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Preliminary results regarding the essential oil extraction yield and the compositional diversity obtained from 5 lavender cultivars growing in Vinga fields, Arad County.

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Abstract:
In this study five cultivars of *Lavandula angustifolia* Mill grown under identical conditions specific to the year 2023 were analyzed and evaluated. They were selected from a collection of 40 different lavender varieties based on phenological traits, environmental resilience, geographical origin, and precocity. The chosen cultivars were Munstead, Silver Mist, Miss Katerina, Grosso, and Edelweiss, the last two being lavender hybrids. The harvested inflorescences were processed under identical conditions for the extraction of essential oil by hydro-distillation, followed by chemical composition analysis using gas chromatography (GC-MS). Silver Mist exhibited the highest extraction yield of 46.66 ml/kg dried flower, while Munstead had the lowest at 21.5 ml/kg dried flower. The chemical composition is varied, with high values determined for the compounds: linalool, 4-Terpineol, linalyl acetate, lavandulol, 4-carvomenthenol, bergamot oil, and camphor. Silver Mist is notable for specific compounds such as beta-Linalool, camphor, and nerol-acetate. The compositional variations among the different cultivars reflect both genetic diversity and the chemical characteristics of lavender. These differences allow for the classification of varieties into distinct chemotypes, which grants each essential oil a specific use, whether in aromatherapy, the cosmetics industry, or pharmaceutical applications.

Keywords: growing parameters, chemotypes, GC-MS, essential oil yield, steam distillation

Introduction

Essential oils extracted from aromatic and medicinal plants have been used since ancient times for their recognized therapeutic benefits. Aromatic and medicinal plants are also a valuable source of essential oils, having been recognized since ancient times for their various therapeutic properties. It is widely used in the cosmetic industry for the manufacture of lotions for skin care and cleansing, scented soaps, and perfumes. [1, 2]. Species Lavandula angustifolia, commonly known as lavender, belongs to the Lamiaceae family and the genus Lavandula, which includes about 30-40 different species. [3, 4]. Lavender is a shrub that reaches a height of 20 to 80 cm and grows in sunny and mountainous regions. Although it was a very widespread plant in antiquity, it is assumed that the Middle East was one of the areas of origin of the first lavender species. Lavender is now found all over the world, from the Canary Islands, throughout Europe, in North and Northeast Africa, to Asia India, and even in the United States. [5, 6]. It is also cultivated in the southwestern part of our country [7]. "The essential oil (EO) of Lavandula angustifolia has been used for centuries for therapeutic purposes to treat various conditions, including as a remedy for ulcers." [8], This is a plant with multiple uses that benefit the human body, including scalds [9], rheumatism [10], and relieving nerve pain. [10, 11]. In addition, it is recommended for treating colds [12], coughs [13], diarrhea [14], and respiratory conditions such as asthma, fatigue or during convalescence [14, 15, 16]. It is also used to alleviate symptoms of depression. [17], but also to reduce stress, anxiety, and insomnia. [18, 19]. It is recognized from a medicinal perspective for its strong antibacterial effects [20, 21], with anti-inflammatory and analgesic properties, being particularly effective in modulating pain and inflammation by inhibiting enzymatic activity [22]. Given the proliferation of resistance and the reduced number of antibiotics under development, the identification of new antibacterial agents has become crucial. [23]. This study focuses on identifying the chemical composition of the essential

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oil (EO) of Lavandula angustifolia, obtained from the aerial part of the plant, extracted at the Interdisciplinary Research Platform at the University of Life Sciences, "King Mihai I" of Timișoara.

This study provides relevant data on the compositional structure of lavender essential oils produced by different genotypes grown under identical conditions, offering the possibility of classifying them into structural chemotypes determined by the predominance of the bioactive compound.

Material and Methods

The study was carried out on the experimental site initiated for lavender cultivation within the family farm "Lavanda De Vis," located in the perimeter of Vinga locality, Arad County (Figure 1). The subject of study was five genotypes of Lavandula angustifolia Mill. cultivated in 2023, under an organic farming system in a randomized complete block design, for which, in the first phase, the yield of essential oil extraction was estimated.

