

Chemical Composition of the Essential Oil from the Aerial Parts of *Satureja hortensis* As a Potent Medical Plant Using Traditional Hydrodistillation

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ABSTRACT: The water-distilled essential oils, which were obtained from the fresh aerial parts of *Satureja hortensis* were analyzed by means of GC and CC/MS instruments. The plant was collected during the flowering stage from Foroomad Mountains, Semnan Province, and heart of Iran. Twenty compounds were identified in the different samples analyzed, representing 100% of the total oil contents. In terms of general categories, monoterpene hydrocarbons dominated the chemical profile of the oils with γ -terpinene (27.4%), carvacrol (23.7%), *p*-cymene (11.1%), α -terpinene (10.2%), α -pinene (5.1%) and myrcene (5.1%) as the main constituent components. The other constituents were found to be α -thujene (3.9%), β -pinene (3.0%), sylvestrene (3.0%), α -phellandrene (1.2%) and (-)-terpinen-4-ol (1.0%).

KEYWORDS: *Satureja hortensis*, Essential oil, Hydrodistillation, γ -Terpinene, Carvacrol, *P*-cymene, Monoterpene hydrocarbons.

INTRODUCTION

The genus *Satureja* consists of about 12 species in Iran among them *S. edmondi*, *S. intermedia*, *S. sahendica*, *S. isophylla*, *S. kallarica*, *S. atropatana*, *S. bachtiarica* and *S. khuzistanica* are endemic to Iran. In addition, other well-known species of *Satureja* involving *S. mutica*, *S. spicigera*, *S. macrantha* and *S. boissieri* grow up in Turkmenistan, Ghafghaz, Anatoly and Iraq, as well [1]. *Satureja hortensis* L. (Lamiaceae) (Figure 1) is widely cultivated or grown in different climatic regions of Iran. It is also well known in Iranian traditional medicine as a remedy for various ailments. In the literature, some

promising properties are related to *S. hortensis* of which the most important ones are as muscle pain reliever, tonic and carminative in treating stomach and intestinal disorders such as cramps, nausea, indigestion and diarrhoea. Furthermore, this plant is used as a proper flavoring agent in cookery [2]. *S. hortensis* is an annual plant (10–35 cm tall) and aromatic herb with lilac, purplish or white flowers and linear to linear-oblongate leaves. It grows wild on rocky or eroded slopes, screes, gravelly places and coastal dunes, fallow fields and roadsides in Turkey [3].

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Figure 1. General picture of *S. hortensis* in the flowering stage
(Sampling area: Foroomad Village, Shahrood City, Semnan Province, Iran)

Diverse species of *Satureja* are frequently prescribed in folk medicine as powerful agents for the treatment of a variety of disorders. In the literature, sporadic reports are found concerning the antigenotoxic [4], antibacterial [5,7], antimicrobial [5, 6, 8, 12], antinociceptive [13, 14], anti-inflammatory [13, 14], antispasmodic and anti-diarrhoeal effect [15], antioxidant [6, 12, 16, 17], insecticidal [18, 19], anti-fungal [20] and other biological activities of *S. hortensis*.

Boskabady and his colleagues have investigated relaxant effect of *S. hortensis* on guinea pig tracheal chains. This report shows a potent relaxant impact of this plant which was comparable to that of theophylline [21]. It is notable that its extract is a novel source of some polyphenolic, flavonoids and antioxidants [22, 24]. Soran and his colleagues have successfully analyzed the thymol contents in the extracts of *S. hortensis* by the aid of high performance thin layer chromatography (HPTLC) [25]. Meanwhile, Uslu and his colleagues have suggested that the extract of *S. hortensis* may have the potential to be used as anti-inflammation agent, and in the treatment of rhinosinusitis diseases in rabbit [26].

In view of many activities of the plants belonging to the *Satureja* genus, the isolation, characterization and determination of their essential oils and volatile components seem highly justified. The main goal of the present report was a careful characterization of the

essential oils separated from the aerial parts of *S. hortensis* using traditional hydrodistillation (HD).

The chemical profiles of hydrodistilled oils of *S. hortensis* have been the subject of several studies. However, our study gave a new profile, dominated primarily by monoterpene hydrocarbons. Furthermore, to the best of our knowledge, there has been no report in the literature concerning the water-distilled oil composition of *S. hortensis* in Semnan Province, Iran.

