Essential oil of *Minthostachys mollis*: extraction and chemical composition of fresh and stored samples

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Abstract

The objective of this research work was to determine the amount of essential oil obtained from the plant species *Mintostachys mollis*, using the method of extraction of water-steam distillation by the cohobation process and the comparison of the chemical composition of the fresh and stored samples of the plant. Two factors were experimentally taken into account for the extraction: humidity of the material and time measured in three levels, according to the response variable, which is referred to the volume of extracted oil. The characterization was carried out by gas chromatography/ mass spectrometer. The extraction process showed that the highest yields of essential oil were obtained under the humidity factor at medium levels and not at low or high levels. The analysis of the volatile fraction of the extracted fresh sample showed a content of 42 compounds in its composition, among them the carvacryl acetate, trans-caryophillene and germacrene D; while, the results of the stored sample essential oil showed a composition 24 compounds highlighting the presence of trans-Caryophyllene and sabinene. These results are interesting for research and are potentially useful in the food and pharmacology industry.

Keywords: *Mintostachys mollis*, extraction, yield, composition, volatile fraction

1. Introduction

The aromatic and medicinal plants are an important group in the town's economy and a source of raw materials for the alimentary, cosmetic and pharmaceutical industry, in the production of spices, essential oils and medicines (Low 2006), (Mimica and Bozin 2008), (Oscar 2013). The industry of essential oils (EOs) stands out as the most widely developed utilitarian segment (Verna et al. 2010). In recent years there have been a series of changes in trends and eating habits in markets with high purchasing power such as the European Union, the United States, Canada and, incipiently Japan, Singapore, Hong Kong and Indonesia that have favored a considerable increase in the production and consumption of fresh products with functional value (Rosello et al. 2011).

The plant genus *Minthostachys*, extends throughout Los Andes mountain range from Venezuela to Argentina. This genus can derive up to twelve species of aromatic herbs (Ben et al. 1994). It is a perennial sub-shrub herbaceous plant, with interesting active ingredients for the research and development of natural products with health benefits derived from essential oils and extracts which are always significant.

Therapeutical properties of this species are reported in the treatment of diseases of the respiratory tract and the digestive system, as well as food preservative qualities due to its antimicrobial properties (Fuertes and Murcia 2001), (Carhuapoma et al. 2009). In particular, the essential oil of the plant species *Minthostachys mollis* belonging to the genus *Minthostachys* commonly known as *tipo* or *muña*, is a woody shrub plant, native of Argentina, Bolivia, Colombia, Ecuador, Peru and Venezuela, used in traditional medicine to treat stomach cramps and certain influenza disorders (Torrenegra 2016), and as infusions, for the treatment of gastrointestinal disorders. It can also be used as antispasmodic, antidiarrheal, aromatic, carminative and sedative (Granados et al. 2012), (Chevel et al. 1998). It is important to highlight that currently the trend of consumers is inclined to the consumption of foods free of synthetic products (Gleiser et al. 2007), so it is interesting to study native plants in this region to recommend its application as an additive for industrial purposes.

In addition, knowledge of the chemical properties of essential oils and other extracts allows us to propose these products as an alternative and natural source of useful compounds in industry and technology (cosmetics, fragrances, food, chemical synthesis). Essential oils are aromatic and volatile oily liquids obtained from plant material such as leaves, flowers, bark, and seeds, among the most important ones. They are not pure substances but are a complex mixture of volatile organic compounds of aromatic character that can be found in several families of plants (Casado 2018). The EOs contain active ingredients that exhibit antioxidant, antifungal, antimicrobial bioactivities, among others (Matiz 2012); some with antifungal effects against strains of *Candida albicans* (Cano et al. 2008).

The main components of the essential oil of *Minthostachys mollis*are the oxygenated monoterpenes pulegone (51.2%) and menthone (30.7%), followed by monoterpenes hydrocarbonates (limonene 10.1%, β-pinene 1.5%, α-pinene and camphene 1%), an oxygenated sesquiterpene (spatulenol 1.1%) and others less than 1% (Gleiser et al. 2007). The composition of M. mollis is 29.34 ± 3.94% neomenthol, 24.00 ± 5.23% menthone, 20.55 ± 3.33% menthol, and 8.96 ± 1.65% piperitone (Ben et al. 1991). In the case of Carvacrol, this is a compound found with relative abundance of 21.24% (Torrenegra 2016). However, previous studies mention some percentages of carvone (65%) as an essential component, pulegone (17%), menthone (9%), limonene (4%), menthone (53%) and pulegone (33%) (Del Valle et al. 1998).

