Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 1, No. 2, 2012, pp. 93 – 104
Online Edition ISSN 2285 - 4177
ORIGINAL PAPER

Application of gas chromatography and liquid sampling mass spectrometry for analysis of essential oil from leaf, bark and pericarp of laurel from Montenegro

Received for publication, october, 1, 2012.
Accepted, November, 15, 2012

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Abstract.
Ten samples of essential oils from leaf, bark and pericarp of laurel, originating from Montenegro were analysed by gas chromatography (GC/FID and GC/MS) and liquid sampling mass spectrometry (LS/MS). Along to the clear chemical differentiation of oil samples from distinct parts of laurel determined by GC/FID and GC/MS, it was shown that similar and even better distinction of tested samples can be achieved by liquid sampling mass spectrometry (LS/MS), followed by strong statistical analysis. Since time required for LS/MS is significantly shorter than that needed to GC, LS/MS technique could be used as powerful screening tool wherever big number of volatile samples should be analysed.

Key words*:* LS/MS, laurel oil, differentiation, analysis.

Introduction

The essential oil of laurel [*Laurus nobilis* L. (Lauraceae)] belongs to the group of important spicy oils widely used in food industry (Burdock G.A., 2002). Main producers of oil obtained by steam distillation from leaves are Mediterranean countries, Russia and China. In Montenegro, the main area for collection of leaves of wild growing laurel is the south-east part of Adriatic coast, where, in practice, instead of leaves, young shoots, as well as other plant parts are used for distillation, affecting to the quality and usability of oil. Nevertheless to lack of internationally recognised specification for the oil of laurel, there are a lot of data, which could be used for this purpose (Burdock G.A., 2002; Lawrence B.M., 1999; Kovačević V., 1993; Flamini G. et al., 2002; Kilić A. et al, 2004). According to these, variations in the contents of selected oil constituents (usually 1,8-cineole, linalool and methyleugenol), are too high to define sharp chromatographic profile, needed for setting appropriate standard specification (Lawrence B.M., 1999; Kovačević V., 1993). From the other side, adoption of proposed limits for too many constituents (29) could significantly

increase difficulties in testing of commercial laurel oil samples (Burdock G.A., 2002).

In our previous paper, steam distilled oil from the shoots, separated leaves and stem as well as from flower of laurel, wild-grown in Montenegro, was analysed by GC/FID and GC/MS (Kovačević N.N. et al., 2007). The aim of the present paper is to make possible better definition of the quality of essential oils from different parts of laurel tree. Simultaneously, the aim was to apply liquid sampling mass spectrometry (LS/MS) to tested laurel oil samples, not only to approve results of their characterisation and differentiation by GC, but to test applicability of LS/MS technique for this purpose too.

LS/MS technique was described and developed in our laboratories for a several years, mainly for the purpose of the fast chemical differentiation of essential oils and related products. In our first contribution, concept of development of new analytical technique for the characterisation of the essential oils has been described and tested. This technique (LS/MS) assumes use of common GC injectors and introduction of liquid samples (LS) into analytical system, as well as mass spectrometric (MS) detection of the sample without prior separation. Testing of LS/MS was focused on its' possible use in differentiation of essential oils. The first results were quite optimistic, approving very wide range of possibilities in the application for these purposes (Ristić M. et al., 2001, 2006, 2007). From the difference of well established and generally adopted screening techniques based on simple analytical gas chromatography (GC), or combination of GC with mass spectrometry (GC/MS) (Sandra P. and Bicchi C.), LS/MS is somewhat closer to those principally consisting only from sampling and detector system. Among them, headspace mass spectrometry (HS/MS) (Martí M. P. et al., 2004; Pérez Pavón J. et al., 2008) and proton-transfer-reaction mass spectrometry (PTR/MS) (Lindinger W. et al., 1998) should be mentioned, not only because of the similarity in the part dealing with detection, but because of specific statistic software needed for the processing of raw analytical data. Specificity of sampling devices used by HS/MS and PTR/MS enable these techniques to be employed for the indirect analysis of essential oils, directly from the plant material, avoiding oil isolation, as a necessary step in the case of LS/MS. From the other side, virtually complete mass spectra of essential oil and other complex mixtures, containing constituents of different volatility, could be obtained by LS/MS, but not by to other mentioned techniques.

2. Materials and methods

Plant material and oil isolation. Plant material was collected in the end of summer 2002, at the locality Herceg Novi (Montenegro), from three trees belonging to the same population. Separated air-dried plant parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. Among ten selected oil samples three originated from leaves (L1 to L3), three from pericarp (P1 to P3), and four from bark (B1 to B3). In the case of sample 2, oil from bark was separated in liquid and solid fraction, whose samples were separately analysed (B2L and B2S, respectively). Isolated oils were dissolved in ethanol to obtain ~ 1 % sample solutions and analysed by GC/FID, GC/MS, and LS/MS technique.