MATERIALS AND METHODS

Chemicals and supplies

Alkane mixtures containing 40 mg/L of C₉ to C₂₄ straight chain alkanes in hexane and toluene were purchased from Fluka (Buchs, Switzerland). All the carrier gases used in GC and GC-MS were of the highest purity. The density of the hydrodistilled oil was assessed by a glass densitometer.

Plant material and botanical identification

The plant material was collected during the flowering stage in April, 2012, in the Foroomad Mountains, Semnan Province, at east longitude 56.45' and north latitude 36.30' at an altitude of 1230 m above sea level. Polyethylene gloves were worn to prevent unwanted contamination. After sampling, the plant was shipped on wet ice, arriving in the laboratory 10 h later. Special care was taken to minimize damage and cross-contamination of plant parts. The plant was identified by a local botanist, and a voucher specimen was deposited at the Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran, for further authentication.

Hydrodistillation method

100-g portions of the air-dried aerial parts of *S. hortensis* were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The essential oils were collected, dried with anhydrous sodium sulphate and stored at 4°C until being used.

Chromatographic analyses

GC analyses were performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio

1:30) injector and a flame ionization detector, both operating at 250°C. High purity nitrogen served as the carrier gas role (1 mL/min); the capillary column used was DB-5 (50 m×0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 °C for 3 min, then heated to 220°C with a 5 °C/min rate and finally kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak areas using a CR5 Shimadzu CR pack without the use of correction factors. In addition, GC/MS analysis were performed using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 µm). The effluent of the GC column was introduced directly into the source of the MS. The column temperature programming was same with GC analysis. The flow-rate of helium carrier gas was 1 mL/min, final temperature 230°C. The detector temperature was set at 250°C; MS was taken at 70eV (E1), electron multiplier voltage 1800 eV; mass range were over the range 30-350 amu and scan time was 2 scans/ sec.

Qualitative and quantitative analyses

Identification and determination of the constituents of each oil were tentatively made by comparison of their mass spectral fragmentation patterns and retention indices (RI) relative to C₉-C₂₄ *n*-alkanes both with those given in the literature and those stored in a MS library (Wiley 275). For further reliability, we utilized some of the spectral patterns as well as Kovatz indices based on previous findings of our research group [27,36]. Relative percentages of the components were calculated from peak areas using a Shimadzu C-R4A

chromatopac on the DB-5 column, without the use of correction factor.

RESULTS AND DISCUSSION

Chemical profiles of the hydrodistilled oil of S. hortensis

Although the chemical profiles of hydrodistilled oils of *S. hortensis* have been reported previously, this study produced a new profile, mainly consisting of monoterpene hydrocarbons. Moreover, quantitative and qualitative analysis of essential oils from the aerial parts of *S. hortensis*, using the HD method, has led to identification of several monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons.

The volatile constituents obtained from aerial parts of *S. hortensis* along with their structures are listed in Table 1, in which both the percentages and retention indices of the components are given. As can be seen, there are negligible differences in numerical values of Kovatz retention indices between the calculated ones and those cited in the literature. Furthermore, a total of 20 components were identified, accounting for 100% of the components (Figure 2). To calculate the Kovatz retention indices of each constituent component, as unique criteria for the characterization of the natural compounds, a mixture of normal alkanes over the range *n*=9-24 was injected into the column under the same conditions and the respective chromatogram is given in the Figure 3.

