

ORIGINAL RESEARCH PAPER

**WILD *ROSMARINUS OFFICINALIS* L. EXTRACTS AS A POTENT
ANTIOXIDANT AGENT**

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Abstract

In this work, was investigated the phytochemical composition and the antioxidant activity of extracts of rosemary collected from the natural site “*Taoura*”, situated in Souk-Ahras, North-Far-East area of Algeria. Two types of extracts were prepared, the first was the methanolic extract obtained by maceration of the aerial parts in methanol, and the second was the essential oil obtained by hydrodistillation using Clevenger device. The phytochemical analysis of the methanolic extract of rosemary revealed that it contained a relatively high phenolic content (168 mg GAE/g) and a strong antioxidant activity, as measured with the DPPH radical scavenging activity test. The yielded essential oil was analyzed by gaz chromatography-mass spectrometry. Forty-seven compounds representing 99.37% of the oil were identified. The oil consisted of oxygenated monoterpenic hydrocarbons, monoterpenes and sesquiterpene hydrocarbons. The main constituents of the oil were: 1,8-cineole (31.75%), camphor (18.94%) and α -pinene (11.13%). In addition, the DPPH radical scavenging activity of rosemary oil was investigated. The SC₅₀ of rosemary essential oil was higher than it's methanolic extract. The radical scavenging capacity of rosemary oil was 7.6 folds greater compared to the methanolic extract.

Keywords: antioxidant activity, essential oil, phenolic content, reducing power, rosemary

Introduction

Rosemary (*Rosmarinus officinalis* L.) belongs to the *Lamiaceae* (*Labiatae*) family, and has long been considered a medicinal plant of great importance. It is commonly

used as a spice and perfume. The leaf and the essential oil (EO) of the plant are also used in balneotherapy and aromatherapy, respectively (Abbaszadeh *et al.*, 2020).

Rosemary is known for its medicinal and cosmetic properties in the civilization of ancient Greece and Rome. In the middle ages, rosemary EO had been used as a medicine and the alcoholic distillate as a popular perfume (Aguilar *et al.*, 2008). The term Rosemary derives from the Latin Ros = dew and Marinus = sea, which means « dew of the sea » (Al-Sereiti *et al.*, 1999).

Rosemary is a shrub that grows spontaneously around the Mediterranean basin. Currently cultivated throughout the world for its culinary uses. In addition, rosemary extracts are used in industry as food preservatives for their antioxidant powers (Borrás-Linares *et al.*, 2014).

It has been scientifically proven that the extracts of this plant (EO, alcoholic and aqueous extracts) play a key role in health protection as anti-carcinogenic (Singletary & Nelshoppen, 1991), antispasmodic (Lis-Balchin *et al.*, 1996), against carcinogenesis, mutagenesis and fertility deficiency (Alkofahi *et al.*, 1997). Other effects including estrogenic (Zhu *et al.*, 1998), anti-hepatotoxic (Fahim *et al.*, 1999), antimicrobial (Mangena & Muyima, 1999), antiulcer (Corrêa Dias *et al.*, 2000), enzyme induction (Debersac *et al.*, 2001), anti-inflammatory (Lo *et al.*, 2002), anti-nephrotoxic (Makino *et al.*, 2002) were reported. Moreover, anti-trypanosomal (Abe *et al.*, 2002), antioxidant (del Baño *et al.*, 2003), osteoclasts (Mühlbauer *et al.*, 2003), immune stimulation (Hur *et al.*, 2004), and diuretic (Haloui *et al.*, 2000) effects were studied.

In the European Union *R. officinalis* is used in foodstuffs as a preservative agent due to the presence of antioxidant compounds such as phenolic acids, flavonoids and diterpenoids (European Commission, 2012).

Several studies have identified the compounds that are primarily responsible for the antioxidant properties of rosemary extracts. It is due to the content of phenolic abietane diterpenes such as carnosic acid and its derivatives, carnosol, rosmadial, rosmanol, rosmanol isomers and methyl carnosate. The occurrence of other phenolic compounds such as flavonoids and phenolic acids, especially rosmarinic acid, also contributes to the bioactivity of this aromatic plant (Fahim *et al.*, 1999; Zhu *et al.*, 1998). Moreover, other components present in the EO, including 1,8-cineol, camphor, α -pinene or borneol have also been related to some bioactivities, mostly antioxidant and antimicrobial activities (Sedighi *et al.*, 2015).