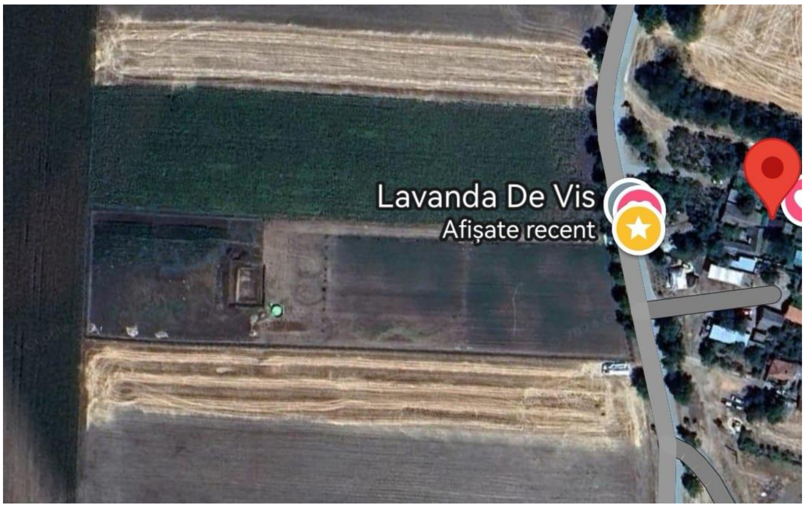

Figure 1. Experience location at Lavanda De Vis farm, 46˚00'37.3" N /21˚07'20.2" E

The experiment was established in 2019, using cuttings planted at 1.4 between rows and 1 m per row, with 5 lavender cultivars belonging to Lavandula angustifolia Mill. And Lavanda X intermedia (hybrid obtained between Lavandula angustifolia and Lavandula latifolia. The experimental design involved altering the five cultivars (1-5) arranged in three blocks with 3 repetitions. The size of the plot was 4.2 m2, figure 2.

Seedling care involves applying fertilization, irrigation, and bush-forming pruning, at least five times in the first year. Although lavender is drought-resistant, we carried out frequent watering in the first weeks after planting. Subsequently, we reduced irrigation, applying a watering rate of 1/month, thus ensuring a balance between water requirements and the plant's adaptation to climatic conditions. Being an ecological crop, we applied exclusively organic fertilizers, using fertilizers organic fertilizers with gradual release of phosphorus and potassium, which support plant growth and flowering. We used well-matured compost between 1-2 kg of compost per square meter, incorporated into the soil before planting, but also in the vegetation around the plant in an amount of 0.5 kg in a thin layer (1-2 cm) thus ensuring a continuous and natural source of nutrients.

To support soil fertility, we sowed clover and turf throughout the area. We periodically mowed the grass and clover, letting the plant debris decompose naturally on the soil, thus providing a uniform green manure and helping to improve the structure and fertility of the land.

The flowers were harvested in the morning when they were about to open, to obtain an intense fragrance and a superior quality of the essential oil. Approximately 70% of the flowers on the inflorescence were open at harvest, thus ensuring a strong aroma and a valuable harvest.

The cultivation of lavender in western Romania, especially in regions such as Banat, has experienced rapid development due to favorable environmental conditions – warm summers, mild winters, and well-drained calcareous soils, similar to those in the Mediterranean area. The increased interest in this crop has led to the creation of plantations in counties such as Timis and Arad, which stand out both on the local market and in exports. Romanian growers have started to introduce varieties adapted to the regional climate, and Romanian lavender essential oil is attracting attention in Europe due to its quality and organic cultivation methods. The soils in western Romania are usually calcareous, well-drained, and have a light texture, which favors root growth and prevents water stagnation, an important aspect of lavender. In addition, the climate of this region, with hot summers and mild winters, provides the necessary conditions for optimal development of the plant, similar to those of the Mediterranean area. These characteristics make western Romania a favorable area for growing lavender and for obtaining quality essential oil.