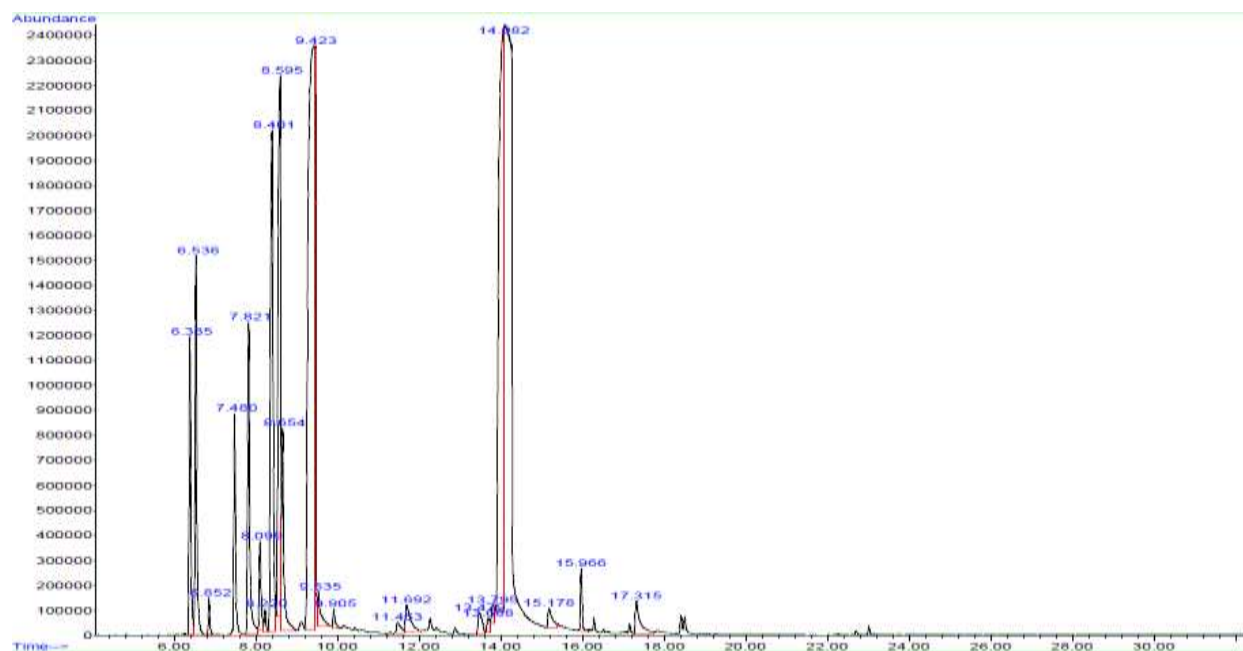


Figure 2. GC-MS chromatogram for the volatile essential oil from aerial parts of *S. hortensis*

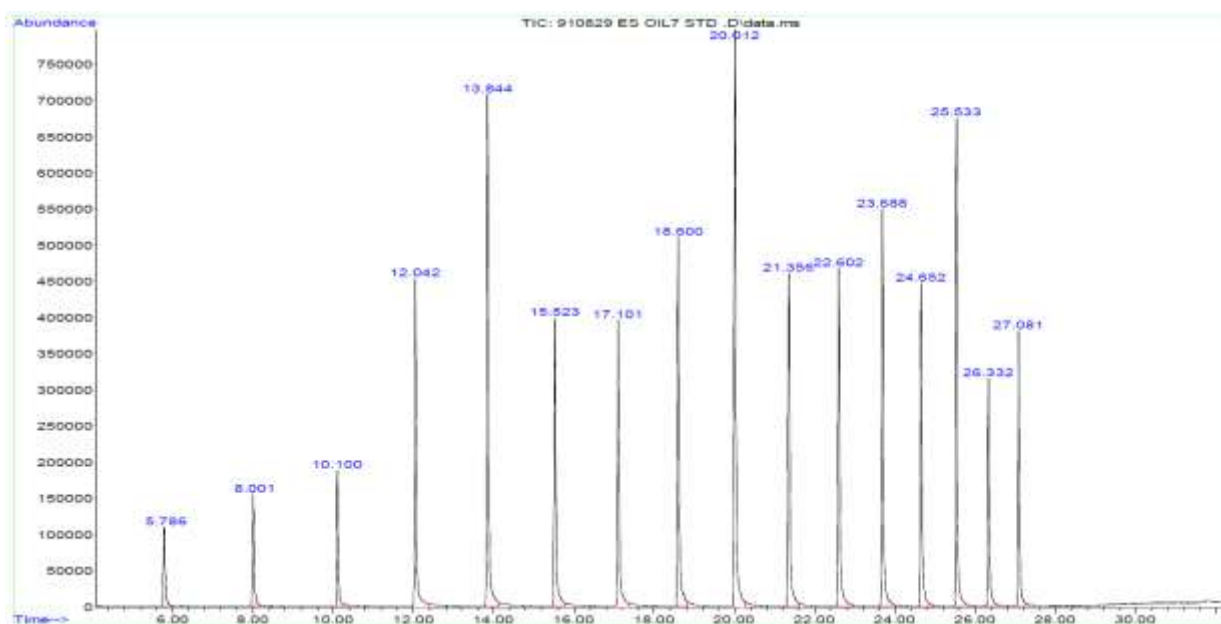


Figure 3. The GC-MS chromatogram of the normal alkanes over the range $n=9-24$.