Rosemary is one of the oldest known medicinal plants in Algeria (Bendif *et al.*, 2017), it is widespread and broadly used in traditional medicine (Boutekedjiret *et al.*, 2004). According to data from the local forest conservation of the wilaya of Souk-Ahras, rosemary is among the most encountered species; its distribution area extends to 9460 Ha in the province (Fedjer *et al.*, 2022).

In Algeria, Rosemary leaf powder is used in the south of the country as a traditional additive to paste of crushed dates in order to preserve it from decay, worms and insects. It is also used by the natives of the Blidean Atlas in culinary preparations, particularly for the preparation, in spring, of a renowned traditional special dish with

health benefits called "*al h'ammama*" made up of around forty local plants including rosemary.

The measurement and use of plant antioxidant have drawn increasing attention in regard of scientific and industrial (dietary, pharmaceutical and cosmetics) purposes. This is largely due to their strong biological activity. Many synthetic antioxidants, however, have possible activity as promoters of health concern.

Hence, there is a need of safe, economic, effective natural antioxidants in place of these synthetic ones. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts (McClements & Decker, 2000). It is an established fact that polyphenolic compounds possess remarkable antioxidant activities. Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers (Zheng & Wang, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Gulcin, 2020; Batiha et al., 2021; Novais et al., 2022). In addition, naturally antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers (Nahak & Sahu, 2010).

Therefore, the aim of this study was to unveil the chemical composition in natural antioxidants, notably phenolic compounds of a wild plant, locally used by natives, and to determine the biological activity of its extracts by mean of tests measuring its antioxidant power.

Materials and methods

Plant Material

R. officinalis samples were harvested in the region of "Taoura", province of Souk-Ahras, about 550 km east of Algiers, at the geographical coordinates 36°07'07.3"N 8°05'45.2"E.

The Phytogenetic Resources Research Division, Algeria's National Institute of Agronomic Research, performed the botanical identification of samples. Fresh leaves were air-dried in shade at room temperature before use.

Preparation of Extracts and Chemical Analysis

Hydrodistillation

Stems and leaves of *R. officinalis* were cut and hydrodistilled in Clevenger-type apparatus using distilled water. Drops of water were discarded to obtain the pure EO. The EO was sealed and conserved at -20°C until use.

Methanolic Extraction

R. officinalis sample (leaves and stems) were ground into powder, and then sieved through 0.5 mm mesh sieve. The plant powder (2 g) was put in an Erlenmeyer-type flask, added 20 ml of methanol 80% (v/v) and let to macerate for 24 hours. The methanolic extract was put apart in another flask and the same volume of solvent was added to the residue and let to macerate for another 48 hours. The two

methanolic phases were combined and evaporated under vacuum using an evaporator (Büchi, Germany). The obtained rosemary methanolic dry extract was re-suspended in distilled water to be lyophilized (Christ alpha 1-2 model, Germany). The obtained lyophilized methanolic extract was sealed and conserved in a dry place until analysis. To perform analysis, the dry methanolic extract of rosemary was re-suspended to the targeted concentration in 70% (v/v) ethanol.

Phytochemical Content of the Methanolic Extract

Total phenolic content was assessed according to the Folin-Ciocalteu method described by Singleton & Rossi, (1965). Through a calibration curve created with gallic acid ($y = 0.0095x + 0.0194$; $R^2 = 0.997$), the total phenolic content of the plant extract was represented as milligrams of gallic acid equivalents per gram sample (mg GAE/g).

Flavonoids content was determined using Aluminum Chloride method (Mazari et al., 2022). Total flavonoids content was deduced from the prepared standard curve ($y = 0.0107x + 0.0054$; $R^2 = 0.998$) and expressed as mg quercetin equivalent per gram (mg QE/g).

For the test of flavonols, the Adedapo et al. (2008) proposed method was operated for estimating the total flavonols in the plant methanolic extract. Total flavonols content was quantified through the quercetin's calibration curve ($y = 0.0164x + 0.0112$; $R^2 = 0.999$) and reported as milligrams of quercetin equivalents per gram sample (mg QE/g). All measurements were made using UV-Vis spectrophotometer (Selecta, Spain).