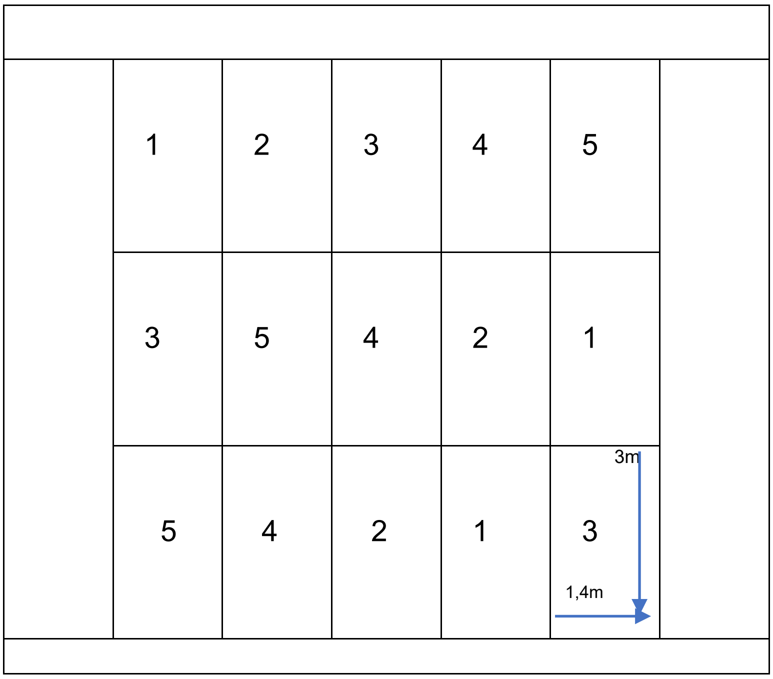

Figure 2. **Location of lavender plots. 1 - cultivar MUNSTEAD; 2 - cultivar SILVER MIST, 3 - cultivar MISS KATERINA, 4 - cultivar GROSSO, 5 - cultivar EDELWEISS**

Changes in minimum, maximum, and average air temperatures and total precipitation were recorded by a weather station located in the Vinga commune, Arad County 46°03′N, 21°17′E.
Temperature and relative humidity: Minimum temperatures in the winter months, such as January and December, are around 0°C or even slightly below zero, while in the summer they remain high, ranging between 15-20°C. Maximum temperatures in the summer season, especially in July and August, reach 30-35°C, and in the winter they remain lower, around 5-10°C.

Precipitation and Clouds: In the summer months, the humidity sometimes drops to values of around 40-50%. In the winter and autumn months, the relative humidity frequently increases to 80-90% or even close to 100%.

Laboratory Methodology

The inflorescence of the *Lavandula angustifolia* species was harvested in July 2023 at full bloom, on sunny days. After the flowers harvested were stored in a dark, well-ventilated, and cool place, to maintain the freshness and quality. Each flower sample, which included only the flowers, without stems and leaves, was collected from a minimum of 10 different plants, and the data presented represents the average of three replicates that were sorted according to each variety and kept in separate containers to avoid mixing varieties. At this stage, each batch was labeled and properly prepared for the distillation process, so that the unique characteristics of each variety were preserved in the extracted oil. This careful preparation method ensures efficient extraction and maintains the high quality of the essential oil obtained. Subsequently, the prepared flowers were subjected to a steam distillation process in a still. In this process, hot steam was passed through the floral mass, gradually releasing the essential oils. The oil-laden steam vapors were then condensed, thus separating the essential oil from the residual water. This process allowed the extraction of pure oils, maintaining the aromatic characteristics specific to each lavender variety.
Steam distillation is the basic method for extracting lavender essential oil, applicable to both the fresh plant and the dried flowers. In the case of dried flowers, the process is similar: steam is passed through the plant mass, releasing the essential oils, which are then collected by condensation. The yield obtained from dried

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flowers can vary slightly compared to that of fresh plants, but generally remains between 1% and 3%, depending on the lavender variety, the quality of the harvest, and the growing conditions.

