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Research Paper

A Comparison Study on Chemical Profile of *Laurus Nobilis* Leaves by Using Different Extraction Techniques

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Abstract: The essential oil of *Laurus nobilis* leaves were analyzed from natural population of Tirana, located in Central Albania by using two different extraction techniques: hydro-distillation with Clevengar apparatus (HD) and Head space technique by using PDMS fiber (HS-SPME). *Laurus nobilis* can be found easy (naturally) in western lowlands of Albania up to altitudes of 1500 m (for areas with warm climates). It is easy to harvest (their leaves), and it is very popular in Albania in culinary and traditional medicine. Essential oils derived from *Laurus nobilis* have valuable pharmacological properties that have been used and continue to be investigated due to their interest properties. The samples of *Laurus nobilis* (their leaves) were taken in different stations (6 stations) of Tirana, at June 2024. The fresh and air-dried samples were the subject of two extraction processes: hydro-distillation by using Clevenger apparatus and Head space technique. The chemical composition of the essential oils for both methods were determined by using a gas chromatograph model Varian 450 GC equipped with split/spitless injector and flame ionization detector. VF-1ms capillary columns (30 m x 0.33 mm x 0.25 um) were used for separation of compounds. Monoterpenes (especially oxygenated monoterpenes) were in higher percentage in all *Laurus nobilis* samples. Terpenes that were found in higher percentage were: Cineole, Linalool, Menthol, Pulegon, Carvone, Piperthone, Thymol, beta-Caryophyllene. The chemical profile between fresh and dry leaves of Laurus nobilis was almost the same. Small differences were observed between two extraction techniques. Head space technique showed a higher percentage for compounds with lower boiling point. Also between stations there were some differences between the main compounds because of atmospheric conditions and soil composition. Chemical profile of *Laurus nobilis L.* samples from Tirana, was similar with other reported studies from Albania as well as Balkan and Mediterranean areas.

Keywords: *Laurus nobilis*, Essential oil, Hydro-distillation, Head space GC/FID

Introduction

Laurel (*Laurus nobilis*) is a large evergreen tree or shrub with green leaves. It is part of the flowering plant family Lauraceae. It grows naturally in the Mediterranean region. Laurus nobilis forests covered most of the Mediterranean countries when the climate of the region was wetter, but with the increase of temperatures during the Pliocene, the laurel forests gradually retreated and were replaced by populations of drought-tolerant plants, which are still found today. Some laurel forests can be still found today in some areas of the Mediterranean (Anzaro *et al*, 2022; Asllani, 2004; Burnie, 1995; Dafera *et al*, 2010; Kathe *et al*, 1999; Mikail *et al.,* 2013). Laurel is one of the earliest plants known and used by humans. In ancient times, the laurel was a symbol of glory, peace and security. Its leaves have been used in culinary spices to give characteristic flavors to foods. Laurel was used widely in traditional medicine because it has for treatment of some illness. Among the compounds present in laurel leaves are Eugenol (1,8-Cineole) and Limonene, which are known as active substances useful due to their antiseptic, antioxidant, digestive and anti-carcinogenic properties (Dafera *et al.,* 2010; Asllani, 2004). Fresh leaves of *Laurus nobilis* are an important source of vitamin C, which is one of the most powerful natural antioxidants capable of combating

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the action of free radicals. Vitamin C also has antiviral effects, stimulates the immune system and promotes wound healing. Also, when laurel leaves are fresh, they are a good source of folic acid, which is important for DNA synthesis and during pregnancy it prevents the appearance of birth defects in children (Anzaro *et al*, 2022; Asllani, 2004; Burnie, 1995; Dafera *et al,* 2010; Dobrosslavic, 2021). Laurel leaves also contain vitamin A, essential for maintaining vision, skin and mucous membranes. Consumption of foods rich in vitamin A protects the lungs from cancer. Also, they contain a mixture of vitamins B, such as niacin and riboflavin. Vitamin B regulates metabolism, contributes to enzyme synthesis and the functioning of the nervous system (Naser *et al*, 2020; Paparella *et al*, 2022). The mineral content it contains should be considered. Among them, in fact, can be found potassium, copper, calcium, manganese, iron, selenium, zinc and magnesium. Potassium is important for keeping blood pressure and heart rate under control. Iron is necessary to produce red blood cells. In herbal medicine, laurel leaves are known as laxative, diuretic and appetite stimulant. Bay leaf tea has been used to relieve stomach pain or abdominal pain [3-7]. The lauric acid content in its leaves is a natural remedy for insects and parasites. The components present in laurel leaves have been used to produce medical products for the treatment of arthritis, muscle pain, bronchitis and flu symptoms. Tea obtained from laurel leaves aids digestion and removes gas from the gastrointestinal tract, and it is also a useful remedy in cases of sciatica (Mikail *et al*, 2013; Stefanova, 2020)

