

Climatic factors as quality determinant of essential oils and phenolics in *Rosmarinus officinalis* L. (Lamiales Lamiaceae) collected from three geographic areas in Algeria

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ABSTRACT

The present work was aimed to evaluate the effect of the climatic factors on quality and quantity of essential oils and phenolic compounds of the aerial parts of *Rosmarinus officinalis* L. (Lamiales Lamiaceae) collected from three different geographic origins in Algeria (humid, semi arid, and arid). Gas-Chromatography/Mass-Spectroscopy analysis of essential oils revealed important disparities, both quantitatively and qualitatively. α -pinene and camphor as major components were found to range from 16.78 to 40.95% and from 11.24 to 36.72%, respectively. The assessment of total phenolics in water and ethyl acetate extracts of the three samples displayed a content ranging from 58.26 to 114.10 mg GAE (gallic acid equivalent)/g of water extracts and from 73.75 to 167.91 mg GAE/g of ethyl acetate extracts. The flavonoids content was found to vary from 14.63 to 28.86 mg QE (quercetin equivalent)/g of water extracts and from 66.2 to 93.1 mg QE/g of ethyl acetate extracts. Moreover, the HPLC analysis of phenolics revealed the presence of many compounds amongst which Hesperidin and Rosmarinic acid have relatively high contents in both extracts and in the three sites.

KEY WORDS

Environment factors; essential oils; flavonoids; *Rosmarinus officinalis*; phenolics.

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INTRODUCTION

Plant secondary metabolites are considered as the major source of supply for pharmaceuticals, food additives, perfumes, savors, and other key compounds in chemistry and bio-chemistry widely

used in everyday life. The role of secondary metabolites continues to be a contentious subject. Unlike the primary metabolites, which govern all fundamental physiological processes necessary to the growth and development of plants, secondary metabolites are mandatory in the survival of the

plant in its environment. They constitute an essential part of the armamentaria used by plants in the fight to survive and proliferate. Their role may centre on defense of the producer against predators (herbivores), pathogens, or competitors, on assistance to pollination or seed dispersal, or on protection against or adaptation to extrinsic abiotic factors and in overcoming stress conditions, or combinations of these functions (Ramakrishna & Ravishankar; 2011; Rhodes, 1994; Borges et al., 2013). Environmental factors such as soil composition, temperature, latitude, humidity, light intensity, rainfall, evaporation, minerals, and CO₂ have a fundamental impact on the growth of a plant and accumulation of secondary metabolites and, hence, influence directly the quality of the plant for medicinal application and therapeutic value. Plants have the ability to overcome biological, physical, chemical, and ecological stresses by acclimatizing the buildup of secondary metabolites. Therefore, researches on the impact of ecological factors on the accretion of secondary metabolites in medicinal plants become a hot subject of prime concern (Gobbo-Neto & Lopes, 2007; Guo et al., 2013; Hanif et al., 2018).

Algeria is characterized by a mild Mediterranean climate along the coast, a transitional climate in the northern hills and mountains, a little more continental and moderately rainy, and finally the desert climate of the vast area occupied by the Sahara. The landscapes of Algeria, from desert, mountains, valley, and plateaus to basins support thousands of floral species, many of which are endemic to the country. This striking biodiversity led us to carry out this investigation on the effect of climatic conditions on the content of essential oils and phenolics in both water and ethyl acetate extracts of a widely used medicinal plant, *Rosmarinus officinalis* L. (Lamiales Lamiaceae), taken in three distinguished geographic locations (arid, semi arid, and humid).

Rosmarinus officinalis is a popular fragrant and medicinal plant. Many authors consider this species as the most used folk species worldwide because its volatile components and phenolics are continuously reported to possess important biological assets. Depending on what has been stated in literature, its secondary metabolites may vary according to the harvesting time, extraction technique, climate conditions, geographic origins,

phenological stages, etc. The plant is traditionally used as an antispasmodic in renal colic and dysmenorrhoea and in relieving respiratory disorders. It has also been used as an analgesic, antirheumatic, carminative, cholagogue, diuretic, expectorant, antiepileptic, and for human fertility. Other uses are as a general tonic in case of excessive physical or intellectual works and in heart diseases. Externally, it is a rubefacient, and a stimulant for the growth of hair and treatment of eczema of the scalp. Moreover, it was reported to relax smooth muscles of trachea and intestine. The most important constituents of rosemary are caffeic acid and its derivatives, such as rosmarinic acid (Tai et al., 2012; Rozman & Jersek, 2009; Singh & Guleria, 2013; Bajalan et al., 2017; Al-Sereiti et al., 1999).

