i>trans</i> - β -ocimene			97	1.86	1042	1044	Monoterpene
15	γ -terpinene	97	0.96	94	0.62	1066	1054	Monoterpene
16	<i>cis</i> -sabinene hydrate			93	0.25	1076	1068	Monoterpenoid
17	Terpinolene	93	0.24	87	0.57	1095	1086	Monoterpene
18	Linalool	97	0.66	98	1.74	1102	1095	Monoterpenoid
19	Phellandral	95	0.45			1114		Monoterpenoid
20	<i>trans</i> - p-menth-2-en-1-ol	93	0.53			1135	1136	Monoterpenoid
21	Camphor	98	44.60	97	0.50	1148	1141	Monoterpenoid
22	Borneol			97	1.06	1167	1165	Monoterpenoid
23	<i>cis</i> -pinocarveol	95	1.42			1178	1182	Monoterpenoid
24	4-Terpineol	96	2.41	94	0.35	1189	1177	Monoterpenoid
25	p-cymen-8-ol	93	0.27			1194	1183	Monoterpenoid
26	α -terpineol	97	0.39	91	0.65	1193	1186	Monoterpenoid
27	<i>trans</i> -piperitol	95	0.23	95	0.40	1214	1027	Monoterpenoid
28	Isobornyl formate			96	6.60	1239	1235	Monoterpenoid
29	<i>trans</i> -chrysanthenyl acetate	96	11.78			1246	1235	Monoterpenoid
30	Chrysanthenone	84	0.35			1262		Monoterpenoid
31	Isobornyl acetat	88	3.13	97	2.60	1289	1283	Monoterpenoid
32	Carvacrol	97	0.52			1301	1298	Monoterpenoid
33	Δ - elemene			93	0.33	1347	1339	Sesquiterpene
34	α - copaene				5.05	1380	1374	Sesquiterpene
35	β -bourbonene			96	1.06	1394	1387	Sesquiterpene
36	β -elemene			96	2.46	1396	1389	Sesquiterpene
37	<i>trans</i> -caryophyllene	95	0.14	97	3.97	1434	1438	Sesquiterpene
38	α -humulene			98	1.71	1469	1452	Sesquiterpene
39	γ - curcumene			85	0.28	1488	1481	Sesquiterpene
40	Germacrene D			94	2.23	1495	1484	Sesquiterpene
41	δ -cadinene			97	1.87	1535	1522	Sesquiterpene
42	Juniper camphor	91	0.2			1688	1691	Sesquiterpenoid
Total			96.86		86.82			

%S: Similarity percentage, RI: Retention index, KI: Kovats index.