GC/FID. A Hewlett Packard, HP-5890 Series II gas chromatograph (Waldbronn, Germany), equipped with a split-splitless injector, HP-5 fused silica capillary column (25 m · 0.32 mm id; 0.5 μm film thickness), and flame-ionisation detector (FID) was employed. Oil solutions in ethanol (~ 1%) were injected in split mode (1:30) using automatic liquid sampler and auto-injector (ALS). Injector was heated at 250°C, detector (FID) at 300°C, while the column temperature was linearly programmed from 40°C-240°C (4°C/min.). Carrier gas was hydrogen at the flow rate of 1 mL/min.

GC/MS. Analyses were carried out on a Hewlett Packard, HP G1800C GCD Series II Electron Ionisation Detector (EID) analytical system (Palo Alto, CA, USA), equipped with split-splitless injector (ALS) and fitted to HP-5MS capillary column (30 m · 0.25 mm id; 0.25 μm film thickness). Carrier gas was helium. The chromatographic conditions were as above. Injector was heated at 250°C, transfer line (MSD) at 280°C, while the column temperature was linearly programmed from 40°C-240°C (4°C/min.). Electron impact mass spectra (EIMS, 70 eV) were obtained in scan mode in m/z range 40-450.

LS/MS (liquid sampling mass spectrometry). LS/MS analyses were accomplished using the same apparatus as for GC/MS, using fused silica capillary (5 m · 100 μm id.) instead of GC column, and working isothermally at 260 °C. Injector and detector were heated at 250 °C and 260 °C, respectively. To enable normal ALS operation duration of analytical runs was extended to 3 minutes. Sample solutions in ethanol (~1 %) were injected by ALS (200 nL, split mode, 1:60). Carrier gas was helium. EI MS (70 eV) of samples were acquired in scan mode, in the m/z range 40-450.

Data processing. GC/MS identification of individual constituents was made by comparison of their retention times with those of analytical standards of available terpenoids, and by computer searching, matching mass spectra with those held in Wiley275 library of mass spectra. Confirmation was done using calibrated AMDIS programme for determination of experimental values for retention indices of recorded constituents and comparing them with those from

the literature. For quantification purposes relative area percentages obtained by GC/FID were used. In the case of LS/MS, obtained mass spectra of tested samples were normalised and put to newly created searchable MS library for further evaluation. For processing of the acquired mass spectra, two types of software were used. Probability merge search (PBM), revision B0.01, and NIST MS Search 2.0, as well as additional software for conversion of data between two mentioned data formats. In addition, data obtained by different analytical techniques were treated by hierarchical cluster analysis (HCA) determining, simultaneously, derived stimulus configuration (according to Euclidean distance model), using statistical software SPSS for Windows, release 10.0.1 (SPSS Inc., Chicago, IL 60606, USA).

3. Results and discussion

Results of characterisation of ten tested samples of the essential oil from different parts of laurel by GC/FID and GC/MS are presented in Table 1.

Table 1. Percentage composition of oil from different parts of laurel tree (GC)*

Constituents	RI_E	RI_L	L1	L2	L3	B1	B2S	B2L	B3	P1	P2	P3
n-heptanal	904.8	901								0.3	0.5	0.4
α-thujene	925.5	924	0.3	0.3	0.1					0.1	tr.	tr.
α-pinene	931.6	932	4.0	3.5	0.5	0.1				1.7	1.0	1.1
camphene	946.0	946	0.6	0.4	0.1					2.4	1.2	1.2
thuja-2,4(10)-diene	953.0	953								0.2	0.1	0.1
sabinene	973.0	969	6.5	6.6	2.1	0.1				0.2	0.3	0.3
β-pinene	975.2	974	3.8	3.4	1.3	0.1				0.7	0.3	0.5
myrcene	992.5	988	0.2	0.3	0.2							
isobutyl 2-methylbutyrate	1005.3	1004	0.1							0.3	0.3	0.2
trans,*trans*-2,4-heptadienal	1014.5	1005	tr.	tr.	tr.					0.5	0.7	1.0
isoamyl isobutyrate	1016.4	1007	0.2	0.2	0.2	tr.				0.1	0.1	0.1
α-terpinene	1017.1	1014	0.4	0.3	0.3	0.1				0.1		0.1
p-cymene	1025.4	1020	0.5	0.5	0.6	0.2			0.1	0.8	1.1	1.0
β-phellandrene	1029.1	1025	2.7	2.6	2.6	0.4	0.1	0.3	0.2			
1,8-cineole	1034.7	1026	43.3	44.0	30.6	5.8	1.2	0.4	2.4	61.1	75.0	68.5
cis-β-ocimene	1040.2	1032								0.1	0.4	0.2
trans-β-ocimene	1050.2	1044								6.4	0.7	0.6
γ-terpinene	1059.9	1054	0.6	0.5	0.4	0.1			0.1	0.3	0.2	0.2
cis-sabinene hydrate	1068.7	1065	0.3	0.5	0.5						0.4	0.3
trans-linalool oxide	1074.3	1177				tr.				0.2	0.1	tr.
α-terpinolene	1089.4	1086	0.5	0.4	0.5	0.2		0.1	0.1	2.3	2.3	2.6
rosefuran	1100.4	1091								0.3	0.3	0.3
linalool	1103.1	1095	0.8	1.0	1.0	0.1				0.2		
nonanal	1107.0	1100								0.4	0.7	0.5
vertocitral C	1117.6	1105								0.3	0.4	0.3
cis-*p*-menth-2-en-1-ol	1123.5	1118	0.1	0.1	0.1						tr.	
α-campholenal	1127.7	1122								0.2	0.1	0.3
trans-*p*-menth-2-en-1-ol	1142.2	1136	0.2	0.2	0.2					0.3	0.3	0.4

