Volume 88(1). Published April, 01, 2022
www.jnsciences.org
E-ISSN 2286-5314

JOURNAL OF NEW SCIENCES
WWW.JNSCIENCES.ORG

Effects of grinding and bioclimatic variation on the quality and composition of the Tunisia rosemary essential oils

*WALID YEDDES[1, 2], HAMZA GADHOUMI1, TAYCIR GRATI AFFES [1], HAMMAMI MAJDI[1] *, SAIDANI-TOUNSI MOUFIDA[1]*

[1]*Laboratory of Aromatic and Medicinal Plants, Borj Cedria Biotechnology Center, BP901, 2050 Hammam-Lif Tunisia.*
[2]*Faculty of Science of Bizerte, University of Carthage, Jarzouna7021Tunisia.*

***Corresponding author:** hammamimajdi@hotmail.com

Abstract –The aim of our investigation is to determine the effects of grinding and bioclimatic variation on the quality and composition of the Tunisian rosemary essential oils. The composition of essential oils of rosemary (Rosmarinus officinalis. L) populations varied significantly according to seasons and bioclimatic stage (sub-humid, upper-semi-arid and upper-arid). Essential oils were extracted by Clevenger from entire or grinded dried rosemary leaves. The composition of essential oils was determined by gas chromatography coupled with mass spectrometry (GC-MS). Our results showed that the main compounds found are: 1.8 cineol, camphor, alpha terpinol, bornyl acetate and linalool. The number of compounds was higher during the flowering stage in January. The grinding of leaves into powder results in increase essential oils yields and releases more rosemary volatile compounds. The heatmaps and hierarchical clustering of total identified volatile compounds in essential oil extracted from rosemary leaves reveals that the sub-humid and the upper-semi-arid climatic regions show the highest yield of 1.8 cineol and the lowest yield of camphor. The opposite result was observed in the upper-arid region.

Keywords: *Rosmarinus officinalis L.*, chemotype, chromatography, essential oil, heatmap

1. Introduction

Aromatic herbs are commonly used as additives in foods to improve their flavor and organoleptic properties. Their medicinal, therapeutic, and aromatic potential is related to their secondary metabolites, such as essential oils, which have antimicrobial, (Almela, Sánchez-Muñoz, Fernández-López, Roca, & Rabe, 2006; Pintore et al., 2002) spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic activities (Bozin, Mimica-Dukic, Samojlik, & Jovin, 2007). Rosemary (*Rosmarinus officinalis* L.) bioactive substances have been cited in phytotherapy and food industries studies (Daferera, Ziogas, & Polissiou, 2000; Faleiro, Miguel, Guerrero, & Brito, 1997; Koschier & Sedy, 2003). Rosemary is an evergreen sclerophyll that colonizes coastal habitats of the Mediterranean "maquis" where it grows on calcareous substrates with scant humus and high aridity (Lo Presti et al., 2005). Rosemary is a kitchen herb used to add a spicy flavor to foods; it was traditionally used as an antiseptic, astringent, and food preservative before the invention of refrigeration. Rosemary's antioxidant properties are still used to extend the shelf life of prepared foods (Cuvelier, Richard, & Berset, 1996; Wada et al., 2004). Rosemary oil (RO) is popular in the Mediterranean region as a culinary additive it has been traditionally used as medicine (González-Minero, Bravo-Díaz, & Ayala-Gómez, 2020) or healthcare supplements to cure arthritis, diabetes or care for memory loss and hair restoration (El Omri et al., 2010). Besides their therapeutically applications, essential oils are widely used in the cosmetic industry to produce various perfume waters, bathing essences, hair lotions and shampoos. They are also used as components of disinfectants and insecticides (Boelens, 1984). Essential oils are found in glandular trichrome located in leaves. They are extracted using various isolation methods, such as hydrodistillation, steam distillation or organic solvent extraction (Lo Presti et al., 2005). The present work analyses yield and quality of rosemary EO extracted from twelve spontaneous populations located in different bioclimatic areas in Tunisia at different stages of their development.