The hydro-distillation technique is one of the most common methods used both in the laboratory and on an industrial scale to produce essential oils from aromatic and medicinal plants. For research purposes and also recognized by the Pharmacopoeia, Clevenger model hydro-distillation apparatus, is the most used. In this apparatus, continuous extraction is applied by continuously enriching the organic phase with the compounds found in the plant being extracted. When the amount of organic matter is small, an organic solvent such as toluene or hexane is used to dissolve the compounds in the organic phase. Now, this method has been modified with gases in a critical state (instead of water), such as carbon dioxide, which is more efficient than water vapor. Other methods used in the extraction of essential oils include maceration, squeezing, ultrasonic baths, and more recently, Head space techniques. These techniques are based on the isolation (extraction) of volatile compounds (flavonoids) from plants or other substances in a bottle closed at its head. Air sampling from this bottle can be done directly using a syringe (classic Head space) or using a high-porosity polymer fiber that binds organic compounds well (absorbs) (Anzaro *et al*, 2022; Asllani, 2004; Burnie, 1995; Naser *et al*, 2020; Paparella *et al*, 2022; Dafera *et al,* 2010; Dobrosslavic, 2021). In this study hydro-distillation (by using Clevenger apparatus) and HS-SPME (by using PDMS fiber) was used to determine the chemical profile of *Laurus nobilis* leaves from Tirana area, Albania.

Material and Methods

Sampling of laurel leaves

The leaves of *Laurus nobilis* were collected at 6 different stations in the Tirana area (Farkë, Mullet, Artificial Lake, Linzë, Sharrë and Kashar). The plants were collected in June 2024. At each station, the leaves of the plant were collected. The leaves were dried in the shade so as not to lose their morphological characteristics. After drying, the plant material was chopped into small pieces of 0.5-2 cm for further analysis.

Materials and reagents

High purity n-Hexane and toluene, suitable for gas chromatography, were provided by Merck (Darmstadt, Germany). Also, the mixture of n-alkanes from n-octane (C8) to eicosanes (C20) that was used to calculate the Kovats indices (KI) was provided by the Merck catalog.

Chemical profile of *Laurus nobilis* by using HS/SPME technique

5 g of the laurel leaves were placed in a SPME bottle with a volume of 10 ml. The bottles are equipped with Teflon stoppers suitable for their analysis by means of the Head-space technique. The manual SPME syringe equipped with a 100 um PDMS (Polydimethyl siloxane) fiber was inserted through the Teflon stopper into the top of the sample. The bottle was placed in Banjo Mari at a temperature of 50oC for 40 minutes. After the adsorption process, the syringe (PDMS fiber) is transferred to the Varian 450 gas

chromatograph equipped with FID detector where their injection is performed (desorption process at 280oC for 20 seconds). The separation of volatile compounds of sage will be carried out in the VF-1ms column (Paparella *et al*, 2022).

Isolation of essential oils by using Clevenger apparatus

Dried and crushed laurel leaves from the Tirana area (50 g of plant material) were subjected to hydro-distillation for 4 hours without interruption using a Clevenger-type apparatus (recommended by European Pharmacopeia, 2014) for the isolation of the essential oil. The essential oil was collected in 2 ml of toluene as the extraction solvent. The extract was dehydrated by adding 1 g of anhydrous sodium sulfate. It was stored in dark vials at +4oC. The essential oil of *Laurus nobilis* was subjected to analysis using the GC/FID technique (Awada *et al*, 2023; Daferra *et al*, 2000; David *et al*, 2010).

Apparatus and gas chromatographic analysis

Gas chromatographic analysis of the essential oil of *Laurus nobilis* was performed on a Varian 450 GC apparatus, equipped with a PTV injector and a flame ionization detector (FID). The injector and detector temperatures were set at 280 ºC and 300 º C, respectively. 2 µl of the essential oil dissolved in toluene was injected in split mode (1:50). Nitrogen was used as carrier gas (1 ml/min) and as 'make-up gas' (25 ml/min). Hydrogen and air were detector flame gases at 30 ml/min and 300 ml/min respectively. A VF-1ms capillary column (30 mx 0.33 mm x 0.25 mu) was used to isolate the essential oil compounds. The identification of the compounds was based on the comparison of the exit times (RT) with the Kovats indices, which together with the literature data were used to identify the main compounds of laurel. The quantitative data of the analyzed compounds are given in % against the total peak areas without calculating the toluene peak area (Adams, 1995; Asllani, 2014; Dafera *et al,* 2000; Koing et al, 1999). Figure 1 shown chromatograms of essential oils for laurel leaves obtained by hydro-distillation and Head space techniques.