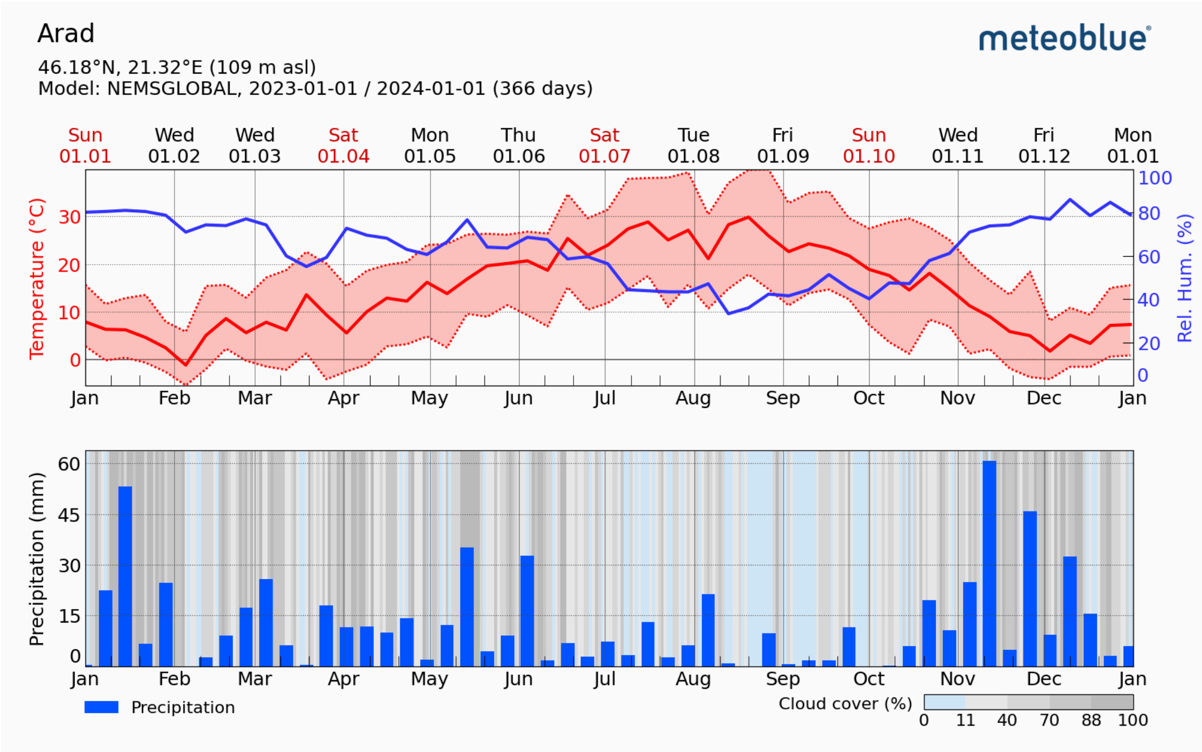

Figure 3. Climatic information for the experimental year, including temperature values (minimum, maximum, and average) and precipitation recorded at the Arad weather station; ***https://ww.meteoblue.com/en/weather/historyclimate/weatherarchive/arad_România_686254

Results and Discussion

The yield of lavender oil varies depending on the cultivar and the time of harvest. Lavandula angustifolia (English lavender) has a lower yield of about 1-1.5% but produces a higher quality oil, valued for its delicate aroma. In contrast, hybrids such as Lavandula x intermedia can reach yields of 3-6%, but the oil obtained contains more camphor, giving it a more intense and distinct aroma than classic lavender.

GC-MS (Gas Chromatography-Mass Spectrometry) is an analytical technique commonly used to identify and analyze the chemical components of Lavandula (lavender) essential oil. The process involves separating volatile compounds from the essential oil by gas chromatography and analyzing them by mass spectrometry, providing a detailed profile of the main chemical constituents, such as linalool, linalyl acetate, camphor, terpinene-4-ol, and cineole.

The essential oil was extracted by distillation of the dried flowers, with three repetitions for each variety. In the table below, we present the average values obtained. The amount of essential oil was calculated relative to 1kg of dried flower, and the results indicated values presented in Table 1.

Table 1. Extraction yield expressed by the amount of essential oil per 300 g or kg of dried lavender flower

Lavender cultivars	Average values for extraction yield ml EOs/300 gr dried flower $x + s_x$	Oil extraction yield ml EOs / kg dried flower $x + s_x$
Lav. ang. MUNSTEAD	6,47 + 0,5	21,57 + 1,6
Lav. ang. SILVER MIST	14,00 + 0,8	46,66 + 2,4
Lav. ang. MISS KATERINA	7,94 + 0,35	26,47 + 1,1
Lav. X int.GROSSO	10,99+ 0,15	36,65 + 0,5
Lav. X int. EDELWEISS	10,48 + 0,33	34,93 + 1,0

From the analysis of the values, it is observed that the Silver Mist variety is the most productive in terms of the amount of oil obtained, the extractive yield being 46.66 ml/kg, followed by Grosso with a yield of 36.65ml/kg, Edelweiss 24.93ml/kg, Miss Katerina 26.47 ml/kg. The lowest yield was obtained for Munstead with 21.57ml/kg.

Regarding the chemical composition of the essential oils, Table 2 presents the compositional structure for each lavender cultivar studied. A total of 79 compounds were separated using GC-MS for the essential oils obtained but the compositional profile is different for each oil.

