Terpenic profile of different *Rosmarinus officinalis* extracts

Neli-Kinga Olah[1,2], Daniela Benedec[3*], Sonia Socaci[4], Claudia Crina Toma[1], Lorena Filip[3*], Claudiu Morgovan[1] and Daniela Hanganu[3]

[1]Vasile Goldi Western University of Arad, Faculty of Pharmacy, 86 L. Rebreanu Street, Arad, Romania
[2]SC PlantExtrakt SRL, 407059, Rădaia, Cluj, Romania
[3]Iuliu Haieganu University of Medicine and Pharmacy, Cluj-Napoca, Faculty of Pharmacy, 12 I. Creangă Street, Cluj-Napoca, Romania
[4]University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Science and Technology, 64 Calea Floresti Street, Cluj-Napoca, Romania

Abstract: The Rosemary (*Rosmarinus officinalis* L.), a well-known medicinal and culinary herb, was studied to compare the terpenic profile of different extracts obtained from dry and fresh herb. There were studied the volatile oil extracted by hydro distillation from dry plant, the hydroalcoholic extracts obtained from fresh respectively dry plant and the glycerol macerate obtained from fresh plant, by GC-MS using headspace injection. The separated compounds were identified using a MS spectra library. The quantitative determination was performed by normalization respectively by calibration curve method for 1,8-cineole, alpha-pinene and D-limonene. The main separated compounds were alpha-pinene, 1,8-cineol, camphene, camphor, D-limonene and cymene. A significant difference was observed between the 4 samples volatile profiles. 1,8-cineole was found major component of the essential oil (VO-21.39%) and glycerol macerate (GM-35.60%), while and α-pinene was detected as the main constituent of the two tinctures (T-46.05%; MT-31.93%). The highest 1,8-cineol content, determined by calibration curve method, was found in the volatile oil, while the fresh plant hydroalcoholic extract was richer in α-pinene and D-limonene.

Keywords: *Rosmarinus officinalis,* terpenic profile, GC-MS, dry plants, fresh plants.

INTRODUCTION

Rosmarinus officinalis L., having the popular name rosemary and belonging to Lamiaceae family, is a very well-known culinary, aromatic, but also medicinal plant. For therapeutic purposes the flowering tips, the young shoots, the dry or fresh leaves can be used. The Rosemary leaves extracts have been shown to contain essential oils, polyphenols (rosmarinic acid, hesperidin, luteolin etc.), diterpenes (carnosic acid), triterpene acids (ursolic acid) and it possess anti-inflammatory antispasmodic, antinociceptive, antimicrobial, hepatoprotective, diuretic properties (Benedec *et al*., 2015; Erkan *et al*., 2008; Jordan *et al.*, 2013; Kontogianni *et al*., 2013; Olah *et al.,* 2016; Wojdylo *et al*., 2007). The essential oil contains as main components the 1,8-cineole (18.9-47.1%), α-pinene (7.6-19.2%), camphor (4,9-29.7%), and also other terpenic compounds like: camphene (3,3-12,8%), borneol (2.2-11.8%), bornyl acetate (0.7-3.8%), ß-caryophyllene, *p*-cymene, D-limonene, verbenone etc. (Erkan *et al.*, 2008; Jordan *et al*., 2013; Zaouali *et al*., 2010). Rosemary essential oil has shown parasiticide, antimicrobial, antifungal, antinociceptive, antioxidant activities (Bernardes *et al*., 2010; Olah *et al.,* 2016). A lot of traditional food receipts and studies demonstrate the possibility to use the rosemary leaves and oil as preservatives in different foods (Klancnik *et al*., 2009; Castano *et al*., 2010; Raiciu *et al*., 2010). The purpose of this study was to compare the terpenic profiles of different Rosemary extracts to highlight the similarities and the differences between the extracts chemical composition in order to obtain high-quality pharmaceutical preparations.

MATERIAL AND METHODS

The vegetal raw material and the preparation of extracts

The Rosemary leaves were supplied by Fares BioVital Laboratories Orastie (Hunedoara, Romania), being harvested from the organic cultures of the company. The fresh leaves were dried in controlled conditions. The Rosemary fresh young shoots were harvested from an organic culture near Oradea, Romania in 2013. The fresh vegetal material was botanical identified in the quality control laboratory of the company PlantExtrakt, Radaia, Cluj, Romania. A voucher sample was retained (Voucher No. 5679).