2. Materials and methods

2.1. Plant material

Wild rosemary (*Rosmarinus officinalis* L.) was collected in summer and winter in twelve sites from northern, central and southern Tunisia (Table 1). Rosemary populations belong to three distinct ecological areas: semi-arid superior, sub-humid and arid superior. The three bioclimatic stages were defined by Emberger (1966) rainfall temperature coefficient, Q2 = 2000P/M2 − m2, in which P is the mean of annual rainfall (mm), M is the mean of maximal temperatures for the hottest month and m is the mean of minimal temperatures for the coldest one. Emberger's coefficient, Q2, is an ecological parameter which has been used as an ecological site descriptor in many studies (Khiari & Boussaid, 2000). Fresh aerial parts of rosemary leaves were left to dry at ambient temperature for one week. Each sample is split in two, one consisting of dried leaves, the second is and grinded using a commercial mixer for 3 minutes.

Table 1 : Collection zones parameters of *Rosmarinus officinalis* populations used in our study

Population Code	Collection region	Bioclimatic zone	Altitude (m)	Rainfall (Mm/year)	Latitude	Longitude
ZG	Dj. Zaghouan		166		36°54'1268N	10°07'712E
HZ	Hammam Zriba	Usa $45 < Q_2 < 70$	255	500-600	36°35'4247N	10°18'9692E
JM	Dj. Mansour		571		36°29'4497N	9°80'9144E
BK	BarrageKhmaeis		443		35°97'9132N	9°46'8709E
JB	Dj. Bargou		865		35°91'0264N	9°46'5738E
FT	Fajj Tfall		763		35°37'6085N	9°17'4437E
JF	Dj. Frigha		742		35°53'3499N	9°02'3661E
JS	Dj. Sammam	Sh 45 < Q2<70	679	400-500	35°24'6227N	8°76'0712E
JC	Dj. Chaambi		935		35°16'8152N	8°67'9429E
CT	Chaab el Tweel	Ua $23 < Q_2 < 35$	234	100-150	33°58'5876N	10°08'2104E
EM	El Amra		305		33°53'0154N	10°09'9477E
TJ	Toujane		490		33°47'1878N	10°13'645E
DT	Dhkilla – Toujane		530		33°45'5145N	10°15'4324E

Sh: sub-humid; Usa: Upper semi-arid; Ua: Upper arid; Q_2: bioclimatic zones were defined according to Emberger (1966) pluviometric coefficient.

2.2. Extraction procedure

A quantity of 100g from rosemary leaves, entire or grinded were put in the distilled water. Essential oils were extracted by hydro distillation using a Clevenger type apparatus for 3 hours after yield kinetic optimization. Essential oils were stored in glass vials with Teflon sealed caps at -20 ± 0.5 °C in darkness.

2.3. Gas Chromatography - Mass Spectrometry (GC-MS) analyses

The analysis was carried out on Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an electronic pressure control injector, a flame ionization detector and an HP Innowax (polyethylene glycol capillary, Agilent Technologies, Palo Alto, CA, USA) column (30 m x 0.25 mm; 0.25 µm). The GC/MS coupling made it possible to identify volatile compounds. The re-leased ions will be classified according to their mass/charge ratio (m/z). The analysis is carried out by a chromatograph coupled to an Agilent (Agilent Technologies, Palo Alto, CA, USA) mass spectrometer (5975C inert XL MSD) and electron impact ionization (70 eV). An HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) coated with 5 % phenyl methyl silicone and 95 % dimethylpolysiloxane (Agilent Technologies, Palo Al-to, CA, USA) was used.