Minthostachys mollis contains volatile fractions such as thymol, thymol acetate, methyleugenol, pulegone, menthone, limonene, linalool, among others, which act in synergism and predominantly an oxygenated fraction of 67.77% (Carhuapoma et al. 2009). Others report a content of 2.14% thymol, 2.12% thymol acetate and 0.11% methyleugenol (Fuertes and Murguía 2001). The real composition of an essential oil must be known to limit its variability or volubility, and thus be able to demonstrate its true benefits or virtues (Bandoni et al. 2009).

Studies of essential oils in plants of the same species and geographical region, show that some variations in their chemical composition may occur due to the stress, height or season. This fact allows to group the essential oils according to the presence of certain major compounds in their composition as a result of genetic variations (Ruiz et al. 2007), (Schmidt-Lebuhn 2008). In addition, the composition of the extracts is subject to other variables such as: technique of extraction, treatment or storage of plant material and geobotanical growth conditions (Stashemko et al. 2004). Some variations of its components are given according to the region and its yield varies from 0.27 and 0.21% (Fuertes and Murguía 2001) and of 0.25 – 4.93% (Elechosa et al. 2007).

Because of their cost and performance, essential oils are mostly extracted by the steam distillation method, where the steam obtained externally enters the extraction body that passes through the plant material and extracts the volatile components by the direct current effect of water vapor. In the distillation with water-steam in the same extraction body, the plant material is suspended over a screen (false bottom) that prevents the contact of plant material with the boiling liquid medium, a process that is developed by a cohobatingsystem, which allows the return of condensed liquid to the extractor body. Once the essential oil is recovered; this system reduces the net load capacity of raw material within the extractor, but improves the quality of the oil obtained, where for most oils, the losses reported using this system do not exceed, more than just for oils rich in phenols, the 0.2% (Elechosa et al. 2007), (Granados et al. 2012), (Casado 2018).

This fact makes it possible to minimize the losses of oxygenated components, particularly the phenols, which have a high solubility in water. The reuse of condensed water will allow it to become saturated with the dissolved constituents, in such a way that it will not be able to dissolve a greater number of components. Therefore, it is very important to carry out studies on the plant species *Minthostachys mollis*, with emphasis on extraction methods that are easy to apply in rural and marginal urban areas of Ecuador and its potential application of the volatile fraction for industrial purposes.

For the above mentioned, the work has a double objective: to determine the amount of essential oil obtained from the plant species *Mintostachys mollis* (*Kunth*), by means of the steam-water extraction method and to compare the chemical composition of the fresh sample and the stored one. The natural products derived from medicinal plants, such is the case of essential oils, are presented as an alternative for research and development of new products for the food, cosmetic and pharmacological industry.

2. Materials and methods

A bibliographic review on the plant species *Minthostachys mollis*, as well as on the extraction methods and chemical composition of the volatile fraction of the essential oil extracted from this plant species was carried out. The vegetal material used in this research work was obtained from the province of Carchi between 2500 and 3500 meters above sea level. For the extraction of the essential oil, a water-steam distillation equipment with application of cohobation (return of condensed liquid from the water) to the body of the extractor was used once the oil was recovered, as shown in figure 1.

Fresh plant material with 67.74% humidity and dehydrated at a temperature of 50 ° C until reaching 51.2 and 30.4% humidity was used. To control the extraction process, factors, levels and response variables were used with factorial 3^2 with two repetitions (Table 1) and the results were analyzed using the Statgraphics Plus software.

The levels determined for the factor time of extraction in the study, were deduced according to values between 1 to 3 hours of extraction at 96 ° C, proposed in a study conducted by the steam-water extraction method of *Mintostachys mollis* (Fuertes and Murguía 2001). The analysis of the components of the essential oils of the fresh and stored samples (Temperature 15-20 °C and Relative Humidity 50-60%) was carried out using the gas chromatograph coupled to a mass detector (GC-MS). The results are shown in figures 5 and 6.