There were prepared 4 different extracts. The volatile oil (VO) was obtained by hydrodistillation in 3h, in a Clevenger-type apparatus, using the dry leaves (Eur. Ph., 2015).

The hydroalcoholic extracts were obtained by cold extraction (maceration). Twenty g of cut dry leaves were mixed with 100ml of 70% vol. ethanol (1:5 - dry plant: solvent). One hundred g of cut fresh young shoots were mixed with 140g of 90% ethanol, according to method described in European Pharmacopoeia. Both mixtures

**Corresponding author:* e-mail: dani_67ro@yahoo.com

were mixed 10 days, 2 times a day, and each time 10 minutes. After 10 days the liquids were separated from the mixtures and the remained plant material was pressed manually. The obtained extracts were preserved 5 days and then filtered thus result the tincture (T) from dry plant and the mother tincture (MT) from fresh plant (Eur. Ph., 2015).

The glycerol macerate (GM), used in Gemmotherapy, was obtained from the cut fresh young shoots. To 50g of vegetal material were added 150g of 96% ethanol and 150 g of glycerol, according to method described in European Pharmacopoeia (1:20- plant: solvent). The extraction was made by maceration (cold extraction) and the mixture was mixed 20 days, 2 times a day, each time 10 minutes. After 20 days the liquid was separated from the mixture and the remained plant material was pressed manually (Eur. Ph., 2015).

GC-MS analysis

The analyses were carried out on a GC-MS QP-2010 (Shimadzu, Kyoto, Japan) model gas chromatograph-mass spectrometer equipped with a CombiPAL AOC-5000 autosampler (CTC Analytics) (Socaci *et al*., 2013). The volatile compounds were extracted (head space extraction) by incubating the samples at 85^{o}C for 15min. After incubation an aliquot from the gaseous phase was injected in the gas-chromatograph injector and the volatile compounds were separated on a Zebron ZB-5ms capillary column of 50m x 0.32mm i.d and 0.25µm film thickness. The column oven temperature program was: 40^{o}C (5min.) to 150^{o}C with 4^{o}C/min, to 230^{o}C with 10^{o}C/min and held for 5min. The carrier gas was helium at 1ml/min flow rate, the ion source temperature and interface temperature were set at 250^{o}C and the MS mode was EI. The mass range scanned was 40-400u. The identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra libraries, NIST27 and NIST147. The quantitative determinations were performed by area normalization respectively by calibration curves method. The calibration curves for 3 identified volatile oil compounds were obtained in the same chromatographic conditions, using a concentration range of 0.5 to 5ml/ml. For the chromatographic analysis, α-pinene, 1,8-cineol, D-limonene purchased from Merck, Germany were used as standards.

STATISTICAL ANALYSIS

For all samples were made three individual determinations and the final results are the mean value of those three determinations. The graph and the calculations were perfomed using the Excel software program.

RESULTS

GC-MS method allows the identification and quantification of volatile compounds from complex mixtures as are the essential oils obtained from plants or the plant extracts. The identification is based on the comparison of MS spectra of the separated compounds with those of standard MS spectra from spectra libraries. The quantitative determination is made by area normalization for each separated compound respectively for 4 compounds by calibration curves method, which is more accurate. The GC chromatograms obtained for the studied extracts (volatile oil, tincture, mother tincture and glycerol macerate) are showed in fig. 1. The calibration curve and MS spectra of standard and separated compounds are presented in fig. 2 and 3. table 1 and 2 showed the identified compounds with retention times respectively the quantitative results.

DISCUSSION

As can be seen there were identified: 19 terpenes in VO, 11 in T, 18 in MT and 15 in GM, a smaller number of compounds was found in tincture. The identification is based on the comparison of MS spectra of the separated compounds with a spectra library, and the similarity percentage being more than 90% in each case.