3. Results and Discussion

3.1. Effect of grinding on identified volatile compounds number

Chromatographic analysis of the different essential oils samples showed remarkable variability in the number of volatile compounds detected. Rosemary powder gave the highest values for the number of volatile compounds. On the other hand, the leaves always give fewer identified compounds. The highest numbers are recorded for oils extracted from crushed rosemary leaves. Indeed, for the HZ region, we could identify 60 volatiles against 35 from the same sample of unground leaves. During flowering stage in January (Figure 1) some rosemary sample showed an important number of compounds such as (JB, JF, and JS). This is in agreement with the work of (Tischer et al., 2017) who showed that the grinding of *Baccharis articulata* could influence the quality of the oil obtained after hydrodistillation. This finding shows that grinding enhanced the number of detected compounds probably by rupturing the glandular trichomes which release their contents (Bousbia et al., 2009).

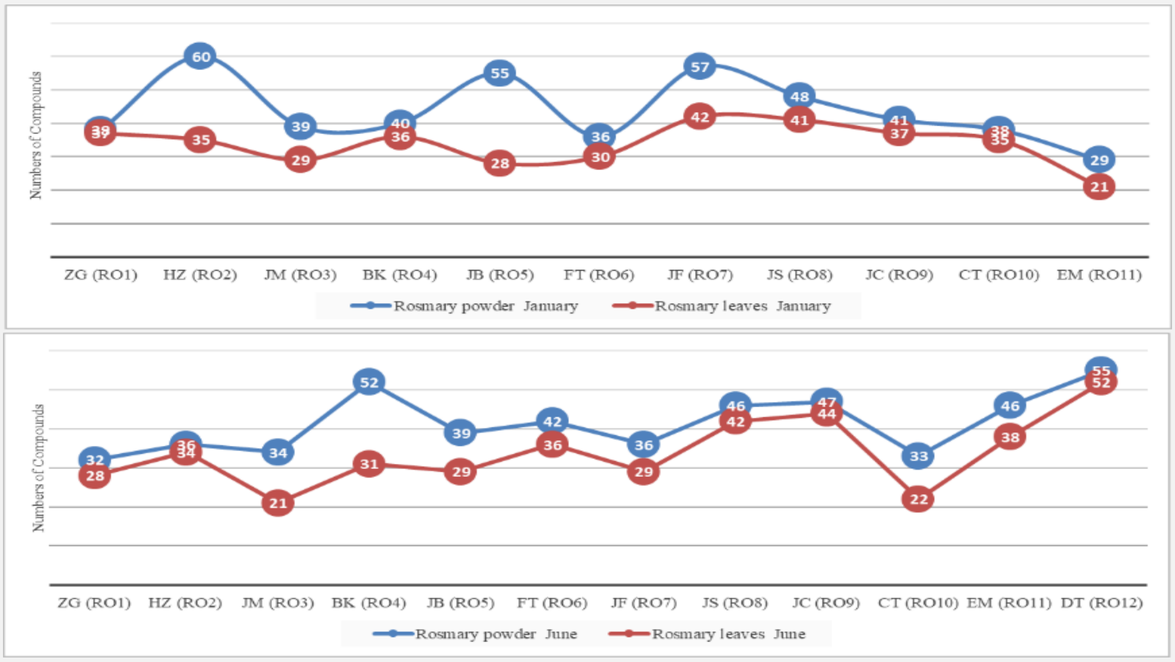

Figure 1. Variation of number of compounds of rosemary essential oils

3.2. Effect of grinding on essential oil yields

Figure 2 clearly shows that the yields of essential oils extracted from the crushed leaves are higher than that unground ones. For the flowering stage, the crushed rosemary leaves collected in the HZ region reached a yield of 2.1%. On the other hand, the best yields are recorded with the regions JC (2.2%) and DT (2.16%) for the samples collected during the summer (June). The lowest yields are recorded with non-crushed leaves for the regions of JM (0.86%), BJ (0.93%) and JB (0.98%). This variation was consolidated by the works of Ben Hassine et al. (2021); Marotti and Piccaglia (1992); Tischer et al. (2017) who found that grinding influences the efficiency of extraction of essential oils from different sources of plant material. Conversely, Hmaied, Bouafif, Magdouli, Braghiroli, and Koubaa (2019) has reported that the pre-treatments of forest products reduce the yields of essential oils in these products.