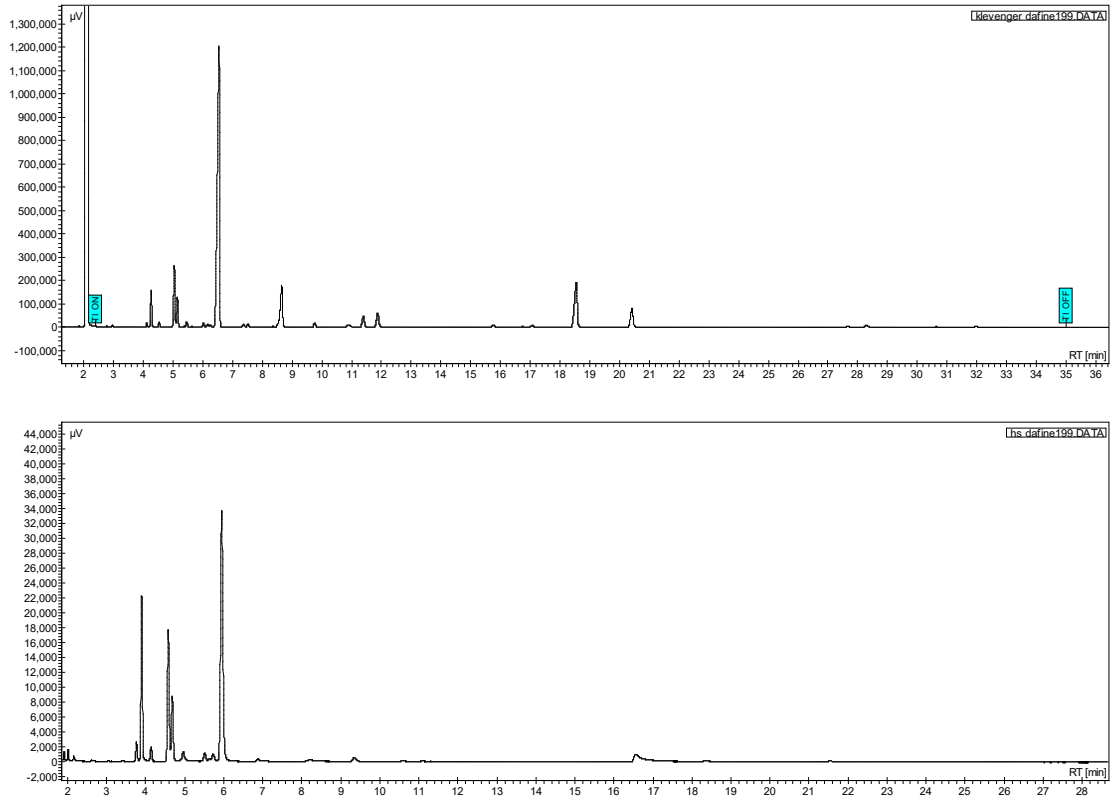

Figure 1. Chromatograms of essential oils for laurel leaves using the hydro-distillation method (above) and Head space (below)

Results and Discussions

The GC/FID analyses of laurel leaves by using the hydro-distillation technique (Clevenger apparatus), it showed that in the chromatograms were 40-60 compounds, while for the same samples but analyzed with Head space technique, their number was 75-100 compounds. The analysis included 28 main compounds that constitute about 95.6% of the total compounds using the Clevenger technique and 93.5% of the total compounds using the Head space technique. The main compounds that were identified in the majority in all samples, for both methods, were: Cineol (Eucalyptol), Sabinene, alpha-Pinene, beta-Pinene, and alpha-Terpinyl acetate. Table 1 gives the average percentages of the components analyzed from laurel leaves for both methods. Piks (compounds) with an area lower than 0.01% were not considered in this study.

Table 1. Average percentages of main components for *Laurus nobilis* leaves from Tirana area