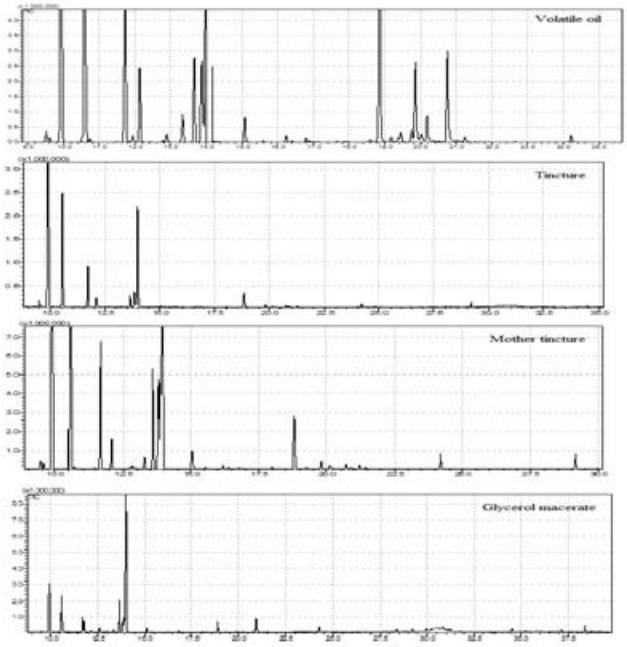

Fig. 1: The GC chromatograms of the volatile compounds in *Rosmarinus officinalis* extracts: (A) GC chromatogram of volatile oil; (B) GC chromatogram of tincture; (C) GC chromatogram of mother tincture; (D) GC chromatogram of glycerol macerate.

The main compounds determined in the studied Rosemary samples were: α-pinene, ß-pinene, 1,8-cineole, camphene and camphor. As can be seen from the tables, there are differences between the two samples of tinctures (T and TM) and the two samples of oil and macerate (VO and GM).

Table 1: The identified terpenes from different *Rosmarinus officinalis* extracts

No.	Compound	Retention time, min.			
		VO	T	MT	GM
1.	α-thujene	9.6	-	-	-
2.	α-pinene	9.9	9.9	9.9	9.9
3.	camphene	10.6	10.6	10.6	10.6
4.	trans-verbenol	10.7	-	10.7	
5.	ß-pinene	11.7	11.7	11.7	11.7
6.	ß-myrcene	12.1	12.1	12.1	12.1
7.	α-phellandrene	12.9	-	12.9	--
8.	α-terpinene	-	-	13.3	13.4
9.	p-cymene	13.7	13.6	13.6	13.7
10.	D-limonene	13.9	13.8	13.8	13.9
11.	1,8-cineole	14.0	14.0	14.0	14.0
12.	γ-terpinene	15.1	-	15.0	15.1
13.	terpinolene	16.2	-	16.2	16.3
14.	camphor	18.9	18.8	18.8	18.9
15.	iso-menthone	19.2	-	19.2	19.3
16.	pinocamphone	19.4	-	-	-
17.	borneol	19.9	19.8	19.8	19.9
18.	terpineol	20.8	-	-	-
19.	verbenone	21.2	-	21.2	-
20.	bornyl acetate	-	24.2	24.2	24.2
21.	caryophyllene	29.2	29.2	29.2	29.2

Table 2: The quantitative determination of identified terpenes from different *Rosmarinus officinalis* extracts

No.	Compound	Concentration (by normalization), %							
		VO		T		MT		GM	
1.	α-thujene (H)	0.12		-		-		-	
2.	α-pinene (H)	13.57		46.05		31.93		15.12	
3.	camphene (H)	11.59		18.36		14.33		11.29	
4.	trans-verbenol (O)	0.09		-		-		-	
5.	ß-pinene (H)	5.57		6.63		7.80		4.49	
6.	ß-myrcene (H)	2.04		1.43		1.76		0.60	
7.	α-phellandrene (H)	0.21		-		-		-	
8.	α-terpinene (H)	-		-		0.69		0.82	
9.	p-cymene (H)	2.60		2.06		6.11		8.57	
10.	D-limonene (H)	2.95		2.41		5.64		3.81	
11.	1,8-cineole (O)	21.39		17.50		17.82		35.60	
12.	γ-terpinene (H)	0.52		-		0.91		1.15	
13.	terpinolene (H)	0.13		-		0.22		0.19	
14.	camphor (O)	9.97		2.47		3.57		2.89	
15.	iso-menthone (O)	0.08		-		0.09		0.14	
16.	pinocamphone (O)	0.12		-		-		-	
17.	borneol (O)	2.64		0.47		0.47		0.29	
18.	terpineol (O)	3.00		-		-		-	
19.	verbenone (O)	0.11		-		0.21		-	
20.	bornyl acetate (O)	-		0.45		0.93		1.21	
21.	caryophyllene (H)	0.26		0.84		1.05		0.66	
	Total (%)	76.95		98.67		93.53		86.83	
		H:39.56	O: 37.40	H: 77.78	O: 20.89	H: 70.44	O: 23.09	H: 46.7	O: 40.13
		Concentration (by calibration curve), µl/ml							
1.	α-pinene	10.30		2.57		10.70		1.82	
2.	D-limonene	1.97		0.47		2.74		0.82	
3.	1,8-cineol	9.70		1.20		6.30		4.04	