MATERIALS AND METHODS

Soil and climatic data

The geographic areas covered in this study are Oum El Bouaghi (site 1: semi arid), el Taref (site 2: humid), and Ouargla (site 3: arid). The climate data during 2015 were obtained from the available climate database of the National office of Meteorology, Algeria (Tables 1–3).

Plant material

The aerial parts of *R. officinalis* were collected from Oum El Bouaghi (site 1: semi arid), El Taref (site 2: humid), and Ouargla (site 3: arid). The plants were identified by Pr. A. Zellagui, Oum El Bouaghi University, Algeria. Voucher specimens (RO1, RO2, RO3) of the three sites respectively were deposited in the Laboratory of Natural Resources and Management of Sensitive Environments, University of Oum El Bouaghi, Algeria

Essential oil extraction

Hydrodistillation is used in the extraction of essential oil. This is the simplest and usually the cheapest distillation procedure. It works better for powders and tough materials. In this method, the plant material is heated, by placing it in water, which is brought to the boil. The heat causes the

cell structure of the plant to burst and break down, thus releasing the essential oils. The essential oil molecules and steam are carried along a pipe and conducted through a refrigerator, where they return to the liquid form and collected in a holder. The rising liquid is a mixture of oil and water, and because essential oils are not water soluble, they can be easily separated from the water and siphoned off. In our case, 300 g of each plant sample was ground and subjected to hydrodistillation for 3 hours in a Clevenger type apparatus.

Water extract

500 mL of distilled water were added to 100 g of finely ground dry plant material for each sample. After 60 minutes, the aqueous extract was filtered and dried under vacuum, weighed, and prepared for HPLC analysis.

Ethyl acetate extract

100 g of dry plant material of each sample was subjected to overnight extraction using ethyl acetate. After separation, the organic phase was evaporated and the crude extract was weighed and prepared for further analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC/MS analyses were obtained on Perkin Elmer mass spectrometer with built-in auto sampler using BPX-20 column (30 m × 0.25 mm × 0.25 µm film). For GC/MS detection, an electron ionization system, ionization energy of 70 eV, was used. Helium was the carrier gas, at a flow rate of 1.3 mL/min. The column temperature was operated under the same conditions as described above.

Identification of the individual components was based on (a) comparison of their GC retention indices (RI) with those of authentic compounds or literature data and (b) computer matching with a mass spectral library and commercial libraries (WILLEY and NIST database/ChemStation data system).

Determination of total phenolic contents

The concentration of phenolics in plant extracts was determined using spectrophotometric method

(Singleton et al., 1999). The methanol solution of the extract at the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanol solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 mL methanol. 2.5 mL 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of NaHCO₃. The samples were thereafter incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the meas-

parameters	Oum El Bouaghi (site 1)	El Taref (site 2)	Ouargla(site 3)
Latitude and Longitude	35°52'31" N, 7°06'48" E	36°46'01" N, 8°18'49" E	31°56'57" N, 5°19'30" E
Altitude (meters above sea level)	925	24	138
Clay %	21.93	35.45	03.27
Silt %	38.42	16.74	0.3
Sand %	39.6	47.8	96.44
Texture	loam	silty clay	silty
EC (electric conductivity)	0.37	0.37	0.30
pH	7.22	7.47	7.86
CaCO ₃ T %	19.78	0.86	1.29
CaCO ₃ A %	14.38	0.00	0.00
OM (organic matter)	5.07	1.69	2.53

Table1. Geographical and soil characteristics of different collection sites.