trans-verbenol	1147.4	1140			tr.					0.2	0.3	0.4
isobutyl hexanoate	1154.1	1149								1.3	2.0	1.5
pinocarvone	1163.3	1160	0.1	0.1	0.1	tr.				0.2	0.2	0.2
borneol	1168.3	1165	0.9	0.7	1.3	0.3	0.2	0.4	0.4	0.4	0.5	0.6
p-mentha-1,5-dien-8-ol	1170.4	1166								0.2	0.2	0.1
terpinene-4-ol	1180.8	1174	2.3	2.0	3.2	1.7	1.0	1.7	1.9	0.6	0.4	0.5
p-methylacetophenone	1189.3	1179				tr.				0.3	0.4	0.3
p-cymen-8-ol	1189.7	1179								0.2	0.2	0.2
α-terpineol	1195.1	1186	1.5	1.2	2.2	1.2	0.6	0.9	0.9	0.2	0.3	0.3
myrtenol	1199.4	1194	0.1							0.4	0.4	0.5
trans-piperitol	1211.4	1207								0.2	0.4	0.3
nerol	1232.3	1227	0.2	0.2	0.2						0.1	
cuminaldehyde	1242.7	1238								0.3	0.2	0.1
carvone	1247.1	1239								0.1	0.3	0.3
2-phenylethyl acetate	1260.2	1254	0.1		0.1					0.9	1.1	0.4
trans-2-decenal	1265.7	1260										0.3
2-*cis*-hexenyl valerate	1282.5	1282								0.2	0.1	0.1
bornyl acetate	1288.1	1287	0.1	0.1	0.1					0.6	0.3	0.4
2-undecanone	1297.7	1293	0.5	0.3	0.9	tr.			0.1	7.1	3.6	5.0
benzyl butanoate	1302.0	1297								0.3	0.2	0.2
δ-terpinyl acetate	1320.6	1316	0.7	0.7	0.9	0.2	0.1	0.2	0.2	0.2	0.2	0.3
δ-elemene	1341.7	1335				0.2	0.2	0.3	0.1			
exo-2-hydroxycineolacetate	1346.8	1354		0.1	tr.					0.1	0.4	0.4
α-terpinyl acetate	1357.7	1346	11.7	12.1	16.3	3.4	2.1	5.2	3.0	0.2	0.5	0.4
α-cubebene	1355.0	1349				10.8	15.0	13.2	5.8	0.4	0.1	0.1
eugenol	1364.9	1356	1.9	1.1	1.8	0.8	0.4	0.3	0.7	0.3	0.2	0.2
α-ylangene	1376.0	1373	0.1	0.2	0.4	1.8	0.5	0.4	2.2	0.6	0.2	0.5
α-copaene	1380.5	1374	0.1	0.1	0.3	3.8	4.0	3.4	3.0	0.1		0.1
trans-β-damascenone	1388.0	1383								0.2	0.1	0.1
β-bourbonene	1389.1	1387		tr.	0.1	0.1	0.1	0.1	0.1			0.1
β-elemene	1396.6	1392	0.4	0.7	1.1	4.5	5.9	6.7	4.0	0.8	0.3	0.5
methyleugenol	1415.0	1403	7.1	6.1	13.2	2.1	1.3	1.9	4.4			
β-caryophyllene	1424.4	1417	1.2	2.4	5.6	3.8	5.2	4.5	4.0	0.5	0.1	0.2
β-copaene	1433.7	1430			tr.	0.2	0.1	0.1	0.1			
α-guaiene	1443.4	1437	0.2	0.2	0.2	0.8	0.6	0.5	0.6	0.2		
6,9-guaiadiene	1448.1	1442	0.1	0.1	0.1	0.9	0.3	0.3	0.9	0.1		
α-himachalene	1453.8	1449	0.1	0.2	0.2	2.6	1.9	2.3	3.0	0.3		0.2
α-humulene	1458.7	1452	0.6	0.7	1.2	2.4	2.7	2.5	2.1	0.3		0.1
allo-aromadendrene	1465.8	1458	0.3	0.7		0.9	0.8	0.7	0.8	0.1		
γ-muurolene	1483.5	1478	tr.	0.2	0.1	3.4	3.7	3.8	3.2			0.2
germacrene D	1486.5	1484	0.3	0.5	0.3	2.4	2.1	2.2	3.1			
β-selinene	1492.1	1489	0.7	0.8	0.7	1.4	1.5	1.4	1.0	0.7	0.1	0.7
bicyclogermacrene	1501.1	1500	0.5	0.8	0.7	5.1	6.7	8.4	6.3	0.3	0.1	0.3
germacrene A	1510.4	1508	0.3	0.4	0.4	1.7	1.4	1.5	1.3	0.2		0.2
γ-cadinene	1520.4	1513	0.2	0.2	0.4	2.8	2.3	3.7	4.7	0.3		0.3
δ-cadinene	1529.4	1522	0.3	0.4	0.5	14.1	14.6	12.6	14.0	0.5		0.5
10-*epi*-cubebol	1536.3	1533		0.2	0.2	1.9	1.9	2.1	1.7			
α-cadinene	1544.8	1537	0.4	0.3	0.6	2.2	2.2	2.1	2.2	0.3		0.3
α-calacorene	1548.4	1544					0.5	0.6				