	Clevenger (Hydro-distillation)				Head space – Solid Phase Micro-Extraction			
	Mean	**Min**	**Max**	**STDEV**	**Mean**	**Min**	**Max**	**STDEV**
alfa-Thujene	0.59	0.34	0.73	0.21	1.17	0.45	1.89	1.02
alfa-Pinene	4.97	3.32	6.21	1.49	9.75	3.63	15.87	8.65
Camphene	0.68	0.51	0.95	0.24	1.03	0.45	1.61	0.82
Sabinene	8.78	6.53	12.01	2.87	7.34	0.02	14.66	10.35
beta-Pinene	2.18	0.88	3.17	1.18	6.95	6.03	7.87	1.30
Myrcene	0.43	0.18	0.80	0.33	1.49	0.83	2.15	0.93
alfa-Felandrene	0.44	0.09	1.12	0.59	0.02	0.02	0.02	0.00
alfa-Terpinene	0.22	0.07	0.52	0.26	0.17	0.11	0.22	0.08
para-Cymene	0.27	0.00	0.58	0.29	1.02	0.67	1.37	0.49
Limonene	0.27	0.00	0.72	0.39	1.14	0.47	1.80	0.94
1,8-Cineole	48.40	44.84	50.49	3.10	31.58	25.38	37.77	8.76
gama-Terpinene	0.59	0.00	0.98	0.52	0.53	0.40	0.65	0.18
Cis-Sabinenehydrat	0.17	0.00	0.47	0.26	0.25	0.25	0.25	0.00
Linaleol	0.38	0.07	0.95	0.50	0.19	0.15	0.22	0.05
Terpinen-4-ol	0.90	0.08	2.36	1.27	0.18	0.12	0.24	0.08
alfa-Terpineol	1.54	0.58	2.21	0.85	0.81	0.35	1.26	0.64
Acetat bornili	0.39	0.12	0.65	0.27	0.97	0.51	1.42	0.64
alfa-Terpinil acetat	8.04	3.94	12.44	4.26	7.34	5.62	9.06	2.43
Timol	0.92	0.31	2.08	1.00	0.32	0.31	0.32	0.01
Eugenol	2.27	1.47	2.80	0.71	0.65	0.14	1.16	0.72
Aceatt Nerili	0.25	0.14	0.33	0.10	0.43	0.25	0.60	0.25
beta-Elemene	1.27	0.47	2.57	1.13	0.27	0.24	0.29	0.04
Metil eugenol	7.96	5.32	10.32	2.51	2.51	0.91	4.11	2.26
beta-Kariofilene	1.71	0.45	4.15	2.11	0.19	0.17	0.21	0.03
Germacene D	0.46	0.11	0.78	0.34	0.16	0.12	0.19	0.05
Biciklogermacene	0.45	0.25	0.56	0.18	0.48	0.11	0.85	0.52
delta-Cadineen	0.16	0.14	0.18	0.02	6.15	1.05	11.25	7.21
Cariophyllen oksid	0.84	0.23	1.93	0.95	10.51	3.21	17.81	10.32
Total	95.57	90.04	99.73	4.99	93.55	87.53	99.56	8.51
Bicyclic monoterpenes	17.20	11.58	23.07	5.99	26.24	10.58	41.90	22.15
Monocyclic monoterpene	1.52	0.16	3.34	1.75	1.85	1.00	2.69	1.20
Aliphatic monoterpene	0.43	0.18	0.80	0.33	1.49	0.83	2.15	0.93
Oxygenated Monoterpene	60.09	49.77	69.90	10.60	41.73	32.63	50.82	12.86
Aromatic Monoterpene	11.42	7.10	15.78	4.51	4.50	2.03	6.96	3.49
Sesquiterpenes	4.06	1.42	8.24	3.78	7.24	1.69	12.79	7.85
Sesquiterpenes oxide	0.84	0.23	1.93	0.95	10.51	3.21	17.81	10.32

Hydro-distillation method with the Clevenger apparatus showed fewer compounds compared to the HS technique (Figure 1). This difference in the analyzed compounds should be mainly related to the selectivity of Toluene, the solvent used for extraction. The Head space technique has lower sample

discrimination because the number of compounds with this technique is 2 – 3 times higher. The HS technique allows the analysis of a larger number of compounds.