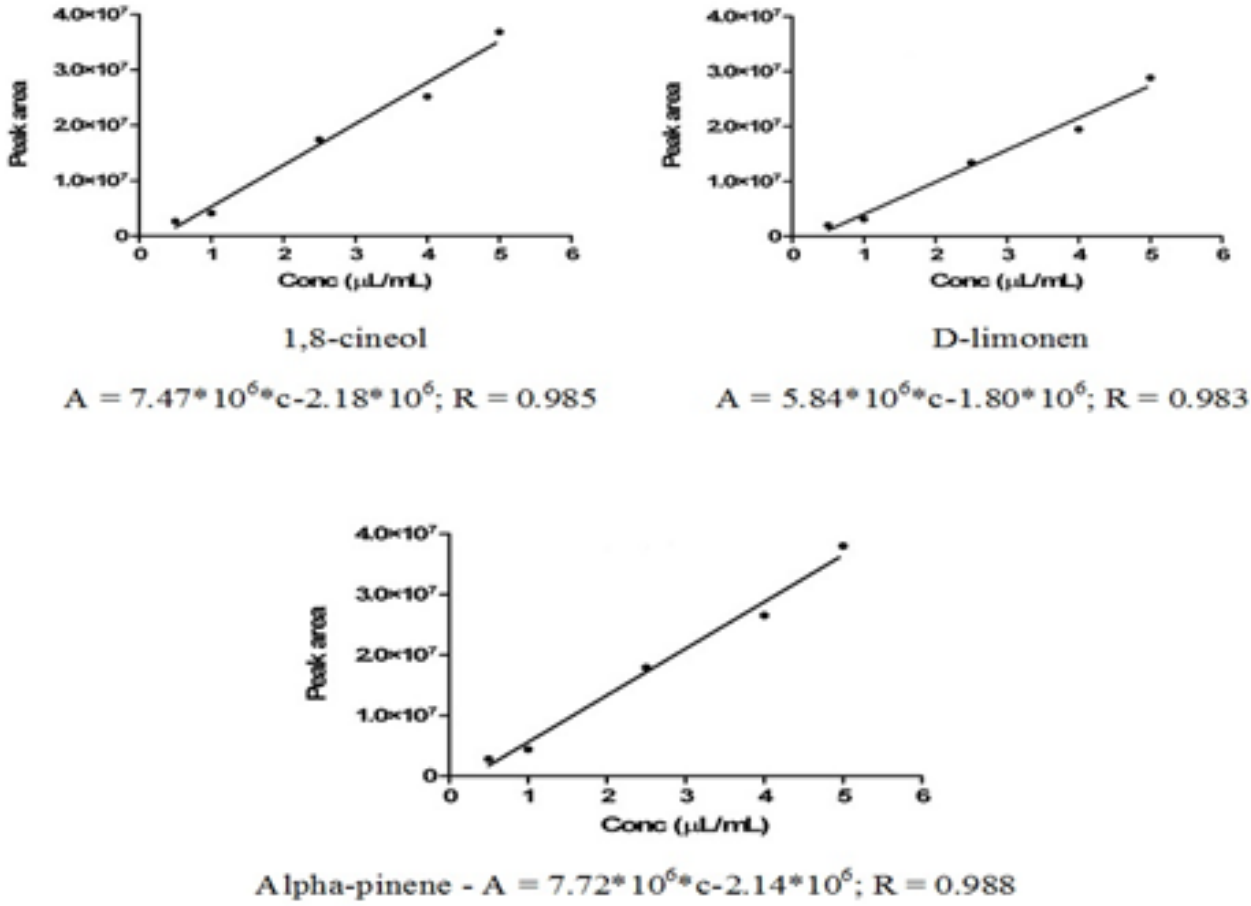

Fig. 2: The calibration curves.