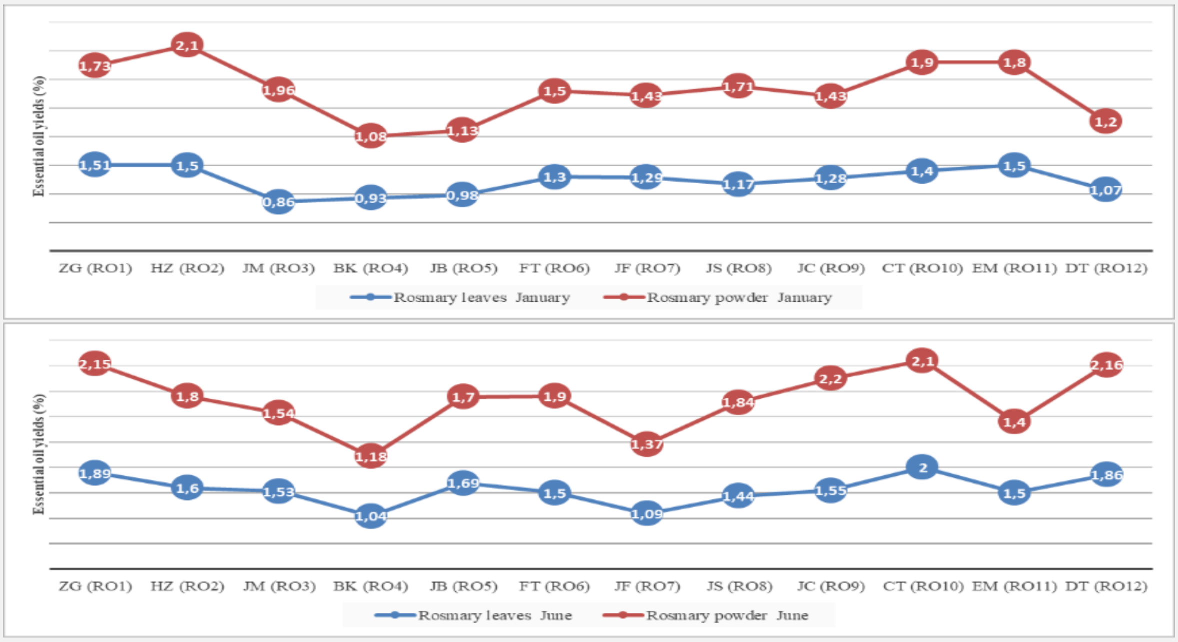

Figure 2. Variation of yields of rosemary essential oils

Volume 88(1). Published April, 01, 2022
www.jnsciences.org
E-ISSN 2286-5314

3.3. Effects of bioclimatic variation on the composition of the rosemary essential oils

The comparative examination of the primary chemical composition of rosemary oils according to the origin is shown in table 2. We propose a comparative study of rosemary oils' main chemical composition. Results showed that the Tunisian rosemary oils was found to be rich in 1.8 cineole (12.1 % - 59.38 %), camphor (9.98 % - 36.3 %), α terpineol (1.75 % - 7.51 %), bornyl acetate (0.03 % - 3.34 %) and linalool (0.11 % - 1.68 %).

They represent the main chemotypes because they have the best yield approved by the GC-MS, and tend to be the chemotypes that specifies the regions studied" to '1.8 cineole and camphor represent the main chemotypes When we consider the variation in chemical composition of the rosemary essential oil and the change of bioclimatic stage, we find that a close relationship that affects the metabolism of the plant studied." to "Our study revealed that a close relationship between the variation in chemical composition of the rosemary essential oil and the change of bioclimatic stage is find.