Antioxidant Activity

The antioxidant activity of *R. officinalis* evaluated through three tests, DPPH radical scavenging activity, reducing power, and total antioxidant capacity were performed according to the protocols detailed in Mazari et al. (2022).

DPPH Free Radical Scavenging Activity Test

The ability of the extracts to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated through the following steps: various dilutions of plant extracts (2 mL) were mixed with 2 mL of a 0.2 mM methanolic solution of DPPH. Reaction mixtures were incubated in a dark place at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm with a UV-Vis spectrophotometer (Selecta, Spain). The discoloration of DPPH was recorded and the percent inhibition of the DPPH radicals by the sample was calculated according to the following formula:

$$\text{DPPH radical scavenging (\%)} = [(A_c - A_s) \div A_c] \times 100 \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

Ferric Reducing Power Test

The reducing power was determined according to a procedure based on the method of Oyaizu (1986). To aliquots (2 mL) of different dilutions of the plant extract (25 ~ 350 µg/mL) were added phosphate buffer (2 mL, 0.2 M, pH 6.6) and mixed with potassium ferricyanide (2 mL, 1%). Then, the mixtures were incubated at 50°C for

20 min. The reaction was terminated by trichloroacetic acid solution (2 mL, 10%) and centrifuged at 3900 rpm for 10 min. Two milliliters of the supernatant was taken out immediately, added 2 mL of pure methanol and mixed with ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a blank. Three replicates were performed on each tested sample.

Total Antioxidant Capacity Assay

Total antioxidant capacity (TAC) of the plant extract was determined using the phosphomolybdenum method as described by Prieto *et al.* (1999). The sample (0.6 mL) was mixed in a test tube with 6 mL reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate). The tubes were incubated for 90 min at 95°C. After cooling, absorbance was recorded at 695 nm against the blank, which was incubated within the same conditions as the treated samples. A standard curve was prepared using increasing concentrations of ascorbic acid. The TAC assay was expressed as milligram of ascorbic acid equivalent per gram (mg AAE/g).

GC-MS Analysis of the Chemical Profile of the Essential Oil

Gas chromatographic analysis of *R. officinalis* essential oil was performed on a Hewlett Packard Agilent 6890 plus gas chromatograph equipped with a 5973 mass spectrometer. For separation of volatiles a HP-5 MS (5% phenyl 95% dimethylpolysiloxane, 30 m, 0.25 mm *i.d.*, 0.25 µm film thickness) capillary column was used. The following temperature program was applied: 8 min at 60 °C, then 2°C min⁻¹ to 250°C, held for 10 min, for a total run of 113 min. Injector temperature: 250°C; carrier gas: He; flow rate: 0.5 mL min⁻¹; split ratio: 1:80; the scan acquisition was performed with electron-impact (EI) mode; ionization voltage : 70 eV. Quadrupole mass analyzer. Ion source temperature 230°C. Temperature of the interface 270°C. The essential oil, 0.2 µL, was injected into GC–MS. For identification of the essential oil components co-injection with commercial standards was used whenever possible, together with correspondence of retention indices (RIs) and mass spectra (MS) with respect to those reported in the literature (Petrakis *et al.*, 2005; Sefidkon *et al.*, 2007). Semi-quantification of essential oil components was made by peak area normalization considering the same GC response of the detector towards all volatile constituents.

Results and discussion

Phenolic Content of the Methanolic Extract

Table 1 shows the phytochemical composition of the methanolic extract of rosemary. The result indicated that the methanolic extract of rosemary was high in terms of phenolic content (168.13 mg GAE/g). Casarotti & Jorge (2012) reported a phenolic concentration in rosemary of 82.03 mg GAE/g extract. Mata *et al.* (2007) obtained 73.5 mg GAE/g of phenolics in ethanolic extract of rosemary, whereas Moreno *et al.* (2006) noticed a higher content of phenolics in the methanolic extract of rosemary, about 120 mg GAE/g extract. Our result is in good agreement with that of Erkan *et al.* (2008) who found a content of rosemary phenolics of 162 mg GAE/g extract. The total flavonoids content of the extract was 24.14 mg QE/g. This concur with

Kontogianni *et al.* (2013) who reported a total flavonoids content of 24.6 mg Rutin equivalent per gram dry extract.