Table 1. Chemical composition of essential oils from the aerial parts of *S. hortensis* obtained by using traditional HD method ^a

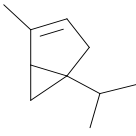
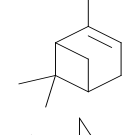
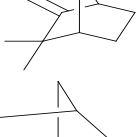
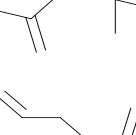
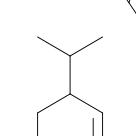
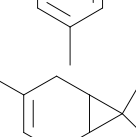
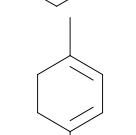
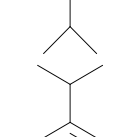
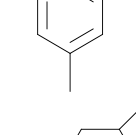
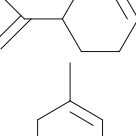
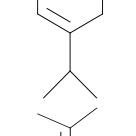
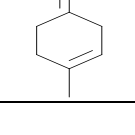
No.	Compound	Structure	Class	R _t ^e	RI (Lit.) ^f	RI (Cal.) ^g	Percentage
1	α -Thujene		MH ^b	6.4	931	927	3.9
2	α -Pinene		MH	6.5	939	933.9	5.1
3	Camphene		MH	6.9	953	948.1	0.5
4	β -Pinene		MH	7.5	980	976.5	3.0
5	Myrcene		MH	7.8	991	991.9	5.1
6	α -Phellandrene		MH	8.1	1005	1004.7	1.2
7	δ -3-Carene		MH	8.2	1011	1010.4	0.3
8	α -Terpinene		MH	8.4	1018	1019.1	10.2
9	p-Cymene		MH	8.6	1026	1028.3	11.1
10	Sylvestrene		MH	8.7	1030	1031.1	3.0
11	γ -Terpinene		MH	9.4	1062	1067.7	27.4
12	α -Terpinolene		MH	9.9	1088	1090.7	0.3

Table 1. (Continued)

No.	Compound	Structure	Class	R _t ^e	RI (Lit.) ^f	RI (Cal.) ^g	Percentage
12	α -Terpinolene		MH	9.9	1088	1090.7	0.3
13	Borneol		OM ^c	11.5	1165	1170.2	0.4
14	(-)-Terpinen-4-ol		OM	11.7	1177	1182	1.0
15	D-Carvone		OM	13.5	1242	1279.7	0.7
16	p-Thymol		OM	13.7	1290	1291.3	0.6
17	Carvacrol		OM	14.1	1298	1314.2	23.7
18	Carvacryl acetate		OM	15.2	1371	1379.5	0.6
19	β -Caryophyllene		SH ^d	16.0	1418	1428.1	0.8
20	β -Bisabolene		SH	17.3	1509	1514.3	1.1
			MH				71.1
			OM				27.0
			SH				1.9
			Total				100

^a The compounds have been sorted according to their retention indices on an HP-5 MS capillary column

^b Monoterpene hydrocarbons

^c Oxygenated monoterpene

^d Sesquiterpene hydrocarbons

^e Retention time (Min.)