Table 2. Concentrations of lavender oil components in the lavender studied cultivars

	COMPOSITION	(1)* Conc.**%**	(2)* Conc.**%**	(3)* Conc.%	(4)* Conc%	(5)* Conc%
1	alpha.-Thujene	0,091	0,091	0,230	0,113	0,229
2	alpha.-Pinene	0,165	0,650	0,389	0,586	0,423
3	alpha -pinene epoxide	-	-	-	-	-
4	Camphene	0,107	0,790	0,069	0,375	0,119
5	1-Octen-3-ol	-	-	-	-	-
6	3-Octanone	0,776	2,311	0,485	0,037	1,220
7	beta-Myrcene	0,667	0,400	0,701	0,645	0,809
8	beta-Pinene	-	0,125	-	-	0,154
9	Butanoic acid, butyl ester	0,134	0,222	0,128	0,028	0,139
10	Acetic acid, hexyl ester	0,488	0,074	0,199	0,186	0,444
11	o-Cymene /o-cymol	0,196	0,227	0,117	0,283	0,139
12	D-Limonene	0,239	0,965	0,430	7,972	-
13	alpha-Limonene diepoxide	-	-	-	-	0,202
14	Eucalyptol	0,542	0,670	0,804	**7,927**	3,122
15	beta.-trans-Ocimene /trans-beta-ocimene	1,167	1,393	0,602	0,139	0,739
16	cis-.beta.-Ocimene /beta-cis-ocimene	0,665	0,144	0,423	0,156	1,175
17	cis-Linalool Oxide	-	-	-	-	0,308
18	2-Furanmethanol, 5ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, trans-	0,237	0,437	0,305	0,242	-
19	Linalool oxide trans	0,108	-	0,069	0,169	-
20	Linalool	**29,496**	-	**29,399**	**29,693**	**20,129**
21	Octen-1-ol, acetate	0,891	0,046	0,830	0,256	1,387
22	3-Octanol, acetate	0,159	-	0,036	ND(W/B)	0,349
23	Camphor	0,175	**18,088**	0,262	6,168	1,335
24	Propanoic acid, 2-methyl-, hexyl ester	0,123	0,135	0,073	0,204	0,078
25	4-Terpineol	4,048	-	**15,631**	3,632	0,072
26	2-Cyclohexen-1-one, 4-(1-methylethyl)-	0,518	-	0,160	0,003	-
27	Butanoic acid, hexyl ester	0,670	1,114	0,428	1,096	0,450
28	Butanoic acid,2-methyl-,hexyl ester	-	0,087	-	-	-
29	Butanoic acid,3-methyl-,hexyl ester	-	-	-	-	0,038
30	Linalyl acetate	**43,240**	-	**28,238**	**32,366**	**41,056**
31	Linalyl anthranilate	-	-	-	-	-
32	Bornyl acetate	0,145	0,179	0,028	0,032	-
33	Bornyl formate cis	-	0,201	-	-	-
34	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate	3,719	-	5,750	3,350	-
35	Epoxy-.alpha.-terpenyl acetate	0,196	-	0,038	0,152	-
36	Epoxy-.alpha.-terpenyl acetate	0,121	-	0,031	0,120	-
37	Nerol acetate	0,170	**12,246**	0,172	0,248	4,070
38	Neryl acetate	-	-	-	-	0,253
39	Geraniol acetate	0,382	0,390	0,344	0,582	-
40	Geraniol acetate trans	-	-	-	-	0,355
41	Hexanoic acid, hexyl ester	0,168	0,067	0,230	0,105	0,125