Figure 2 shows the chemical profile of the main compounds obtained by the hydro-distillation and Head space methods for dried laurel leaves from the Tirana area, July 2024. It is noted that the main compound found for both methods was Cineol. It was found in a higher percentage in the hydro-distillation extracts with 48.4% compared to 31.6% obtained with HS. The compounds found in higher percentages by the HD method were: Cineol, Sabinene, alpha-Terpinyl acetate, Methyl eugenol and alpha-Pinene while their profile for the HS technique was: Cineol, alpha-Pinene, Caryophyllene oxide, Sabinene, beta-Pinene, alpha-Terpinyl acetate and delta-Cadinene. It is noted that HS provides higher levels not only for compounds with low boiling points but also for compounds that have relatively high molecular mass (high boiling points) compared to HD. This should also be related to the discrimination of water vapor or toluene used during HD. Figure 2 provides chemical profiles for the classes of terpenes found in *Laurus nobilis* leaves from the Tirana area. For both extraction techniques, the highest level was for oxygenated terpenes, respectively with 60.1% for HD and 41.7% for HS. Their high level was due to the high percentage of Cineole. For HD the profile of the terpene classes was: Oxygenated monoterpenes, bicyclic monoterpenes, aromatic monoterpenes, sesquiterpenes (monocyclic, aliphatic and oxygenated sesquiterpenes) were at levels lower than 1%). For the HS technique their profile was different because after the oxygenated and bicyclic monoterpenes were oxygenated sesquiterpenes, sesquiterpenes and aromatic monoterpenes (also for this technique the percentages of monocyclic and aliphatic monoterpenes were lower than 1%). This is related to the preference/discrimination that the different extraction techniques have for some of the compounds and the classes to which they belong. In Figure 4 the respective percentages for bicyclic monoterpenes (alpha-Thujene, alpha-Pinene, Camphene, Sabinene and beta-Pinene) for both HD and HS methods are given. For the HS method it should be said that there is a higher percentage of them of 26.2% compared to HS with 17.2%. This is expected because these compounds are the first to appear in the chromatogram due to their low boiling points and have high evaporation which favors the HS technique. Their profile was also different, for HD it was: Sabinene, alpha-Pinene, beta-Pinene, camphene and alpha-Thujen while for HS it was: alpha-Pinene, Sabinene, beta-Pinene, alpha-Thujen and Camphene. These differences are also not directly related to their percentages but to the extraction techniques, their preferences/discrimination towards some compounds and the way of reporting the result in percentage against the total points (We recall that for HD the number of identified compounds was smaller than HS). Similarly, differences were also observed in the profiling of monocyclic monoterpenes (Figure 5). Their profile for HD was: gamma-Terpinene, alpha-Phelandrene, Limonene and alpha-Terpinene while for HS their profile was: Limonene, gamma-Terpinene, alpha-Terpinene and alpha-Phelandrene. Methyl Eugenol was found as the most abundant aromatic monoterpene both in the HD technique and in the HS technique. Its presence in HS was much greater, a fact that can be justified by the solubility of phenols in water while their boiling points are relatively high and could be less preferred in the HS technique which is obvious from the levels of Thymol and Eugenol. The situation changes for para-Cymene which has a lower boiling point and does not have an OH group making it more preferred for HS versus HD. The polarity that the OH group gives to the molecule makes it more attractive for HD. This is also reflected for the oxygenated monoterpenes (Figure 7) which have higher levels of Cineol, alpha-Terpinyl acetate, alpha-Terpineol, Terpinen-4-ol, linaleol, Neryl acetate, Sabinen hydrate. Also the presence of this group affects the increase of boiling points making them less volatile and more discriminated by the HS technique. The increase of molecular mass for sesquiterpenes makes them less volatile and in general (beta-Elemene, beta-Caryophyllene, Germacene, Bicyclogermacene) appear in higher percentage in the HD technique. The only difference is delta-Cadinene identified at a higher level for the HS technique. As for Myrcene (representative of aliphatic terpenes) it was found in an amount less than 0.5% for both methods and without a substantial impact on the overall profile. Data obtained from HD and HS analyses of dried laurel leaves from the Tirana area showed that their chemical composition was the same as those reported in other works from other areas of Albania, the Balkans or the Mediterranean (Asllani, 2014; Caputo et al, 2017; Daferra et al, 2000; Hajlauoni et al, 20008; Fantasma et al, 2024; Fidan et al 2019; Stefanova et al 2020; Naser et al, 2020).

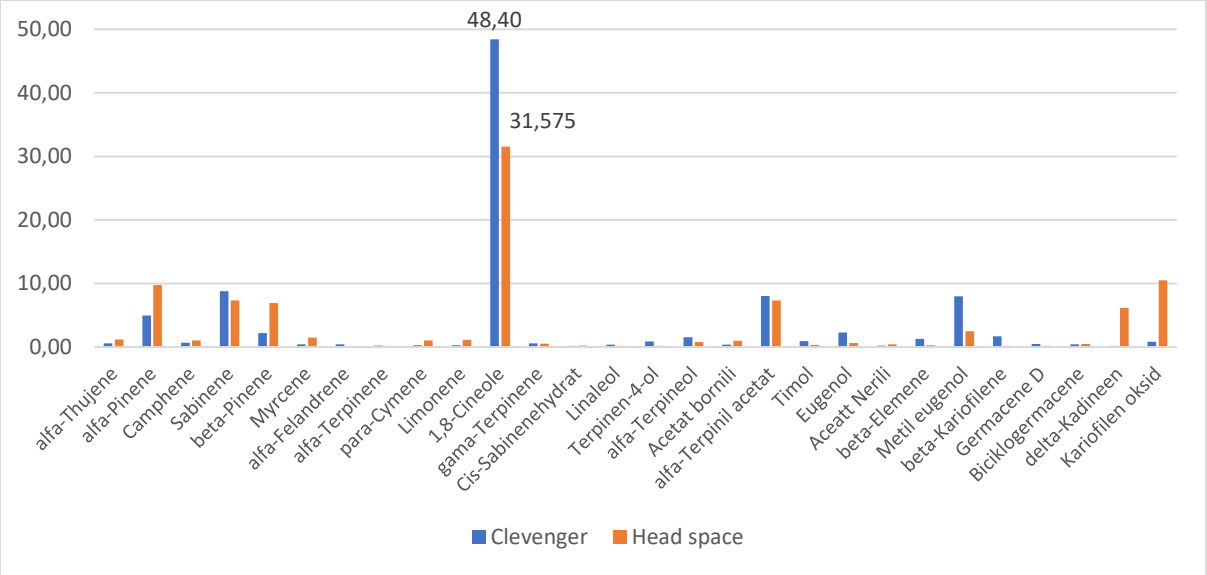

Figure 2. Chemical profile of terpenes in laurel leaves from Tirana area, June 2024