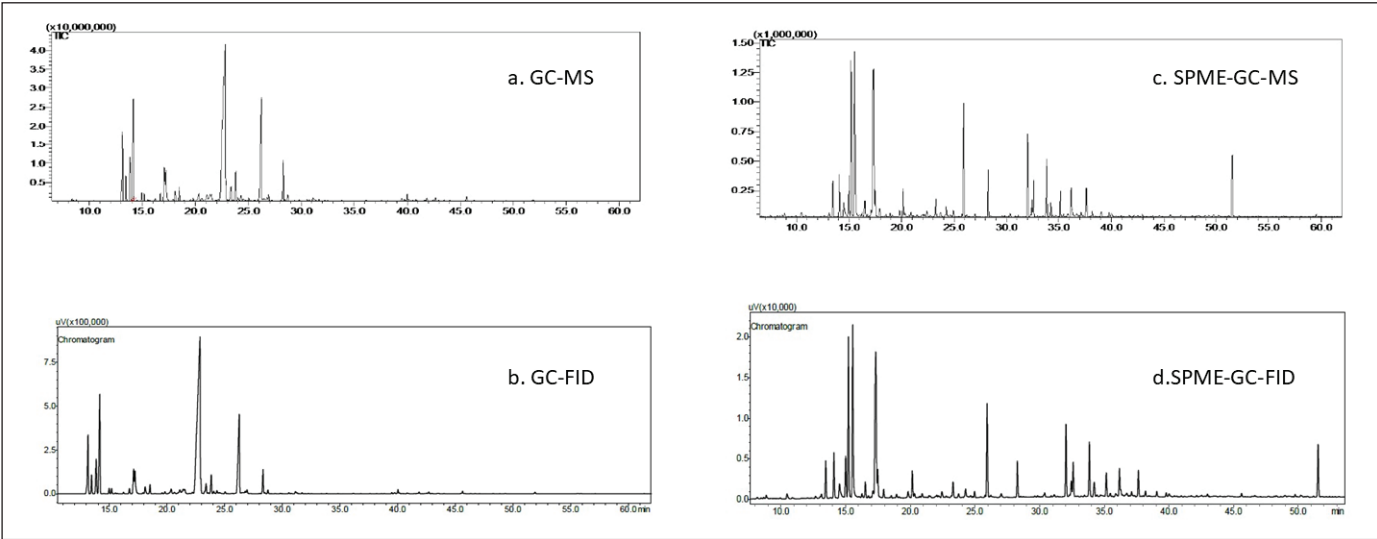


Figure 2. GC-MS/FID and SPME-GC-MS/FID chromatograms.

Table 2. Chemical class distribution of major compounds found as a result of GC-MS/FID and SPME- GC-MS/FID analyses

Compound Class	GC-MS/FID				SPME-GC-MS/FID			
	Number of Compound (Total)	Main Component	% Area	RI	Number of Compound (Total)	Main Component	% Area	RI
Monoterpene	11 (27.03 %)	Camphene	9.64	946	12 (53.71 %)	β-phellandrene	16.28	
Monoterpenoid	14 (69.49 %)	Camphor	44.60	1246	9 (14.15 %)	Isobornyl formate	6.60	
Sesquiterpene	1 (0.14 %)	trans-caryophyllene	0.14	1434	9 (18.96 %)	α-copaene	5.05	
Sesquiterpenoid	1 (0.20 %)	Juniper camphor	0.20	1688	-	-	-	-
Total	96.86%				86.82 %			

terms of main components, of *L. laxiflorus* consists of 12 monoterpenes (53.71%), 9 monoterpenoids (14.15%), 9 sesquiterpene (18.96%).

As a result of this study, 86.82% of the components were elucidated with the SPME-GC-MS/FID method. With the SPME-GC-MS/FID β-Phellandrene (16.28%) is found most abundant compound in *L. laxiflorus*.

By using multiple methods together, it becomes easier to determine what is present in what proportions in the structures of plants that contain thousands of natural compounds. The volatile oils in the plant content cannot be determined accurately due to leaks that may occur with Clevenger extraction. With the analysis performed with the solid phase microextraction method, the adsorption of volatile molecules directly attached to the fiber reduces the possibility of leakage and effectively ensures the correct determination of the content.

3.3. Antimicrobial Activity Results

It is seen in Table 3 that the essential oils obtained from the species have good antimicrobial activity. The antimicrobial activity of *L. laxiflorus* essential oil was evaluated against a panel of Gram-positive and Gram-negative bacteria, as well as one fungal strain. The results indicated a selective inhibitory effect. The strongest activity was observed against *E. coli* (33 mm) and *B. cereus* (15 mm), while moderate inhibition was detected against *Y. pseudotuberculosis* (9 mm) and *M. smegmatis* (15 mm). Interestingly, *S. aureus* and *E. faecalis*—both Gram-positive bacteria—were not inhibited, nor was *C. albicans*. In the antimicrobial activity study of bacterial and fungal species, it was observed that the oil obtained from whole *L. laxiflorus* exhibits stronger inhibitory potential against Gram-negative bacteria than Gram-positive strains

Table 3. Antimicrobial activity results of essential oil.

Sample	Conc. (µg/mL)	Microorganisms and inhibition zones						
		Ec	Yp	Sa	Ef	Bc	Ms	Ca
<i>L. laxiflorus</i>	33	9	18	-	-	-	15	-
Ampicillin	10	10	18	35	10	15	10	-
Fluconazole	10	-	-	-	-	-	-	10

Ec: *Escherichia coli* ATCC 25922, **Yp:** *Yersinia pseudotuberculosis* ATCC 911, **Sa:** *Staphylococcus aureus* ATCC 25923, **Ef:** *Enterococcus faecalis* ATCC 29212, **Bc:** *Bacillus cereus* RSKK709, **Ms:** *Mycobacterium smegmatis* ATCC607, **Ca:** *Candida albicans* ATCC 60193.