3. Results

3.1 Results of oil obtained in the extraction process according to factors and levels

In Table 2 shows results about the volume of essential oil obtained according to factors of: sample humidity and extraction time. The volume of extracted oil depends on the humidity of the plant material, where higher yield (0.285%) is achieved with samples that have suffered moderate dehydration (medium level). Extraction values of 0.1% are achieved with samples subjected to severe dehydration (low level) and for fresh samples a yield of 0.19% was achieved. Those values are also influenced by the efficiency of the equipment, method and time of extraction and conditions of plant material. Figures 2, 3 and 4 show results of the behavior of the process, according to the experimental design studied.

In the figures before referred, the variable humidity has greater significance on the volume of extracted oil and to a lesser extent affects the time variable. Figure 3 shows that the humidity variable affects significantly at the medium level, but not with the time variable for the same level. Figure 4 shows the behavior of the volume of extracted oil affected by humidity and influenced by of extraction time; consequently, the efficiency of the extraction process will be determined by the humidity of the material. The adjusted mathematical model obtained statistically projects the behavior of the extraction process according to factors and levels to which the material has been experimentally subjected, where the humidity of the plant material has a greater incidence in the behavior of the process.

The adjusted mathematical model shows that moisture is a determining factor in the amount of oil obtained at intermediate levels for an R-square of 98.9 %.

$A_t = 5{,}82778 + 0{,}775*X_1 + 0{,}125* X_2 - 2{,}39167*X_1^2 - 0{,}025*X_1*X_2 - 0{,}391667*X_2^2$

It is a linear regression, which is conditioned according to the value of the factors and the factor of greatest significance is the humidity and is affected when it decreases to extreme values.

3.2 The results of the chemical composition of *Minthostachys mollis* oil

In the essential oil of the fresh sample *Minthostachys mollis*, 42 components were found (Table 3 and Figure 5), and for its quantity the carvacryl acetate stands out in 18, 95%, followed by trans-caryophyllene in 10.05%, of Germacrene D in 9.46% and finally Carvacrol in 7.60%, 6.69 in Limonene, 6.46 in Ortho-cimene, 5.37 in Gamma-terpinene, 5.33 in Bicyclogermacrene and other components present less than 5%; previous research indicated that these components have shown remarkable biological properties as antifungal, antibacterial and antioxidant agents (Muñoz et al. 2009), (León et al. 2015).

The presence of compounds such as carvacryl acetate and carvacrol, determine that the essential oil has antioxidant properties that can be used in the food preservation industry for its antimicrobial and cosmetic properties. In addition, the conclusion is reached that carvacrol is a potent inhibitor of the growth of the A549 load cell, which is related to human lung cancer (Andersen 2006), (Baser 2008), (Arunasree 2010), (El Babili 2011).

The essential oil of *Minthostachys mollis*, has 2.14% of thymol, 2.12% of thymol acetate and 0.11% methylenegenol. These compounds are of phenolic type and they are possibly the main compounds responsible for antimicrobial activity (Fuertes and Murguia 2001).

On the other hand, the results of the essential oil after being stored at room temperature show a different composition in percentage with respect to the oil extracted from the fresh sample (Table 4 and Figure 6) standing out the compounds: trans-Caryophyllene (13.88%), Sabinene (10.1%), Germacrene-D (6.69%), Carvone (6.5%), alpha. -Copaene, Benzene 1-methyl-2- (1-methylethyl) 5.7%) and (+) spathulenol (5.55%) and others less than 5%.

Trans-Caryophyllene acts as an antimicrobial (Astani et al. 2011) and has an analgesic activity (Chavan et al. 2010), it activates the endocannabinoid system (Gertsch 2008). The trans-caryophyllene compound also has a well-documented anti-inflammatory action (Medeiros et al.2007), (Fernandez et al. 2012). In addition, trans-caryophyllene is effective in the functioning of the intestinal smooth muscle, blocking the electromechanical and pharmacomechanical coupling of excitation-contraction (Leonhardt et al. 2010). It also has properties that are considered as a potential antispasmodic agent for the tracheal smooth muscle. Several studies have clearly shown that trans-caryophyllene has pharmacological effects and its antispasmodic activity in the tracheal muscle is important, a substance worthy of future research that anticipates its pharmacotherapeutic use (Pinho da Silva et al. 2012).