Analysis parameter	Methods
Particle size (%)	pipette method ROBINSON ISO, 11277
pH Rapport ½.5 at 25°C	Soil referential; 1995
EC (dS/m) rapport 1/5 at 25°C	FAO, 1985
CaCO ₃ T (%)	G.E.P.P.A
OM (%)	Brochure OLIB

Table 2. Analyses Methods.

ured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Determination of total flavonoids

The content of flavonoids in the examined plant extracts was determined using spectroscopic method (Quettier et al., 2000). The sample contained 1 mL of methanol solution of the extract at a concentration of 1 mg/mL and 1 mL of 2% AlCl_3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at $\lambda_{\text{max}} = 430$ nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE /g of extract).

HPLC-TOF/MS analysis

Phenolic content of the plant extracts was determined using Agilent Technology of 1260 Infinity. HPLC System was coupled with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100 mm, 3.5 μm) column. Mobile phases A and B were ultra-pure water with 0.1% formic acid and acetonitrile respectively. Flow rate was 0.6 mL min^{-1} and column temperature was 35°C. Injection volume was 10 μL . The solvent program was as follow: 0–1 min 10% B; 1–20 min 50% B; 20–23 min 80% B; 23–30 min 10 % B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C. Nitrogen gas flow was 10.0 L min^{-1} , nebulizer of 40 psi, capillary voltage of 4000 V, and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered passing through a PTFE (0.45 μm) filter by an injector to remove particulates.

RESULTS AND DISCUSSION

Essential oils

The GC-MS fingerprints of essential oils of the three samples were established. Table 4 shows the essential oil composition of *R. officinalis* from the three precited sites, RO1, RO2, and RO3, respectively.

16 compounds were identified in sample RO1, 21 in sample RO2, and 21 in sample RO3, making up a total of 25 different compounds in all samples representing more than 99% of the total oils. All the identified components have been reported previously. The constituents: α -pinene, camphene, limonene, eucalyptol, linalool, camphor, isborneol, and verbenone account more than 82% of weight in the three samples.

The major compounds in RO1 are α -pinene (40.95%), verbenone (11.90), camphor (11.24%), camphene (8.08%), limonene (5.49%), and isborneol (3.96%). RO2, instead, involves a quite different quantitative order of the major components, namely camphor (19.26%), α -pinene (16.78%), isborneol (12.29%), verbenone (12.19%), eucalyptol (11.88%), limonene (3.52%). Sample RO3, in turn, contains distinguished chemotypes explicitly: camphor (36.72%), α -pinene (17.16%), eucalyptol (13.88%), iso-borneol (7.58%), camphene (4.92%), and Verbenone (3.44%). All major components showed significant discrepancies between samples. There are many reports regarding the variation of rosemary essential oil according to geographic origin. It was reported that camphor (14.3–17.5%), Eucalyptol (11.0–21.6%), and α -pinene (10.2–12.5%) were the major essential oil components in populations growing in Turkey (Gurbuz et al., 2016). Morocco rosemary was found to be rich in Eucalyptol (47.44%), α -pinene (12.51%), and camphor (7.9%), while α -pinene was the major component of rosemary collected in Spain (24.7%) and France (35.80%) (Chalchat et al., 1993). In Algeria, it was reported that the plant contains Eucalyptol (52.4%), camphor (12.6%), and β -pinene (5.7%) (Boutekdjiret et al., 1998). Pino reported the composition of rosemary from Cuba to be: camphor (34.80%), borneol (11.6%), Eucalyptol (11%), and α -pinene (8.17%) (Pino et al., 1998). Balkan Peninsula rosemary was found