elemol	1555.2	1548		tr.	0.2							0.2
elemicin	1562.8	1555				0.7	0.7	0.7	0.8			
spathulenol	1583.7	1577	0.6	0.5	1.1	0.8	1.2	1.2	1.0		0.2	0.3
caryophyllene oxide	1587.6	1582	0.6	0.6	1.4	1.0	1.0	1.2	1.9	0.5	0.2	0.6
gleenol	1591.8	1586				0.2	0.3	0.3			0.1	
β-oplopenone	1600.9	1608			0.1	0.3	0.3	0.3	0.6			0.2
ledene oxide II	1608.3		0.1	tr.	0.1	0.2	0.2	0.2	0.2			
humulene epoxide II	1614.0	1608	0.1		0.2	0.8	0.5	0.5	1.4			0.2
junenol	1624.1	1618	0.1	0.1	0.2	0.2	0.2		0.4			
1-*epi*-cubenol	1636.6	1627			0.3	3.1	4.0	3.4	4.1			0.1
caryophylla-4,8-dien-5*β*-ol	1642.3	1639	0.3	0.3	0.4	3.6						
cubenol	1651.1	1645			0.2		4.2	3.2	4.7			
β-eudesmol	1657.2	1649	0.5	0.5	0.9	2.2	3.0	2.3	2.9	0.2		0.6
α-cadinol	1663.3	1652				0.2	0.3	0.3				
trans-calamenen-10-ol	1676.3	1668	0.1	0.1	0.3	1.1	1.4	1.1	0.5			0.2
cadalene	1683.5	1675							0.8			0.2
germacra-4,5,10-trien-1*α*-ol	1692.5	1685	0.1			0.8	1.0	0.8	1.3			0.2
10-*nor*-calamenen-10-on	1708.9	1702							0.3			
nootkatol	1716.8	1714							0.4			
curcumenol	1730.6	1733			0.1	0.1	0.3	0.2	0.3			
cis-lanceol	1747.7	1760							0.2			
β-costol	1774.5	1763					0.2					

*RIE=Kovat's (retention) index experimentally determined (AMDIS); RIL=Kovat's (retention) index (literature data); tr.=traces (<0.1 %).

According to the results of GC analyses, tested samples contained 99 constituents. Oils originated from leaf, bark and pericarp contained 57-64, 47-59, and 57-73 constituents, respectively. Number of over-one-percent ones, in average, was the smallest in pericarp oil (7), somewhat higher in leaf oil (11) and the biggest in the bark oil (27). In three samples of pericarp oils, the most abundant were 1,8-cineole (68.2%), 2-undecanone (5.3%), *trans*-*β*-ocimene (2.5%), *α*-terpinolene (2.3%), followed by camphene (1.6%), isobutyl hexanoate (1.6%) and *α*-pinene (1.3%). Simultaneously, in the same number of leaf oil samples, major constituents were 1,8-cineol (39.3%), *α*-terpinyl acetate (13.3%), methyleugenol (8.8%), sabinene (5.1%), *β*-caryophyllene (3.0%), *β*-pinene (2.8%), *α*-pinene (2.7%), *β*-phellandrene (2.6%) and terpinene-4-ol (2.5%), followed by *α*-terpineol (1.6%) and eugenol (1.6%). Main constituents of four oil samples isolated from bark were sesquiterpenes and their oxygenated derivatives (among these, 23 were present in concentrations ranging between 1-13.8%), and four further terpenoids: *α*-terpinyl acetate (3.4%), 1,8-cineole (2.5%), methyl-eugenol (2.4%) and terpinene-4-ol (1.5%).

Detail analyses of composition of tested oils, accomplished by GC technique, pointed at big dissimilarity between oil samples originating from different plant parts. From corresponding normalised chromatograms (GC/FID) of tested samples P1, L1 and B1 (Figure 1), it could be concluded that similarity between oils from leaf and pericarp is bigger than that of each of these samples in comparison to the bark oil.

Simultaneously, chromatographic profile of oils originating from the same plant parts, were always more similar to each other, than to remaining ones.