5. Conclusions

The extraction of essential oil, by steam-water distillation method in a single body by cohobation, is a viable alternative, easy to apply in rural areas.

In the same way, the variables control in the process allows to determine the best yield of extracted essential oil from the vegetable species according to factors and levels.

Finally, assessing the chemical composition of the volatile fraction of both fresh and stored samples of the species, constitute the basis for further research aimed to relate the chemical composition with activities for immediate application in production sectors of biotechnology and industry.

Table 1. Experimental design according to factors, levels and response variable

Factors	Levels			Units	Response variable
	Low	Medium	High		
Humidity of the material (X1)	30.4	51.2	67.74	%	Volume of the essential oil (mL)
Time (X_2)	2.5	3	3.5	Hours	

Table 2. Volume of extracted oil according to factors and levels of the extraction process of *Minthostachys mollis*

BLOCK	Humidity (X_1)	Time (X_2)	Volume of essential oil (A_t)
	%	Hours	mL

1	-1	-1	2
1	0	-1	5.4
1	1	-1	3.8
1	-1	0	2.6
1	0	0	5.7
1	1	0	4.2
1	-1	1	2.4
1	0	1	5.6
1	1	1	4
2	-1	-1	2.2
2	0	-1	5.3
2	1	-1	3.6
2	-1	0	2.8
2	0	0	5.8
2	1	0	4.3
2	-1	1	2.4
2	0	1	5.6
2	1	1	3.8

Table 3. Volatile composition of fresh essential oil

Component Number	**Retention time**	**% Composition**	Assignment
1	6.21	1.12	beta-myrcene
2	6.63	0.84	alpha-Terpinene
3	7.26	6.69	Limonene
4	7.53	0.97	beta-falandrene
5	8.64	0.93	cis-ocimene
6	8.95	5.37	gamma-terpinene
7	9.28	2.48	trans-ocimene
8	9.92	6.46	ortho-cymene
9	10.36	0.29	alpha-terpinolene
10	11.23	0.20	Unknown
11	18.09	1.86	alpha-cubebene
12	18.56	0.28	Sabinene trans-hydrate
13	19.35	0.89	Mentone
14	19.49	1.69	Alpha-copaene
15	21.50	0.56	3-menthene
16	21.75	2.36	beta-cubebene
17	22.79	2.23	Linalool L
18	24.26	10.05	Trans-cariofillene
19	26.35	0.46	Pulegona
20	26.49	0.30	Unknown
21	26.86	0.36	Unknown
22	27.39	1.92	Alpha-humulene
23	28.59	0.71	(-)-mirtenil acetate
24	29.25	9.46	Germacrene D
25	30.29	5.33	Bicyclogermacrene
26	30.64	1.04	Beryl Acetate

27	31.49	1.00	Delta-cadinene
28	31.64	0.23	Alpha-farnesene
29	31.98	0.98	Geranyl acetate
30	32.34	1.11	Unknown
31	32.44	0.29	Beta-citronellol
32	33.76	0.38	Nerol
33	35.80	0.86	Timilo acetate
34	36.24	1.15	Unknown
35	36.77	18.95	Carvacryl acetate
36	39.05	0.48	Unknown
37	40.17	0.20	Caryophyllene oxide
38	41.83	0.24	Unknown
39	44.30	0.23	Unknown
40	46.01	1.09	(+) spathulenol
41	48.91	0.39	Thymol
42	49.83	7.60	Carvacrol
TOTAL*		95.95	