Table 2. Comparative study of the main chemical composition of rosemary oils

			ZG	HZ	JM	BK	JB	FT	JF	JS	JC	CT	EM	DT	TJ
1,8 Cineole	**Leaves**	**January**	49.43	32.13	50.57	52.57	47.41	38.08	58.86	35.35	57.32	26.11	33.52	31.3	32.5
		June	43.73	38.13	53.71	33.14	38.04	42.88	42.33	38.98	37.84	29.16	21	20.08	21.3
	Powder	**January**	53.9	27.29	50.96	52.39	46.54	38.86	58.58	35.51	55.85	28.4	33.12	29.78	32.36
		June	45.84	48.03	53.71	28.39	49.39	59.38	47.2	34.13	43.57	26.9	12.1	21.2	10.96
Camphor	**Leaves**	**January**	14.24	15.03	15.83	11.7	19.61	15.3	9.98	18.19	15.85	29.35	28.42	36.3	32.69
		June	16.4	13.18	12.86	14.42	12.1	13.83	13.39	20.25	12.9	34.14	22.48	20.31	22.36
	Powder	**January**	14.3	14.41	15.8	13.4	22.23	18.23	12.05	20.07	17.09	35.31	28.48	31.01	32.6
		June	17.51	15.67	12.86	15.45	10.49	12.95	14.56	18.66	13.57	33.44	21.64	21.43	21.6
Alpha. Terpineol	**Leaves**	**January**	4.08	4.89	4.24	4.62	4	5.64	4.65	5.9	3.84	2.19	2.3	3.3	3.2
		June	2.87	-	4.96	4.03	4.89	6.3	6.3	6.09	6.27	1.75	3.46	3.51	2.3
	Powder	**January**	3.8	5.03	4.18	4.81	4.32	5.83	5.29	7.51	-	-	2.61	2.8	2.9
		June	3.49	3.49	4.96	4.32	4.18	4.65	5.42	6.32	6.28	2.03	3.6	3.73	3.22
Bornyl acetate	**Leaves**	**January**	0.96	-	0.56	0.65	0.82	0.7	0.78	0.03	0.13	2.63	1.47	2.32	2.36
		June	0.79	1.13	0.57	-	-	-	-	-	-	2	-	-	0.32
	Powder	**January**	1.03	-	0.56	0.82	0.72	-	0.79	-	0.13	3.34	1.44	2.12	1.22
		June	1.007	0.65	0.57	-	0.5	0.45	-	-	-	2.62	-	2.89	2.69
Linalool	**Leaves**	**January**	0.6	0.77	0.51	0.45	0.3	1.25	0.76	1.06	0.81	-	0.35	0.11	0.21
		June	0.74	0.93	0.52	0.75	0.63	0.97	1.11	1.68	0.94	0.14	0.13	0.14	0.13
	Powder	**January**	0.5	0.7	0.5	0.49	0.41	1.22	0.82	1.17	0.78	0.12	0.29	0.13	0.12
		June	0.69	0.88	0.52	0.95	0.48	1.14	1.05	1.56	0.96	0.15	0.13	0.16	0.18

3.4. Heatmap and hierarchical clustering of total identified volatile compounds in essential oil

For the whole leaves harvested in January, the classification showed that the upper-arid stage was well identified by the grouping of three regions EM, CT and DT. The other two floors were not classified according to the bioclimatic floor. For untreated leaves harvested in June, a similar trend was noted. EM, TJ and DT came together into one cluster and CT withdrew to create a single cluster. The crushing of leaves harvested in January influenced the classification of essential oils. A remarkable cluster was recorded by the regrouping of two regions belonging to the Upper arid stage (CT and TJ). The other regions are not classified by an order respecting their belonging to the bioclimatic stages. The classification of essential oils obtained from leaves harvested in June and crushed before hydrodistillation did not show any classification according to bioclimatic stages except for the CT and TJ regions. The composition of rosemary essential oils is endowed with great variability. Clustring showed a similarity between regions which do not belong to the same bioclimatic stage. This similarity is due to other pedological factors such as the quality of the soil (Yeddes et al., 2018), costal position (Farhat, Hammami, Cherif, & Nasraoui, 2020),