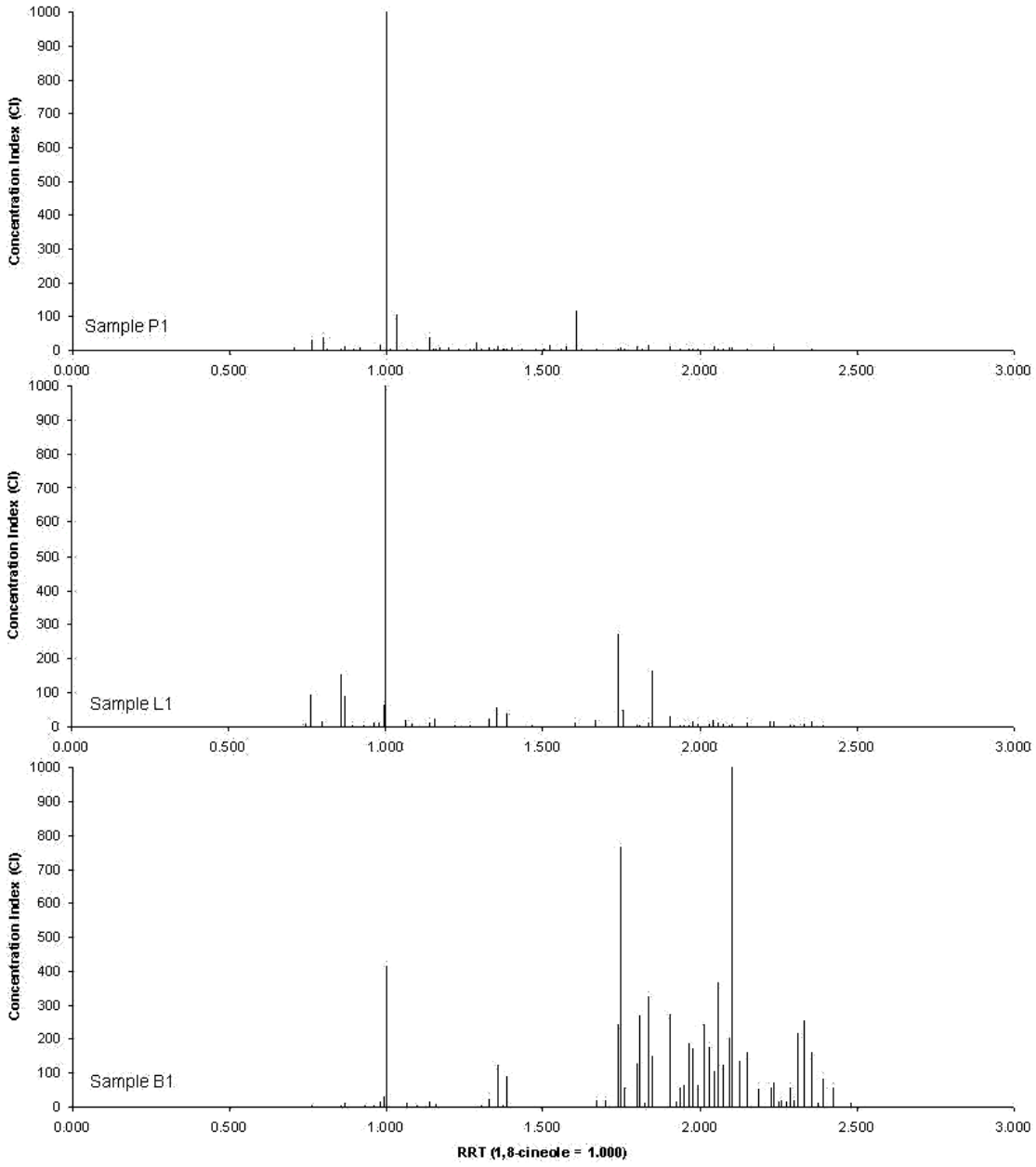

Fig. 1 - Normalised chromatograms of samples P1, L1 and B1 obtained by GC/FID.

Appropriate results obtained by LS/MS technique for same samples, are their mass spectra presented in Figure 2.