Table 4. Composition of the volatile fraction of stored essential oil

Component Number	**Retention time**	**% Composition**	**Component**
1	3.7358	1.82	alpha.-Pinene
2	4.6156	10.1	Sabinene
3	4.721	3.29	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S).
4	6.1825	5.87	Benzene, 1-methyl-2-(1-methylethyl)-
5	6.3279	3.34	dl-Limonene
6	6.4879	1.52	Eucalyptol
7	9.2691	2.53	Linalool L
8	12.5193	1.42	Terpinen-4-ol
9	15.3878	6.5	Carvone
10	17.1911	3.34	Anisole
11	17.6637	2.4	(-)-trans-Pinocarvyl acetate
12	18.8598	3.05	(-)-Myrtenyl acetate
13	19.6923	2.5	alpha.-Cubebene
14	20.7975	5.86	alpha.-Copaene
15	21.3829	3.64	betha.-Cubebene
16	21.4992	1.79	beta.-Elemene
17	22.5244	13.83	trans-Caryophyllene
18	23.9605	2.92	alpha.-Humulene
19	25.0475	6.69	Germacrene-D
20	25.6256	3.69	bicyclogermacrene
21	26.5963	1.57	alpha.-Cubebene
22	26.6763	1.8	delta.-Cadinene

23	28.8394	5.51	(+) spathulenol
24	28.9485	3.61	Caryophyllene oxide

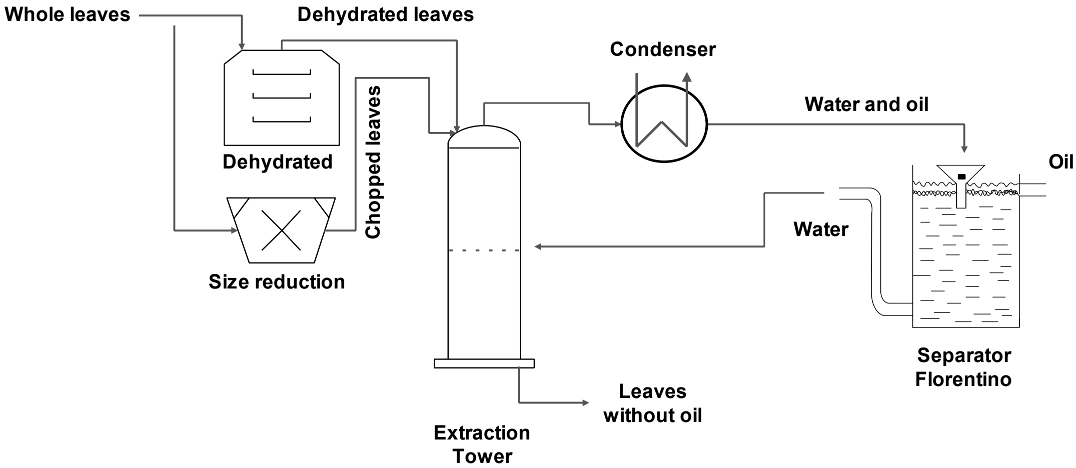

Figure 1. Process of obtaining essential oil.

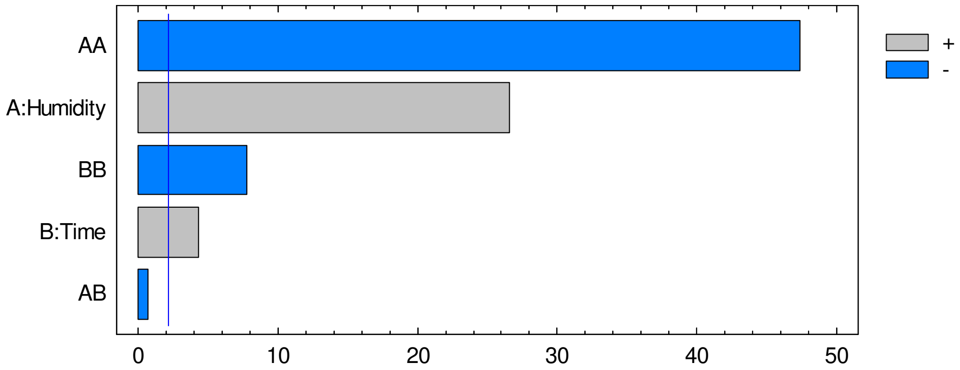

Figure 2. Pareto diagram for essential oil volume.