JOURNAL OF NEW SCIENCES
WWW.JNSCIENCES.ORG

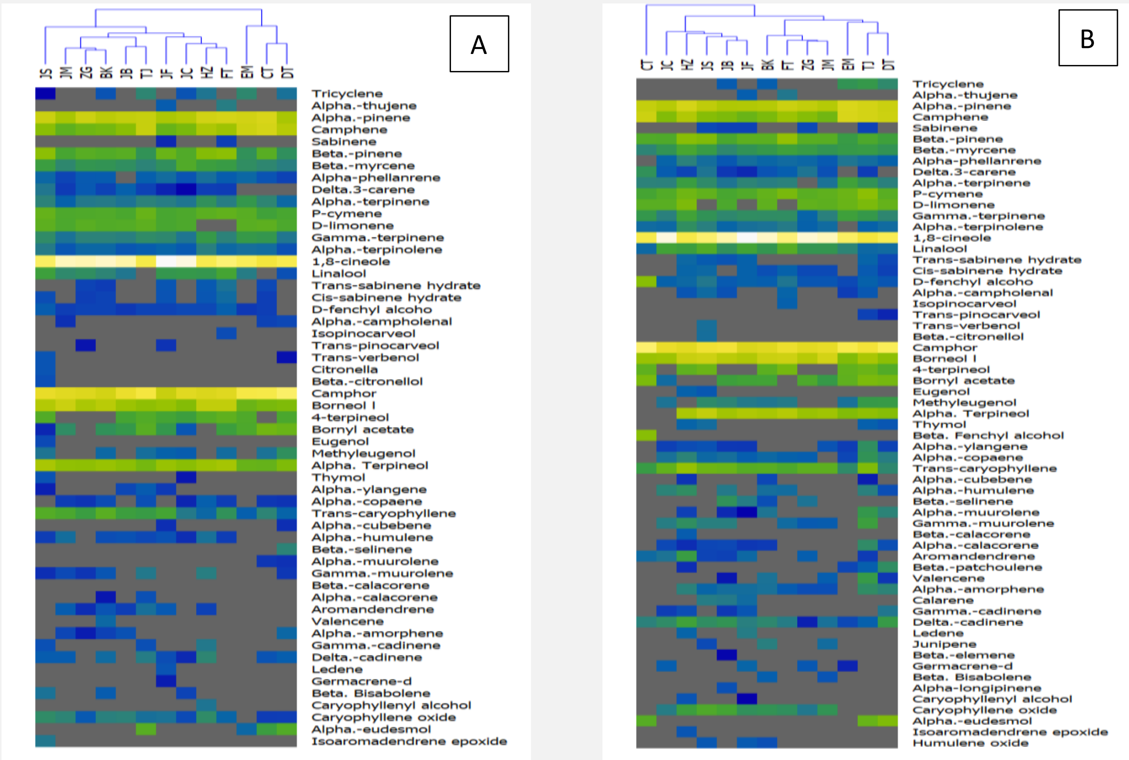

Figure 3. Heatmap and hierarchical clustering of total identified volatile compounds in essential oil extracted from rosemary leaves, A for January and B for June, the color varies from blue to yellow for high values to low values, the gray color indicates the absence of this compound.