Month	Average Mean Temperature °C			Average Humidity, rate %			Relative precipitation in mm			Relative Evaporation in mm	
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2
Jan	5.9	11.3	11.4	81	85	52	39	124	1	49	27
Feb	5.7	11.8	14.2	80	83	56	52	82	24	41	19
Mar	9.6	13.7	17.4	71	73	47	22	68	4	86	40
Apr	14.3	15.5	24.4	60	74	36	3	53	0.0	114	31
May	19.1	19	28.9	54	70	38	16	39	0.0	189	38
June	22.4	23	32.3	52	68	30	10	16	0.0	250	48
July	26	25.7	34.3	46	63	30	27	3	0.0	259	52
Aug	25.9	26.5	36	51	65	38	68	5	0.0	208	51
Sept	22.2	24.5	30.9	56	67	45	6	37	1	162	41
Oct	17.4	20.2	24.8	58	73	45	46	72	0.0	184	31
Nov	11.2	16	17.7	77	80	56	44	90	0.0	51	19
Dec	7.8	12.4	12.5	69	82	54	43	105	0.0	48	10
average	15.62	18.30	23.73	62.91	73.58	43.91	31.333	57.83	2.5	136.75	33.91

Table 3. Climatic data of the three selected sites in 2015.

N	Compound	RT	RO1(Site 1)	RO2 (Site 2)	RO3 (Site3)
1	P-xylene	10.775	-	0.69	0.67
2	α -pinene	12.752	40.95	16.78	17.16
3	Camphene	13.271	8.08	2.46	4.92
4	β -pinene	14.179	1.34	0.67	1.09
5	Myrcene	14.346	-	0.76	0.54
6	Delta -3-carene	15.208	-	1.26	
7	P-cymene	15.675	3.24	1.21	0.60
8	Limonene	15.846	5.49	3.52	2.80
9	Eucalyptol	16.025	1.30	11.88	13.88
10	Gamma-terpinene	16.843	1.29	0.66	0.52
11	Terpinolene	17.917	-	1.23	0.77
12	Linalol	18.169	2.87	3.91	1.42
13	Cyclopentadiene	19.319	-	-	0.54
14	Camphor	20.252	11.24	19.26	36.72
15	Iso Borneol	20.873	3.96	12.29	7.58
16	Bicyclo(3,1,1) heptan-3-one	21.175	-	3.16	-
17	Carvomenthenol	21.185	1.42	-	1.38
18	Alpha-terpineol	21.601	1.38	1.68	1.46
19	Bicyclo(3,1,1)hept-2-ene-2-ethanol	21.957	-	0.92	1.34
20	Verbenone	22.406	11.90	12.19	3.44
21	Urea, phenyl	23.505	-	1.14	-
22	Aceticacid	24.845	-	2.96	-
23	Bornylacetate	24.855	2.64		1.42
24	Beta-caryophyllene	29.510	1.81	1.36	1.26
25	Alpha-Humulene	30.575	1.10	-	0.48

Table 4. *Rosmarinus officinalis* essential oils composition (from the three sites).

sites	water extract	ethyl acetate extract
Site 1	81.63±0.16	123.22±0.31
Site 2	114.10±0.15	167.91±0.35
Site 3	58.26±0.31	73.75±0.07

Table 5. Total phenolics in the two extracts of *Rosmarinus officinalis* (mg GAE /g Dry Weight) (see text).

sites	water extract	ethyl acetate extract
Site 1	28.86±0.31	66.2±0.19
Site 2	28.78±0.02	93.1±0.07
Site 3	14.63±0.08	83.32±0.07

Table 6. Total flavonoids in the two extracts of the two species (mg EQ/g Dry Weight) (see text).

extract compound	W1	W2	W3	EA1	EA2	EA3
Gentisic Acid	4.953	2.025	20.233			
Chlorogenic Acid	135.152	31.174	97.451	6.339	3.084	0.323
4-Hydroxy Benzoic Acid	119.621	39.320	583.822			
Protocatechuic Acid	14.246	16.941	12.645	1.011	1.402	0.510
Caffeic Acid	11.867	160.719	87.937			
Vanilic Acid		14.015	26.788	0.934	1.529	0.515
Rutin	7.889	2.471	24.260			
P-Coumaric Acid			5.557		1.461	0.134
Chcoric Acid			11.582	1.225	1.173	
Ferulic Acid	7.021	0.370	10.667		3.509	0.498
Hesperidin	8963.114	5412.587	14679.306	611.031	348.168	60.900
Apigenin-7-Glucoside						
Rosmarinic Acid	1587.827	1664.396	1653.592	1927.5692	1314.146	41.347
Salisilic Acid			52.754			
Cinnamic Acid	60.850			4.700		