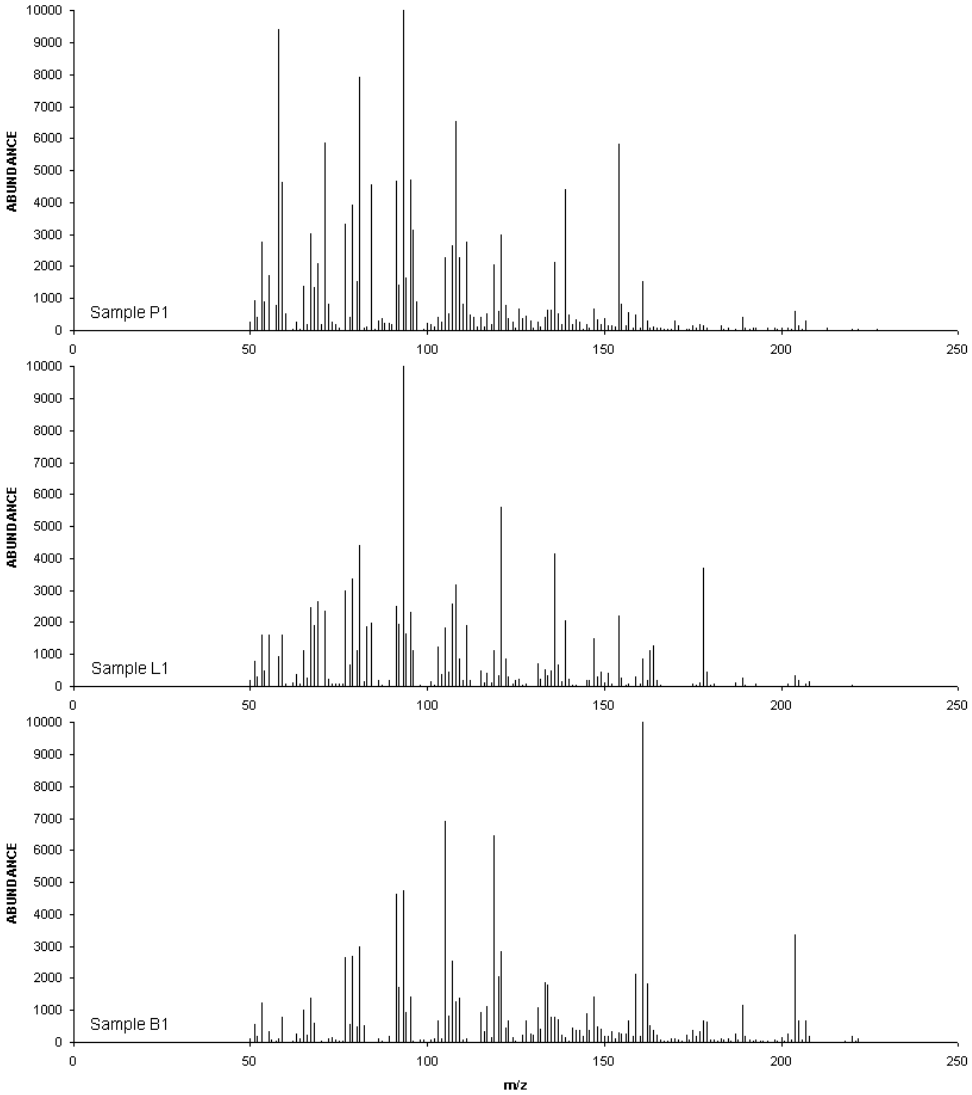

Fig. 2 - Mass spectra of samples P1, L1 and B1 obtained by LS/MS.

MS of samples P1, L1 and B1 were rather dissimilar, but highly informative and usable for preparation of searchable MS library. Testing of that library by available search engines (PBM, NIST) approved its usability in differentiation of our ten samples according to their origin. Nevertheless to their mutual big similarity, samples of the same origin (plant part) were also possible to differ by these common search engines.

Statistical processing of results by hierarchical cluster analysis (HCA), along with determination of derived stimulus configuration (according to Euclidean distance model) is summarised in Figure 3 and Figure 4. Resulting dendrograms certified clear and clean differentiation of tested oil samples by both used analytical techniques (Figures 3a and 3b). From the other side, derived stimulus configuration determined for the same samples and techniques (Figures 4a and 4b) pointed at the more homogeneous grouping of results obtained by LS/MS in comparison to those obtained by GC.

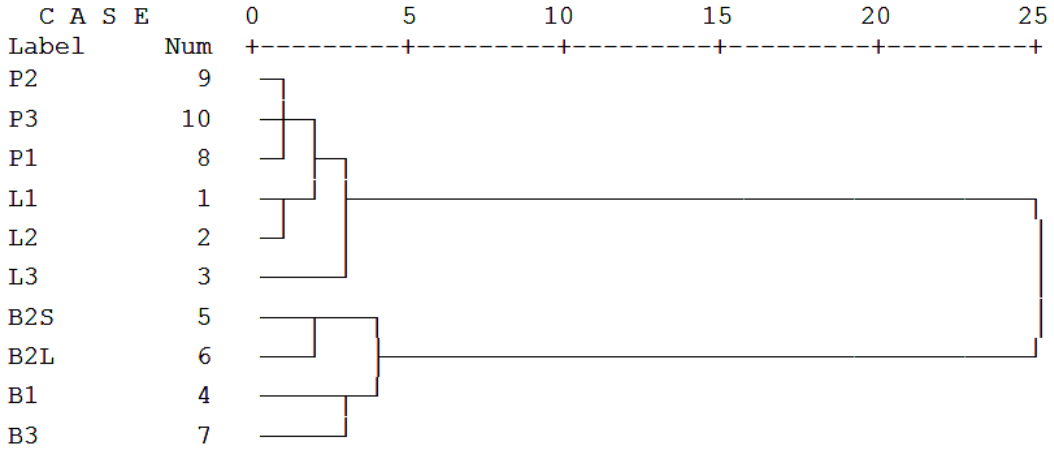

Fig. 3a - Dendrogram obtained by HCA of laurel essential oil analysis by GC/FID & GC/MS (average linkage between groups).