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42	3,7-Nonadien-2-ol, 4,8-dimethyl-	0,063	-	0,013	0,105	-
43	Caryophyllene	**4,105**	2,067	5,909	1,102	3,645
44	Caryophyllene oxide	1,421	0,822	0,615	0,491	0,604
45	alpha.-Bergamotene	0,156	0,233	0,161	0,112	0,132
46	alpha.-Caryophyllene	0,086	0,053	0,149	0,030	0,136
47	alpha -Phellandrene	-	-	-	-	0,102
48	alpha- Bergamotene	-	0,233	-	-	0,132
50	beta.-Farnesene	3,514	-	5,066%	0,828	
51	(Z)-beta.-Farnesene	-	1,576	-	-	2,764
51	Germacrene D	0,561	0,702	1,122	0,193	0,659
52	beta.-Sesquiphellandrene	0,074	-	0,090	0,025	-
53	gamma.-Cadinene	0,131	-	0,199	0,206	0,070
54	gamma-Terpinene	-	-	-	-	0,257
55	2-Pentadecanone, 6,10,14-trimethyl-	0,085	-	0,073	ND(Peak)	-
56	Tricyclene	-	0,040	-	-	-
57	3-Carene	-	0,096	-	-	0,067
58	3-Nonanol 1, 2	-	-	-	-	0,167
59	Terpinolene	-	0,095	-	-	0,072
60	cis-Linalool Oxide	-	0,282	-	-	-
61	beta-Linalool	-	**21,286**	-	-	-
62	Lavandulol	-	**8,887**	-	-	-
63	Borneol	-	1,798	-	-	-
64	Carvomenthenol	-	6,239	-	-	-
65	4-Carvomenthenol	-	-	-	-	**11,422**
66	p-Cymen-8-ol	-	0,149	-	-	-
67	verbenone	-	0,063	-	-	-
68	Geraniol formate trans	-	0,038	-	-	-
69	(+)-Carvone	-	0,082	-	-	-
70	Bergamiol	-	**8,200**	-	-	-
71	Isopulegol acetate	-	0,055	-	-	-
72	beta-Bisabolene	-	6,047	-	-	0,085
73	alpha-Hexylcinnamic aldehyde	-	0,139	-	-	-
74	Alpha -Humulene	-	-	-	-	0,079
75	alfa hexil cinamaldehid	-	-	-	-	0,075
76	Sabinene	-	-	-	-	0,095
77	Cryptone	-	-	-	-	0,477
78	Cumaldehyde	-	-	-	-	0,110
79	Geranyl propionate	-	-	-	-	0,131
	Total constituents	99,99	99,579	96,346	99,968	98,867

***1** -Cultivar MUNSTEAD; **2** - SILVER MIST, **3** - MISS KATERINA, **4** - *GROSS*, **5** –*EDELWEISS*

At first glance, it is observed that the main component is linalool, the concentration varying from 20.129% to 29.693%, and other significant compounds are linalyl acetate 28.238- 43.240% eucalyptol 0.542-7.927%, camphor 0.175- 18.088%,

For the MUNSTEAD cultivar, 40 compounds were identified representing 99.99%, of which the majority is Linalyl acetate (43.240%) followed by linalool (29.496%) and caryophyllene (4.105%).

For the cultivar (2) Silver mist, 46 compounds were identified representing 99.579%. As can be seen, beta-Linalool (21.286%), Camphor (18.088%), Lavandula (8.887%), and Bergamiol (8.200%) are found only in the Silver Mist variety with high values. Also, Nerol acetate is found only in the Silver Mist variety in a concentration of 12.246%, while in the other varieties, it is below 4%.

For the cultivar (3) MISS KATERINA some 40 compounds were identified representing 96.346%, we can see Linalool at 29.399%, 4-Terpineol at 15.631%, and Linalyl acetate at 28.238%.

For cultivar (4) GROSS, 40 compounds were identified representing 99.968%, we observe Linalyl acetate at 32.366%, Linalool at 29.693%, and Eucalyptol (7.927%).

For (5) Edelweiss, several 46 compounds were identified representing 98.867%, the most prevalent being Linalyl acetate (41.056%), Linalool (20.129%), which are found in all 4 cultivars followed by 4-Carvomenthenol with 11.422%, not found in any genotype.

Conclusions

Of the five varieties analyzed in this work, the SILVER MIST cultivar is best suited to produce lavender essential oil, as it has a rich inflorescence and a high content of volatile oil, but also with different compounds compared to the other varieties. The analysis of the chemical composition of lavender essential oil, carried out by the GC-MS method, revealed a total of 79 different compounds, of which linalool was identified as the predominant component, with significant values varying between 20.129% and 29.693% being determined in 4 studied cultivars. This compound plays an essential role in the characterization of the perfume and therapeutic properties of lavender. Other relevant compounds, such as linalyl acetate and camphor, showed variations, thus contributing to the aromatic and functional diversity of the essential oil.

The Silver Mist cultivar demonstrated a distinct chemical profile, with the presence of compounds such as nerol-acetate and beta-linalool, and camphor in significant proportions, suggesting that this variety is part of a distinct chemotype, with the potential to offer unique aromatic and therapeutic characteristics. Our research highlights the importance of genetic diversity in lavender cultivation and its implications in the cosmetic industry and alternative therapies.

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