Thus, the essential oil (VO) and the glycerol macerate (GM) were found to be rich in 1,8-cineole (21.39% and 35.60% respectively), α-pinene (13.57% and 15.12% respectively) and camphen (11.59% and 11.29% respectively). The amount of 1.8-cineole was 1.66 times greater in GM that in VO, this compound has been synthesized since the youthful phase. If we compare the results obtained by other researchers for Rosemary volatile oil we observe that our VO has comparable amount of α-pinene, 1,8-cineole, camphene and α-terpineol, but less borneol and camphor (Jordan *et al*., 2013; Erkan *et al.*, 2008; Zaouali *et al*., 2010). Concerning the chemical composition of tinctures, it was found that: α-pinene was the main constituent (T - 46.05%, MT - 31.93%), and other compounds were found in similar quantities were: 1,8-cineole (17,50% and 17,82% respectively) and camphene (18,36% and 14,33% respectively). Camphor (bicyclic monoterpene) was only found in large amounts in VO (9.97%). The found terpenes can be classified in 2 main classes: hydrocarbons (H) and oxygenated hydrocarbons (O). The tinctures (T and MT) contain more terpenic hydrocarbons (77.78% in T; 70.44% in MT) than the oxygenated ones (20.89% in T; 23.09% in MT), compared to the other samples (VO, GM), where the ratio between hydrocarbons and oxygenates is around 1.

It could be seen similarities in hydrocarbons – oxygenated hydrocarbons ratio at hydro alcoholic extracts due by the use of the same type of solvent, the ethanol, which extracted in similar way the volatile, lipophilic compounds, both from dry or fresh plant. In VO and in GM extract the ratio was almost 1 to 1, the hydrocarbons being a sensitive higher amount. In tinctures, the ratio was inverted in favor of terpenic hydrocarbons.

The results obtained showed that the oxygenated compounds suffer changes during the heating of plants at drying or hydro distillation.

The qualitative and quantitative chemical analysis of the terpenes from the four studied samples highlights: the influence of the solvent, the way of extraction, the processing of the plant product and the harvesting phase. Knowledge of these parameters is important for obtaining pharmaceutical preparations with an adapted chemical composition to certain therapeutic uses.

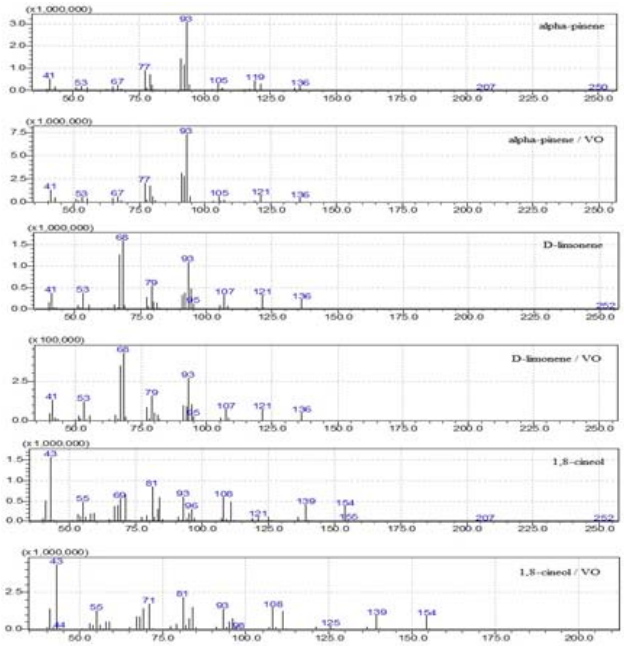

Fig. 3: The MS spectra of standards and components separated from VO.

CONCLUSIONS

The volatile oil, glycerol macerate, tincture and mother tincture were analyzed by GC-MS using headspace injection and then the terpenic profile of the 4 samples was compared. Although, the chemical study revealed that the same more important identified terpenes in these four samples, the percentage composition of major compounds were different in these samples. The major components of the essential oil and glycerol macerate were 1,8-cineole, α-pinene and camphen and the tinctures were found to be rich in α-pinene. Thus, the evaluation of the qualitative and quantitative differences of the most important terpenes may allow the selection of those extracts suitable from the chemical composition point of view correlated with a certain therapeutic action.

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