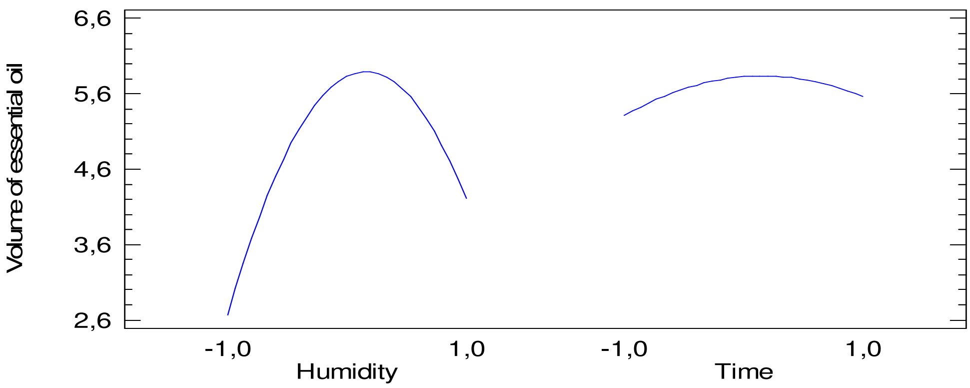

Figure 3. Graph of main effects for oil.

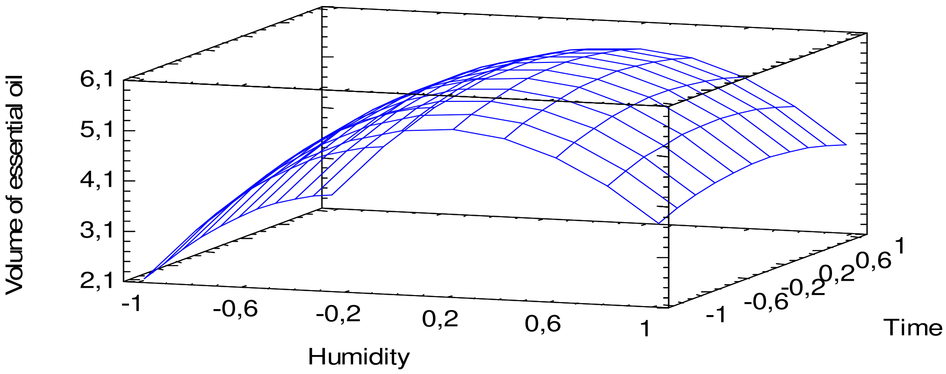

Figure 4. Estimated response surface for the oil.