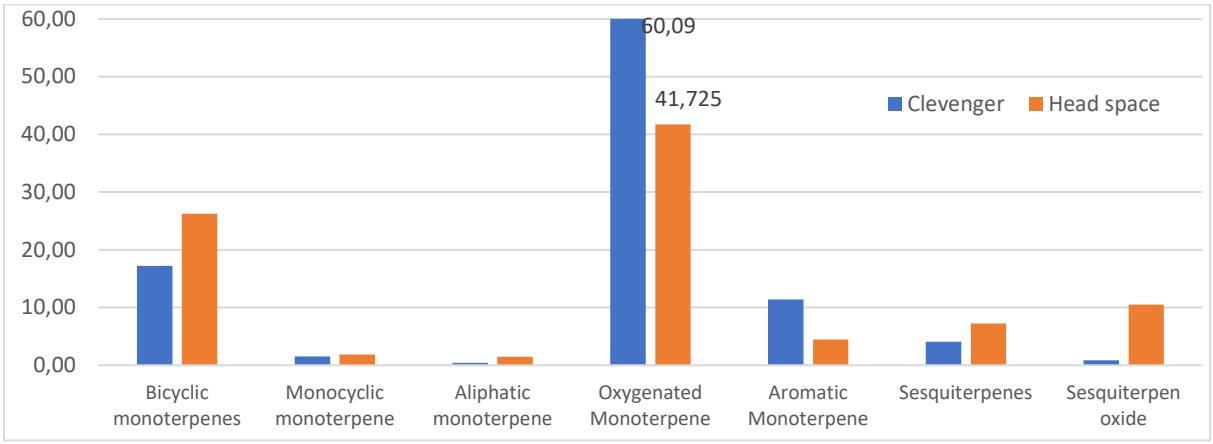

Figure 3. Terpene classes in laurel leaves from Tirana area, June 2024

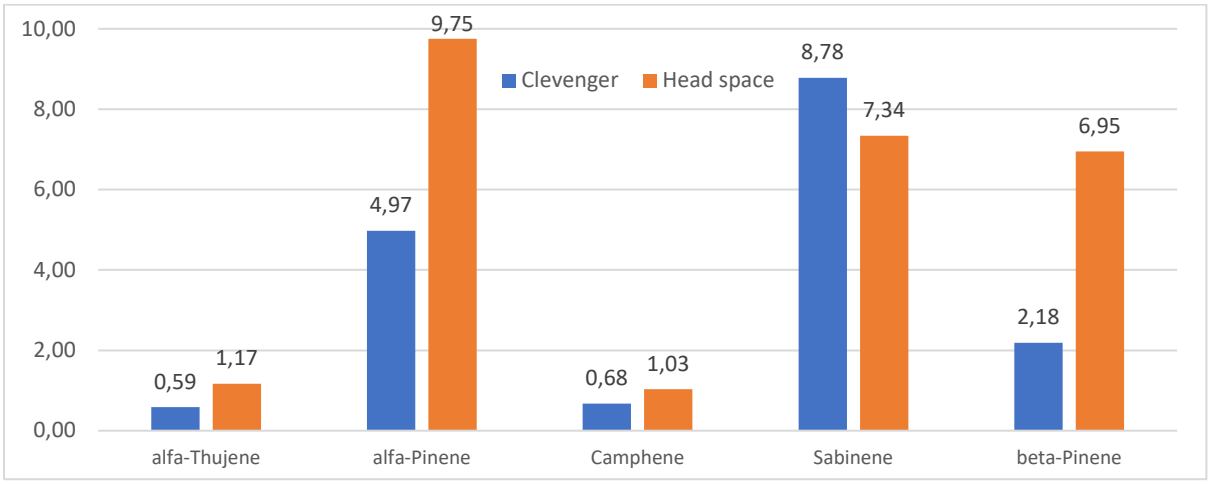

Figure 4. Bicyclic monoterpenes in laurel leaves from Tirana area, June 2024

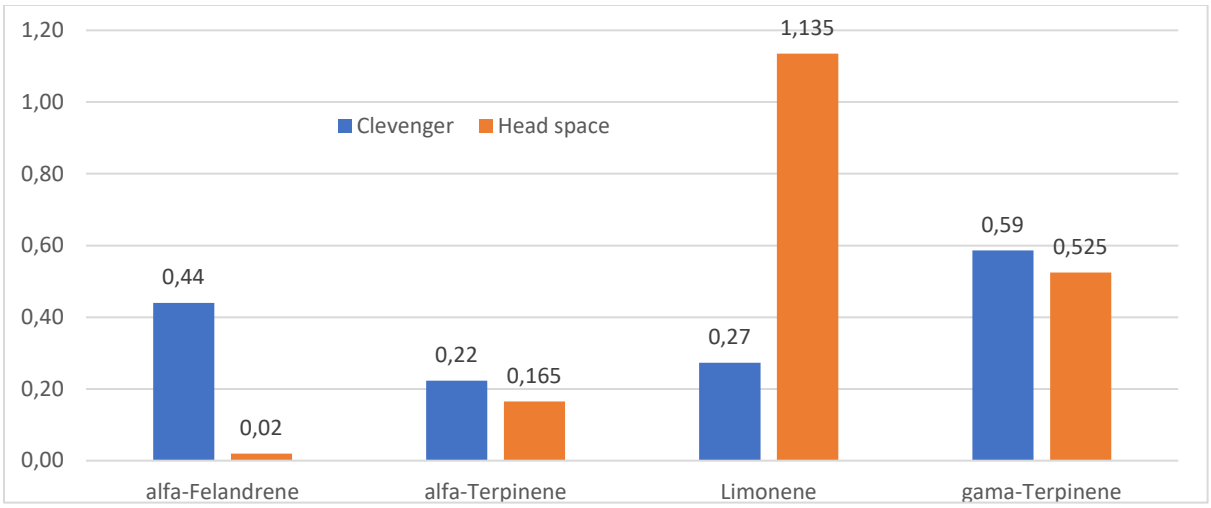

Figure 5. Monocyclic monoterpenes in laurel leaves from Tirana area, June 2024

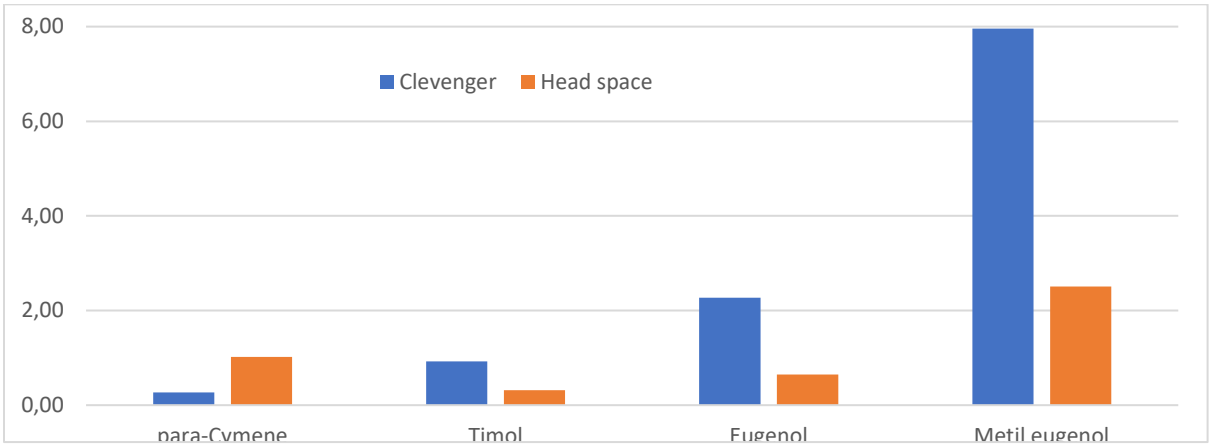

Figure 6. Aromatic monoterpenoids in laurel leaves from Tirana area, June 2024

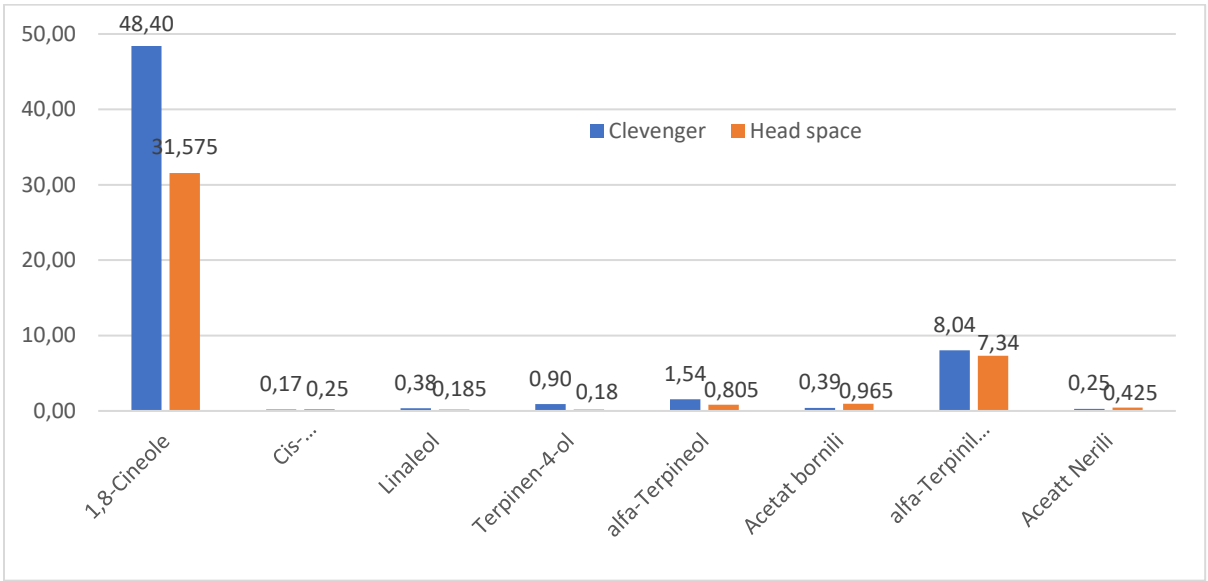

Figure 7. Oxygenated monoterpenes in laurel leaves from Tirana area, June 2024

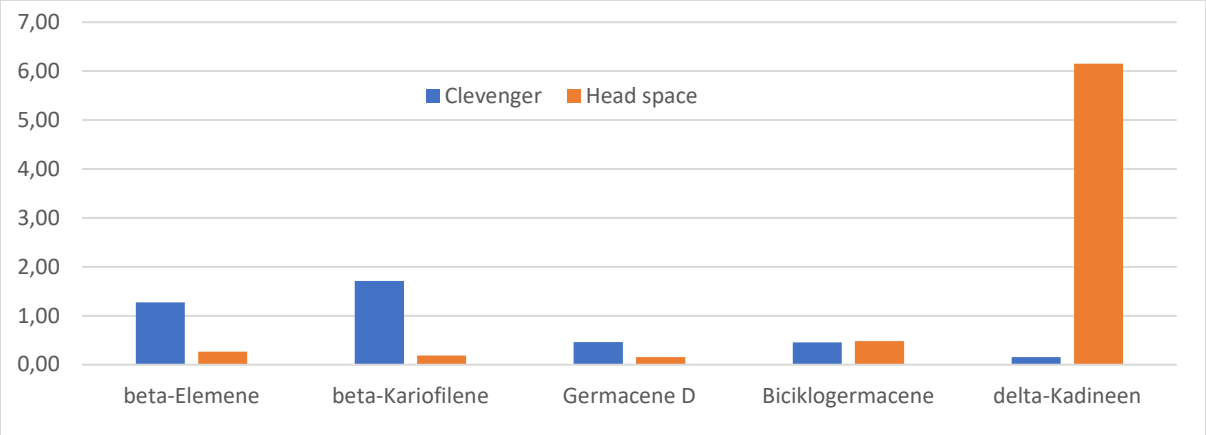

Figure 8. Sesquiterpenes in laurel leaves from Tirana area, June 2024

Conclusions

In this study, leave samples of *Laurus nobilis* L. from the Tirana area were analyzed. Laurel is an aromatic/medicinal plant widely used in cooking as a spice and its active compounds have been found to have very good pharmacological effects and are therefore