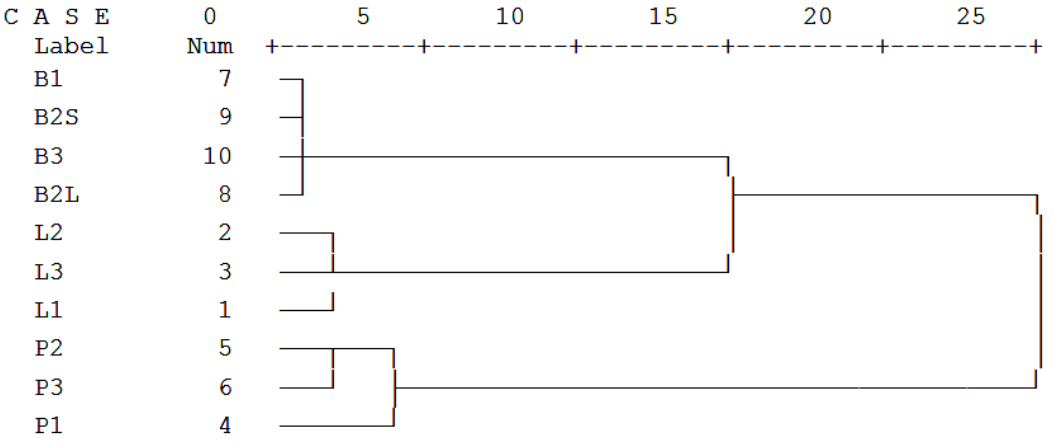

Fig. 3b - Dendrogram obtained by HCA of laurel essential oil analysis by LS/MS technique (average linkage between groups).

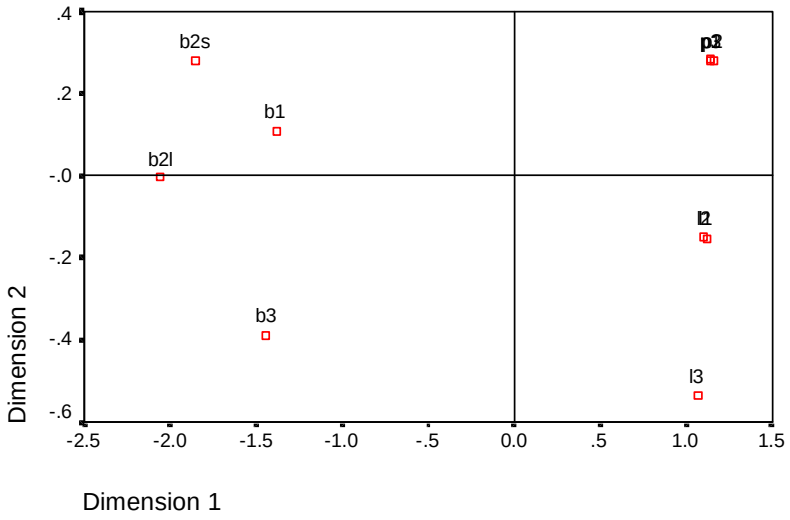

Fig. 4a - Derived stimulus configuration of results obtained by laurel essential oil analysis by GC/FID & GC/MS (Euclidean distance model).

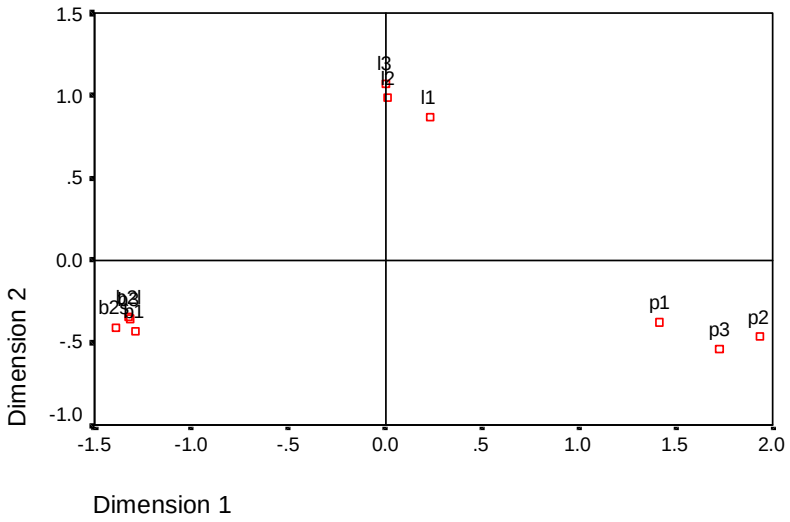

Fig. 4b - Derived stimulus configuration of results obtained by laurel essential oil analysis by LS/MS (Euclidean distance model)

Along with the high content of 1,8-cineole (30-44%), major characteristic of tested leaf oils was the biggest content of α-pinene, sabinene, β-pinene, β-phellandrene, linalool, terpinene-4-ol, α-terpineol, α-terpinyl acetate, eugenol and methyleugenol. Simultaneously, the biggest content of 1,8-cineole (61-75%),

camphene, *trans*,*trans*-2,4-heptadienal, *p*-cymene, *trans*-β-ocimene, α-terpinolene, isobutyl hexanoate, 2-phenylethyl acetate and 2-undecanone were recorded in pericarp oils. The most abundant constituents in bark oil samples were sesquiterpenes and their oxygen derivatives. With the exception of significantly lower content of linalool, content of other important leaf oil constituents were comparable to those from the literature.[1-6] According to the composition and the content of the most abundant constituent, tested oils from different parts of laurel can easily be differed.