^f Kovatz retention indices given in the literature

^g Calculated Kovatz retention indices

Using the HD method (Table 1 and Figure 2), twenty components could be identified in the oil from the aerial parts: Twelve monoterpene hydrocarbons (71.1%), six oxygenated monoterpenes (27.0%) and two sesquiterpene hydrocarbon (1.9%). Specifically, the major components in the hydrodistilled oil of the aerial parts were found to be γ -terpinene (27.4%), carvacrol (23.7%), *p*-cymene (11.1%), α -terpinene (10.2%) and myrcene (5.1%). Moreover, natural components including α -thujene (3.9%), β -pinene (3.0%), sylvestrene (3.0%), α -phellandrene (1.2%), (-)-terpinen-4-ol (1.0%) occurred in the oil structure with lower quantities. The percentage yield of essential oil, in terms of the weight of the collected oil per gram of dried plant, was 0.38% (w/w) for three replicate distillations. In Figure 4, the relative percentages of the various classes of the oil constituting compounds are compared. From this figure, it is immediately evident that in this oil monoterpene hydrocarbons constituted the main components, with the second largest class being oxygenated monoterpenes. In summary, the rank order of classes of compounds in the profiles of the oils obtained by the HD approach is: monoterpene hydrocarbons> oxygenated monoterpenes> sesquiterpene hydrocarbons.

Furthermore, to give a deeper insight into the fragmentation patterns, the mass spectra of four dominant compounds have been represented in Figure 5. As this figure shows most of the frequencies appear

over the $\frac{m}{z}$ range 90-150.

Chemical composition of S. hortensis in similar reports

There have been several reports on the constituents of essential oils from *S. hortensis* in different parts of the world. Adiguzel and his colleagues have reported, based on GC and GC-MS analyses, that the main components of water-distilled volatile oils from the dried fruits of *S. hortensis* were thymol (40.54%), γ -

terpinene (18.56%), carvacrol (13.98%), and *p*-cymene (8.97) [3]. In a related study, Baser and his colleagues have identified the compositions of the essential oils of twenty wild and cultivated samples of *S. hortensis* in Turkey. Accordingly, the oils from cultivated forms contained carvacrol (42-63%) as the major constituent while thymol (29-43%) was the main component in the wild samples [37]. In similar works, carvacrol was characterized as the most frequent compound [5, 9, 19, 38, 41]. Moreover, in the oil and extracts of *S. hortensis* cultivated in Iran by using both supercritical carbon dioxide and hydrodistillation methods, the main extracted components were γ -terpinene, thymol and carvacrol [42]. On the other hand, Saharkhiz and co-workers have inspected the influence of growth phase on the hydrodistilled essential oil composition of *S. hortensis*. This study highlighted the presence of γ -terpinene as the major compound of the essences at all developmental stages, except the ripened fruit stage in which carvacrol was the most abundant compound [43]. Finally, Soran and his colleagues have employed three different techniques, namely maceration, ultrasonic and microwave solvent assisted extraction to isolate of essential oils from *S. hortensis* prior to HPTLC determination and identified thymol, carvacrol, α -pinene (mixture), limonene, linalool, citronellol, and geraniol as the major constituents [44].

CONCLUSION

The present work was mainly focused on inherent ability of a traditional extraction technique, namely, hydrodistillation (HD) to separate the essential oils from the aerial parts of *S. hortensis*, prior to subsequent analysis by GC and GC/MS. By using the HD method, we were able to identify 20 components representing 100% of the total oil components.

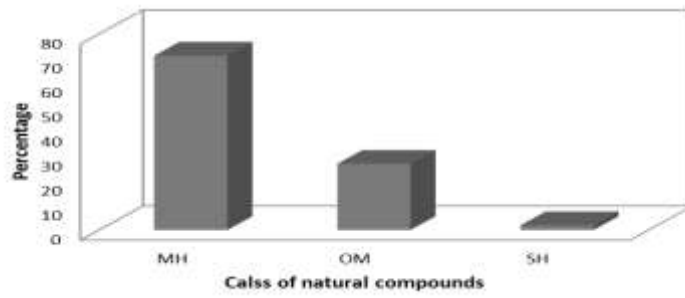


Figure 4. percentages of the classes of natural compounds found in the oils from aerial parts of *S. hortensis* by the use of the HD method. (MH= Monoterpene hydrocarbon; OM= Oxygenated monoterpene; SH= Sesquiterpene hydrocarbon)