often used in traditional medicine to treat many symptoms and to increase the quality of life. The chemical analysis of the essential oils extracted from these plants was performed using two different extraction techniques, hydro-distillation and Head space followed by GC/FID quantification. The HS technique had a greater advantage in the number of identified compounds (up to 100) compared to the HD technique (up to 60). This should be related to the preference/discrimination of compounds and their different classes from water vapor, toluene or from their boiling points. This is also evident from the fact that the bicyclic monoterpenes that appear in the first part of the chromatogram due to their low boiling points are in higher percentage in HS while alcohols and phenols (polar compounds, with higher solubility in water and higher boiling points) are in higher percentage in HD technique. However, it should be said that the chemical profile for both methods was similar where Cineole, alpha+beta-Pinenes, Sabinene, alpha-Terpinyl acetate and Methyl Eugenol were the main compounds. Both methods are suitable for determining the chemical profile of essential oils including dried laurel leaves. Some advantages of HS-SPME method are: it is a green method (organic solvents are not used), has good reproducibility and repeatability (low values for STDEV in parallel measurments), low cost, the analyze time is shorter, etc. The chemical profile for *Laurus nobilis* was the same as those reported in other works from other areas of our country and wider from the Balkans and the Mediterranean.

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***Conflict of Interest:** The authors declare that they do not have any conflict of interest.*

***Change of Authorship:** The author has read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors and is aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.*

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