Gas chromatographic analysis of tested samples reviled significant differences between oil samples originating from different part of laurel tree. These differences were very clear regarding the nature and the content of registered constituents, approving superiority of GC technique, whenever detail chemical characterisation of essential oil is needed. It should be noticed, however, that applied regimes required at least 60 minutes per sample for acquisition of raw chromatographic data, for each type of detection used (FID or MS). Mass spectra of tested samples acquired by LS/MS were also highly informative, enabling their differentiation upon the oil origin, but giving not even provisional information on the chemical composition of these oils. Time needed for MS acquisition could be estimated to up to 3 minutes per sample or at least 20-fold shorter in comparison to that needed for GC.

In comparison of GC and LS/MS in the characterisation of our samples, which were complex mixtures of terpenoids and related derivatives, resolution power of used chromatographic column was directly compared with resolution of MS detector employed. Each of normalised chromatograms presented in Figure 1 was designed on the basis of less than 100 pairs of data, since for that purpose, in the case of appropriate mass spectra given in Figure 2, 180 pairs of data were used. Furthermore, results of HCA of data obtained by GC and LS/MS (Figures 3a and 3b), point at sharper and cleaner clustering of samples of different origin just in case of LS/MS. Finally, determined derived stimulus configuration, set for different analytical approaches used, gave better (sharper) grouping of tested samples in the case of LS/MS technique (Figures 4a and 4b).

Conclusions

Differences in the composition of essential oils from different parts of laurel trees, wild growing in Montenegro, were determined for the first time. Dissimilarity of tested oils of different origin was not approved only by GC using two types of detectors, but by LS/MS followed by strong statistical analysis, too. It was approved once again that LS/MS technique presents unique powerful tool.

Acknowledgements*:*

The authors are grateful to the Ministry of Science and Technological Development of the Republic of Serbia who supported this study, through the project 173021.

References

Burdock G. A., Ed. (2002) - Fenaroli's Handbook of Flavour Ingredient, 4th Edition, CRC Press, Boca Raton, London, New York, Washington DC, p. 996.

Lawrence B.M., (1999) - Progress in essential oils – Oil of laurel, Perfumer & Flavorist, 24**,** 60-63.

Kovačević V., (1993) - Proučavanje osobina lovora (*Laurus nobilis*) u cilju proizvodnje etarskog ulja (PhD Thesis), Faculty of Agriculture, University of Belgrade, Belgrade.

Flamini G., Cioni P.L., Morelli I., (2002) - Differences in the fragrances of pollen and different floral parts of mail and female flowers of *Laurus nobilis*, J. Agric. Food Chem., 50, 4647-4652.

Kilic A., Hafizioglu H., Kollmannsberger H., Nitz S., (2004) - Volatile constituents and key odorants in leaves, buds, flowers and fruits of *Laurus nobilis* L., J. Agric. Food Chem. 52, 1601-1606.

Kovačević N. N., Simić M. D., Ristić M. S., (2007) - Essential oil of laurel (*Laurus nobilis* L., Lauraceae) from Montenegro, Chemistry of Natural Compounds, 43, 408-411.

Ristić M., Đoković D., Stevanov-Pavlović D., (2001) - Evaluation of a new analytical technique for characterisation of essential oils, Lekovite sirovine 21, 37-44.

Ristić M.S., Vuković G.L., Arsić I.A., Kovačević D.L., Đorđević S.M., (2006) - Building of database for the fast screening of flavours and fragrances by LS/MS technique, Proceedings from the Fourth Conference on Medicinal and Aromatic Plants of Southeast European Countries (IV CMAPSEEC), Iaşi (Romania), May 28-31, 2006 [Eds. Ghiorghiţă G., Stănescu U., Toma C., Alma Mater Publishing House, Iaşi (Romania), 2006], 499-504.

Ristić M.S., Đorđević S.M., Đoković D.D., Tasić S.R., (2007) - Setting a standard for the essential oil of chamomile originating from Banat, Acta Horticulturae, 749, 127-140.

Sandra P. and Bicchi C., Ed., (1987) - Capillary Gas Chromatography in Essential Oil Analysis, Dr. Alfred Huethig Verlag, Heidelberg, Basel, New York, 435 pp.

Martí M. P., Busto O., Guasch J.,(2004) - Journal of Chromatography A, 1057 (1-2), 211-217.

Pérez Pavón J., García Pinto C., Guerrero Peña A., Moreno Cordero B., (2008) - Headspace mass spectrometry methodology: application to oil spill identification in soils, Analytical and Bioanalytical Chemistry, 391, 599-607.

Lindinger W., Hansel A., Jordan A., (1998) - On-line monitoring of volatile organic compounds at pptv levels by means of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) medical application, food control and environmental research, International Journal of Mass Spectrometry and Ion Processes, 173, 191-241.