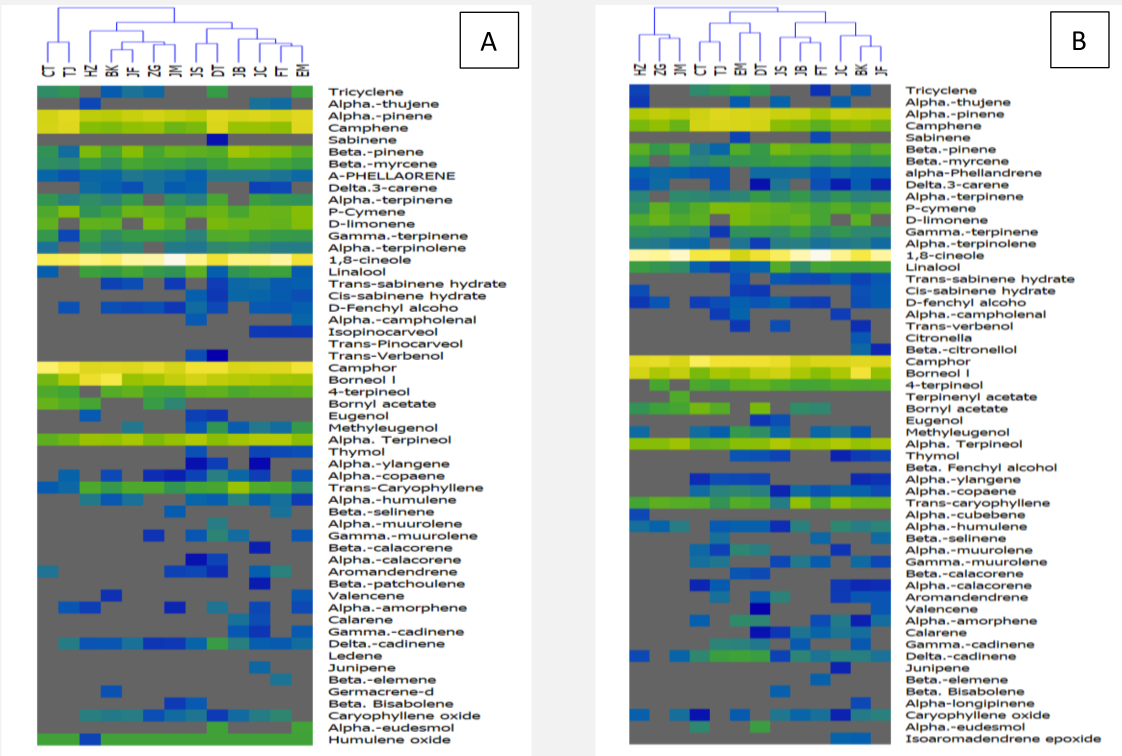

Figure 4. Heatmap and hierarchical clustering of total identified volatile compounds in essential oil extracted from crushed rosemary leaves, A for January and B for June, the color varies from blue to yellow for high values to low values, the gray color indicates the absence of this compound.

4. Conclusion

Pre-treatment of rosemary leaves prior to hydrodistillation increased efficiency the number of volatile compounds identified and in yields. Grinding these leaves improves the essential oils yield while maintaining a good quality of these oils, likewise, we can conclude that rosemary essential oils extracted from plants samples collected from three different bioclimatic stages in Tunisia showed a significant difference in their chemical composition. This variation was related to climate distinction, the pedological composition which induce an important change in the synthesis pathways involved in the biosynthesis of some bioactive compounds such as volatile compounds. The essential oil yield of rosemary samples ranged from 0.89 to 2.16 %, with 60 volatile compounds identified, the majority of oils contains 1,8-cineole, camphor, alpha-pinene, camphene, and borneol. The first separation of two population groups in accordance with the varietal subdivision was revealed by hierarchical clustering done on essential oil contents of all these samples. Depending on this composition, each cluster was subdivided into subclusters.

5. References

Almela, L., Sánchez-Muñoz, B., Fernández-López, J. A., Roca, M. J., & Rabe, V. (2006). Liquid chromatograpic–mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. Journal of Chromatography A, 1120(1-2), 221-229.

Ben Hassine, D., Kammoun El Euch, S., Rahmani, R., Ghazouani, N., Kane, R., Abderrabba, M., & Bouajila, J. (2021). Clove Buds Essential Oil: The Impact of Grinding on the Chemical Composition and Its Biological Activities Involved in Consumer's Health Security. BioMed Research International, 2021.

Boelens, M. (1984). Essential oils and aroma chemicals from Eucalyptus globulus Labill. Perfumer flavorist, 9(6), 1-14.

Bousbia, N., Vian, M. A., Ferhat, M. A., Petitcolas, E., Meklati, B. Y., & Chemat, F. (2009). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. Food chemistry, 114(1), 355-362.

Bozin, B., Mimica-Dukic, N., Samojlik, I., & Jovin, E. (2007). Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. Journal of agricultural food chemistry, 55(19), 7879-7885.