Table 7. Phenolic compositions of *Rosmarinus officinalis* for the 3 sites. W: water extract, EA: EtOAc extract. Values are given as mg of phenolic compound per kg of dry weight of plant material.

to contain eucalyptol and camphor as the major compounds (Lakušić et al., 2012). Essential oils from 21 localities from Iran were found to contain variable percentages of major components such as: α -Pinene (14.19–21.43%), camphene (3.25–7.58%), 1,8-cineole (5.32–28.29%), camphor (1.58–25.32%), borneol (0.16–9.42%), piperitone (4.21–7.71%), and bornyl acetate (4.14–6.95%) (Bajalan et al., 2017). The Tunisian rosemary from 4 geographic origins was reported to be rich in 1,8-cineol (33.08–37.75%), camphor (13.55–18.13%), α -pinene (8.58–9.32%), α -terpineol (6.79–8.17%), camphene (5.07–5.58%), borneol (4.08–5.48%), limonene (3.19–3.04%), and p-cymene (2.42–3.11%) (Hcini et al., 2013).

Total phenolics

The content of the total phenolics is determined from a calibration curve of gallic acid taken as standard with a correlation coefficient ($y = 0.0113X + 0.0686$, $R^2 = 0.9984$).

The contents of total polyphenols are reported in micrograms of gallic acid equivalent per milligram of dry weight extract (mg GAE/g DW) in Table 5.

The results revealed important fluctuations in total phenolics. Site 2 (humid region) exhibits significant phenolic contents (114.10 and 167.91 mg GAE/g DW). This is certainly due to environmental factors, especially water and nutrients favorable

for the biosynthesis of such elements. Site 3, in turn displays less contents especially in water extracts (58.26 mg GAE/g DW).

Flavonoids content

The flavonoid content, expressed in microgram of quercetin equivalent per milligram of dry weight extract (mg QE/g DW), was determined from the regression curve whose equation is: $y = 0.0299 X + 0.0979$, $R^2 = 0.9746$.

The flavonoids contents seem to be less location-dependent for the case of water extracts in sites 1 and 2. However, in site 3 the plant displays only half of the other sites content (14.63 mg QE/g DW). Nonetheless, for ethyl acetate extracts, quite important oscillations were observed (66.2–93.1 mg QE/g DW) in site 3 (Table 6).

High performance liquid chromatography analysis

Recognition and quantification of phenolic compounds were carried out using analytical HPLC-TOF-MS and the results are shown in Table 7 expressed as mg of phenol per kg of dry weight of plant material.

Previous reports stated clearly the drastic dependence of these chemical contents with their geographic origins. For example, but not exclusively, the ethanol dry extract from Romanian *Rosmarinus officinalis* contains 3.22% of flavonoids (expressed as rutin equivalent), 34.30% phenol carboxylic acids (expressed as chlorogenic acid equivalent), 31.86% total phenols (expressed as tannic acid equivalent), and 3.29% rosmarinic acid (Gird et al., 2017). In Iran, the total phenolics were found to be 4.99 ± 0.054 g of gallic acid equivalent /100g dry leaves instead of 4.99 ± 0.054 g as gallic acid equivalent /100g dry leaves as reported by Tavassoli & Emam Djomeh (2011).

CONCLUSIONS

The increased demand of natural products as substituent of synthetic drugs has rehabilitated concern in large-scale production. Environmental factors have impact on availability of active principles and affect remarkably the secondary metabolites

contents in higher plants in medicinal plants, hence, therapeutic value also get influenced. It is well apparent that climate change, soil nature, aridity, and other factors considerably influence water availability, salinity, and several unfavorable circumstances having direct attitude on secondary metabolites yields and qualities. The present work, in the same context, highlights the effect of some abiotic aspects on essential oil and polyphenolic contents of *Rosmarinus officinalis*. Nevertheless, further studies are required to deepen the knowledge towards the establishment of a relationship leading to the use of such stresses as tools to increase the health-related properties of medicinal plant.

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