4. Conclusion and Suggestions

A total of 42 volatile compounds were identified, predominantly belonging to the monoterpene and sesquiterpene classes, while diterpenes were detected in trace amounts. Among these, camphor (44.60%, Clevenger) and β -phellandrene (16.28%, SPME) were determined as the major constituents, representing the first detailed report of such compounds as dominant in this species. The volatile profile was mainly composed of monoterpene and sesquiterpene compounds, with diterpenes occurring at very low levels. While several studies have addressed phytochemical profiles, antioxidant properties, and morphological traits of *Lathyrus* species, essential oil research on this taxon remains notably scarce. The present study provides the first comprehensive report on the essential oil composition of *Lathyrus laxiflorus* subsp. *laxiflorus* using both Clevenger hydro distillation and SPME-GC/MS methods in parallel, alongside antimicrobial activity screening against a broad spectrum of microorganisms. Strong inhibition was recorded against *E. coli* (33 mm) and *B. cereus* (15 mm), while moderate effects were observed on *Y. pseudotuberculosis* (9 mm) and *M. smegmatis* (15 mm). No inhibition was noted against *S. aureus*, *E. faecalis*, or *C. albicans*. These findings indicate that the oil has a stronger inhibitory potential against Gram-negative bacteria compared to Gram-positive strains and fungi.

Previous phytochemical investigations on *Lathyrus* have largely focused on phenolic compounds, fatty acid composition, seed storage proteins, and flavonoid profiles (Bağcı and Şahin 2004, Kıran Acemi 2020). For instance, (Porter et al. 1999) examined flavonoid patterns within the genus, while (Bağcı and Şahin 2004) performed a chemotaxonomic evaluation of fatty acids, reporting linoleic and oleic acids as major components. However, these works did not explore volatile terpenoids.

Studies involving volatile analyses in the genus are extremely limited and mostly restricted to floral scents (Porter et al. 1999) or targeted metabolite profiling of seeds via GC-MS

(İbrahim et al. 2021). In contrast, the present work identified 27 compounds via hydro distillation and 30 compounds via SPME, classifying them into monoterpenes, monoterpeneoids, sesquiterpenes, and sesquiterpenoids. Notably, Camphor (44.60%, Clevenger) and β -phellandrene (16.28%, SPME) emerged as dominant constituents—compounds either absent or present in trace amounts in earlier *Lathyrus* reports.

From a methodological perspective, this dual extraction-comparison approach (Clevenger vs. SPME) has not been previously applied to *Lathyrus* species, offering new insight into how extraction technique influences volatile profile representation. This is especially important for detecting low-boiling-point compounds more efficiently with SPME, as shown by the higher monoterpene proportion in SPME results.

In terms of biological activities, while antimicrobial effects of *Lathyrus* extracts have been reported—often linked to phenolic or alkaloid content—no prior study has documented essential-oil-based antimicrobial activity for *L. laxiflorus*. The present findings demonstrate selective inhibition, particularly against Gram-negative bacteria, aligning with general trends observed for certain volatile-rich plant oils, but differing from most phenolic-rich *Lathyrus* extracts which tend to favor Gram-positive inhibition.

Recent studies have shown that combining hydrodistillation and SPME-GC-MS provides a more complete picture of plant volatiles. Hydrodistillation delivers the bulk essential oil suitable for chemical and biological testing, whereas SPME captures headspace volatiles, especially low-boiling compounds often missed in distillation. Beyond chemical profiling, these approaches have also been applied to enzyme inhibition assays, demonstrating activities against acetylcholinesterase and butyrylcholinesterase (linked to neuroprotection), tyrosinase (cosmetic and food relevance), and α -amylase/ α -glucosidase (antidiabetic relevance) (Şahin et al. 2024, Şener et al. 2023, 2024).

Our results on *L. laxiflorus* highlight the benefit of applying both methods in parallel. Although enzyme inhibition assays were not performed in this study, the essential oil showed selective antimicrobial activity, particularly against Gram-negative bacteria. This suggests that adding enzyme-based evaluations in future work could broaden the functional profile of *Lathyrus* essential oils.

Taken together, the findings position *L. laxiflorus* as a potential natural source of bioactive volatiles. Future studies should move beyond compositional analysis to include activity-guided isolation of major compounds and clarification of their mechanisms, with possible applications in pharmaceuticals, cosmetics, and food technology.

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Author contribution: Nevin Ulaş Çolak: Conceived and designed the experiments, performed experimental studies, analyzed data, and wrote/edited/revised the manuscript. Ahmet Yaşar: Contributed to the conceptualization of the study. İrem Kurt: Assisted in carrying out experimental procedures. Kamil Coşkunçelebi: Provided and identified the plant material.

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