Cuvelier, M. E., Richard, H., & Berset, C. (1996). Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society, 73(5), 645-652.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. Journal of agricultural food chemistry, 48(6), 2576-2581.

El Omri, A., Han, J., Yamada, P., Kawada, K., Abdrabbah, M. B., & Isoda, H. (2010). Rosmarinus officinalis polyphenols activate cholinergic activities in PC12 cells through phosphorylation of ERK1/2. Journal of Ethnopharmacology, 131(2), 451-458.

Emberger, L. J. B. d. l. S. B. d. F. (1966). Réflexions sur le spectre biologique de Raunkiaer. 113(sup2), 147-156.

Faleiro, L., Miguel, G., Guerrero, C., & Brito, J. (1997). Antimicrobial activity of essential oils of Rosmarinus officinalis L., Thymus mastichina (L) L. ssp Mastichina and Thymus albicans Hofmanns & link. Paper presented at the II WOCMAP Congress Medicinal and Aromatic Plants, Part 2: Pharmacognosy, Pharmacology, Phytomedicine, Toxicology 501.

Farhat, I., Hammami, M., Cherif, M., & Nasraoui, B. J. J. o. E. O. R. (2020). Chemometric analysis of geographic origins and compositions of Citrus sinensis (L.) Osbeck var 'Maltaise demi sanguine'essential oil. 32(3), 216-226.

González-Minero, F. J., Bravo-Díaz, L., & Ayala-Gómez, A. (2020). Rosmarinus officinalis L.(Rosemary): An Ancient Plant with Uses in Personal Healthcare and Cosmetics. Cosmetics, 7(4), 77.

Hmaied, M., Bouafif, H., Magdouli, S., Braghiroli, F. L., & Koubaa, A. (2019). Effect of Forest Biomass Pretreatment on Essential Oil Yield and Properties. Forests, 10(11), 1042.

Khiari, D., & Boussaid, M. (2000). Diversité phénotypique de quelques populations de romarin (Rosmarinus officinalis L.) en Tunisie. Tropicultura, 18(4), 203-207.

Koschier, E. H., & Sedy, K. A. (2003). Labiate essential oils affecting host selection and acceptance of Thrips tabaci Lindeman. Crop protection, 22(7), 929-934.

Lo Presti, M., Ragusa, S., Trozzi, A., Dugo, P., Visinoni, F., Fazio, A., . . . Mondello, L. (2005). A comparison between different techniques for the isolation of rosemary essential oil. Journal of separation science, 28(3), 273-280.

Marotti, M., & Piccaglia, R. (1992). The Influence of Distillation Conditions on the Essential Oil Composition of Three Varieties of Foeniculum vulgare Mill. Journal of Essential Oil Research, 4(6), 569-576.

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., . . . Casanova, J. (2002). Chemical composition and antimicrobial activity of Rosmarinus officinalis L. oils from Sardinia and Corsica. Flavour Fragrance Journal, 17(1), 15-19.

Tischer, B., Vendruscolo, R. G., Wagner, R., Menezes, C. R., Barin, C. S., Giacomelli, S. R., . . . Barin, J. S. (2017). Effect of grinding method on the analysis of essential oil from Baccharis articulata (Lam.) Pers. Chemical Papers, 71(4), 753-761.

Wada, M., Kido, H., Ohyama, K., Kishikawa, N., Ohba, Y., Kuroda, N., & Nakashima, k. (2004). Evaluation of quenching effects of non-water-soluble and water-soluble rosemary extracts against active oxygen species by chemiluminescent assay. Food chemistry, 87(2), 261-267.

Yeddes, W., Aidi Wannes, W., Hammami, M., Smida, M., Chebbi, A., Marzouk, B., & Saidani Tounsi, M. (2018). Effect of environmental conditions on the chemical composition and antioxidant activity of essential oils from Rosmarinus officinalis L. growing wild in Tunisia. Journal of Essential Oil-Bearing Plants, 21(4), 972-986.