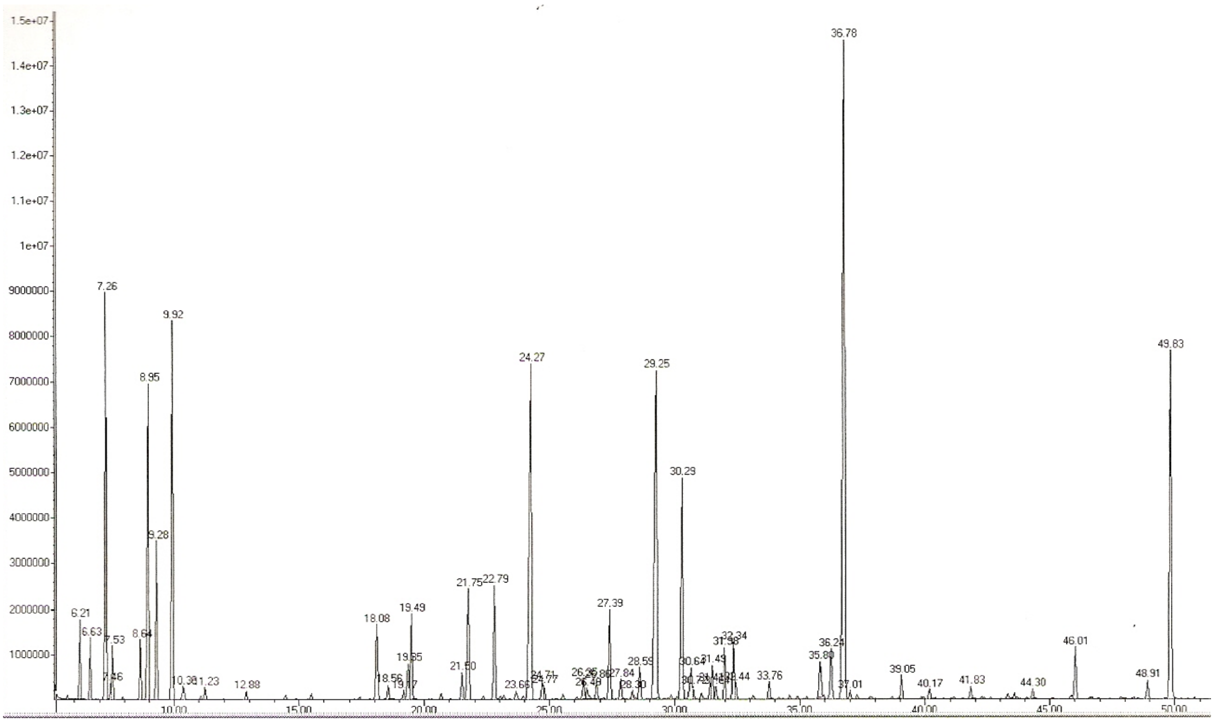

Figure 5. Chromatographic profiles of the components of the fresh sample of essential oil of *Minthostachys mollis.*

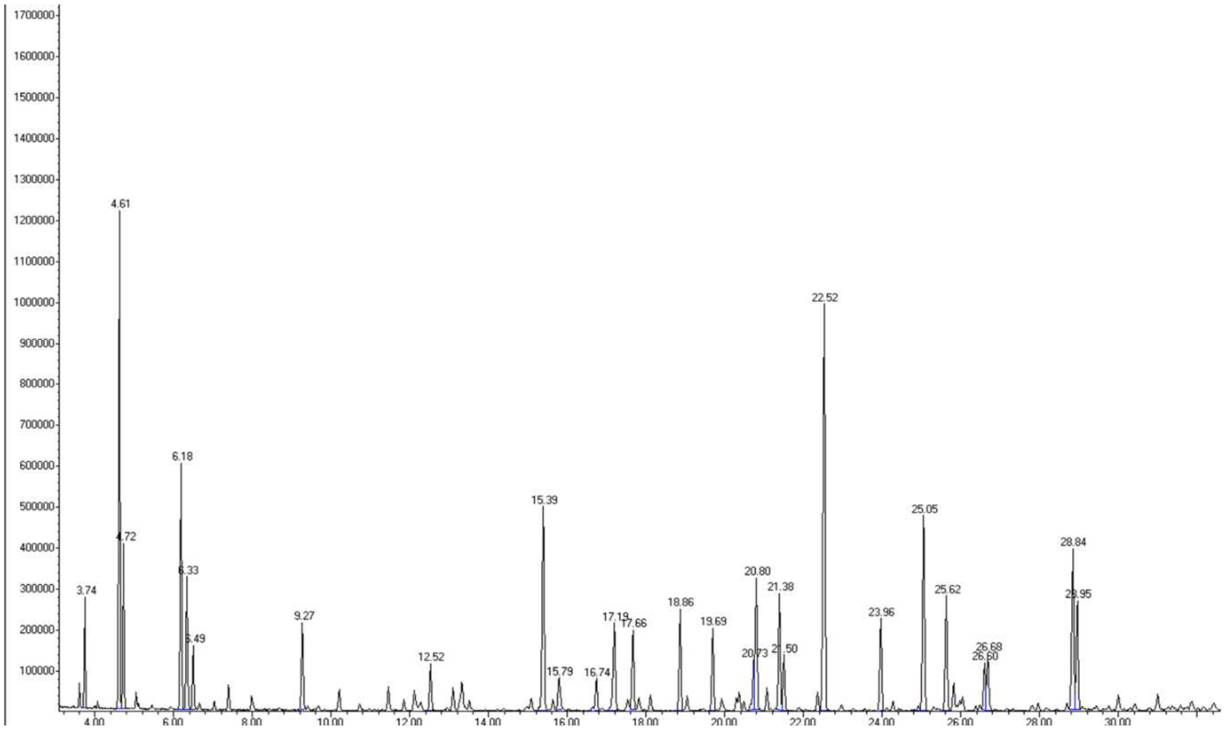

Figure 6. Chromatographic profiles of the components of the stored essential oil of *Minthostachys mollis.*

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