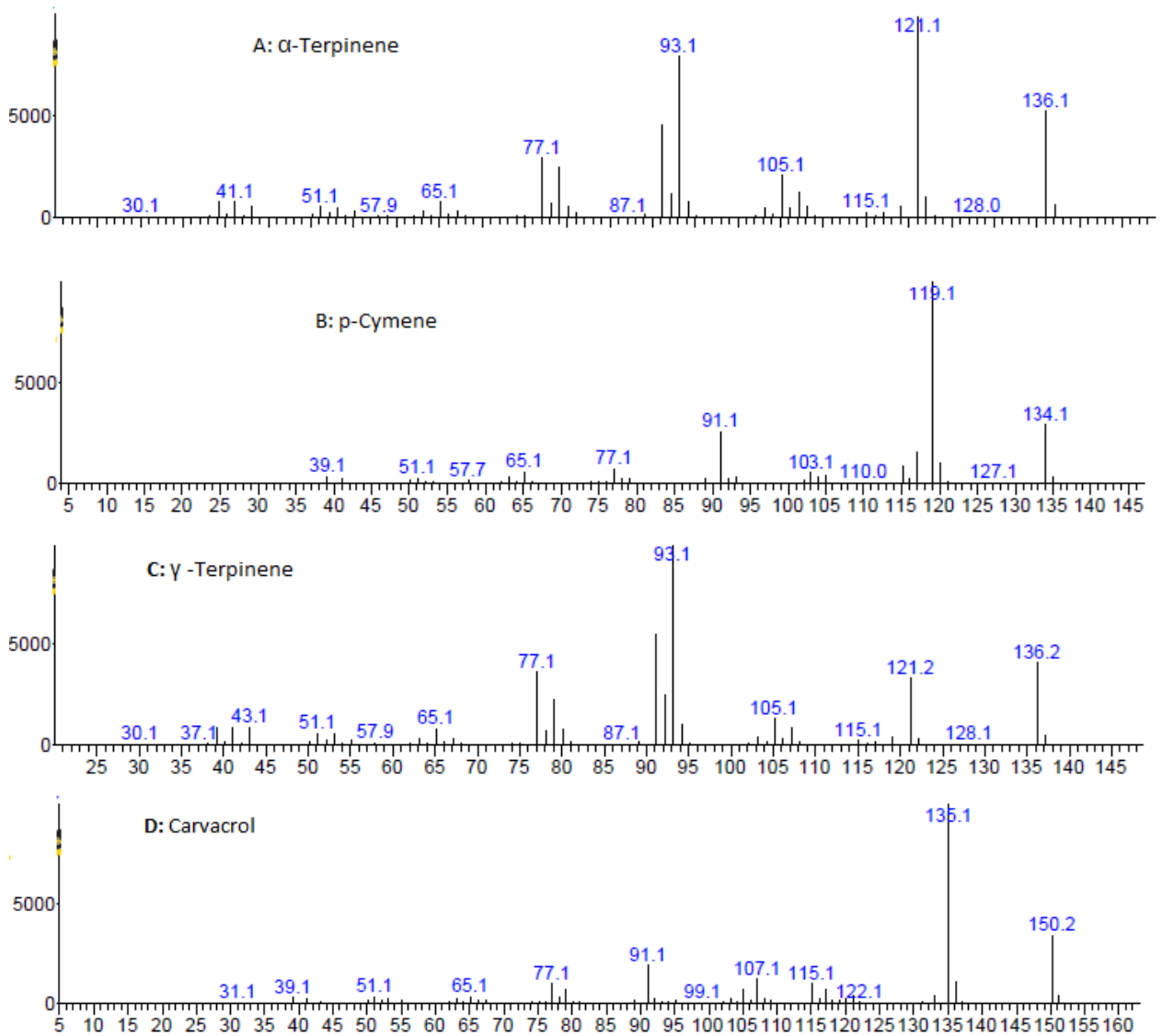


Figure 5. Mass fragmentation patterns for the major constituents of the essential oil from the aerial parts of *S. hortensis*.

The water-distilled profile of the plant composed of twelve monoterpene hydrocarbons, six oxygenated monoterpenes, and two sesquiterpene hydrocarbons respectively containing 71.1%, 27.0% and 1.9% of the total oil composition. The main components of hydrodistilled oil from aerial parts of *S. hortensis* were γ -terpinene (27.4%), carvacrol (23.7%), p-cymene (11.1%), α -terpinene (10.2%) and myrcene (5.1%) followed by α -thujene (3.9%), β -pinene (3.0%), sylvestrene (3.0%), α -phellandrene (1.2%), (-)-terpinen-4-ol (1.0%) with lower percentages.

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