Richheimer *et al.* (1996) analyzed the chromatograms of rosemary leaves of worldwide sources; they reported the principal antioxidant constituents carnosic acid, carnosol and 12-methoxycarnosic. Moreno *et al.* (2006), examined different organs of rosemary; they detected the main compounds rosmarinic acid, carnosol and carnosic acid. In the study performed by Almela *et al.* (2006) they classified the compounds present in rosemary into three groups: diterpenes, flavonoids and phenolic acid. The structures of diterpenes were related with carnosic acid. The flavones apigenin and luteolin were the source of flavonoids and rosmarinic acid was the sole identified phenolic acid. Moreover, Kontogianni *et al.* (2013) identified 17 phenolic compounds among which only one hydroxycinnamic derivative, rosmarinic acid, and the following flavonoid compounds: isorhamnetin-3-*O*-hexoside, homoplantagin, and aglycon hispidulin.

Flavonoids are efficient antioxidants that help preventing oxidative and chronic diseases. They can prevent coronary heart disease. According to previous reports, flavonoids may operate as antioxidants by scavenging free radicals like lipid peroxy radicals, superoxide anion radicals and hydroxyl radicals, singlet oxygen quenchers, and through metal ion chelating. Flavonoids play a key role in the bioavailability of metal ions found in low concentrations in the body, such as aluminum, as well as in the detoxification of heavy metals like Cr, Cd, Sn, and Pb. Toxic metal ions are strongly bind to chelating agents generating complex structures, which are easily evacuated from the body (Gulcin, 2020).

Phenolic compounds accumulation being a reaction to harsh environmental conditions. Under such conditions, plants develop an antioxidant system to neutralize the increased activated oxygen species that may cause cell photodamage (del Baño *et al.*, 2003; Hidalgo *et al.*, 1998; Munné-Bosch & Alegre, 2000).

Table 1. Phenolic content and antioxidant activity of the methanolic extract of rosemary.

Phytochemical content			Antioxidant Activity		
Phenolics (mg GAE/g)	Flavonoids (mg QE/g)	Flavonols (mg QE/g)	SC ₅₀ (µg/ml)	EC ₅₀ (µg/ml)	TAC (mg AAE/g)
168.13±11.76	24.14±1.03	18.55±1.48	46.57±0.90	133.75±02.60	97.43±4.20

Values were expressed as mean of triplicate determinations ± standard deviation.

GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAC: Total antioxidant capacity; AAE: Ascorbic acid equivalent.

Antioxidant Activity of the Methanolic Extract

The antioxidant activity of rosemary extract was estimated through the application of three tests: the DPPH radical scavenging activity, the ferric reducing power and the total antioxidant capacity (TAC) tests.

Figure 1 shows the effect of the methanolic extract of rosemary on DPPH radical scavenging activity and ferric reducing power. The radical scavenging activity and

the reducing power increased concomitantly with the increase in extract concentration. The rosemary methanolic extract scavenged more than 80% of the DPPH radicals at the level of 75 µg/ml.

The antioxidant parameters of the studied extracts were reported on Table 1. The SC₅₀, which is the concentration at which 50% of the DPPH radicals were scavenged, was determined as 46.57 µg/ml for the rosemary methanolic extract. This result is in accordance with those of Moreno *et al.* (2006); Erkan *et al.* (2008); Casarotti and Jorge (2012); and Kontogianni *et al.* (2013). Pandey *et al.* (2014), evaluated the DPPH radical scavenging of neem leaves extract, they reported an IC₅₀ of 110 (µg/ml). Moreover, compared to the methanolic extract of *L. stoechas*, Ceylan *et al.* (2015) had put forward an IC₅₀ of 300 µg/ml, which is quite weaker than SC₅₀ of rosemary extract of the present study (Table 1).

Similarly, the determined ferric reducing power efficiency, set as EC₅₀, that is the concentration corresponding to the spectrophotometric absorption value of 0.5, was determined as 133.75 µg/ml (Table 1).

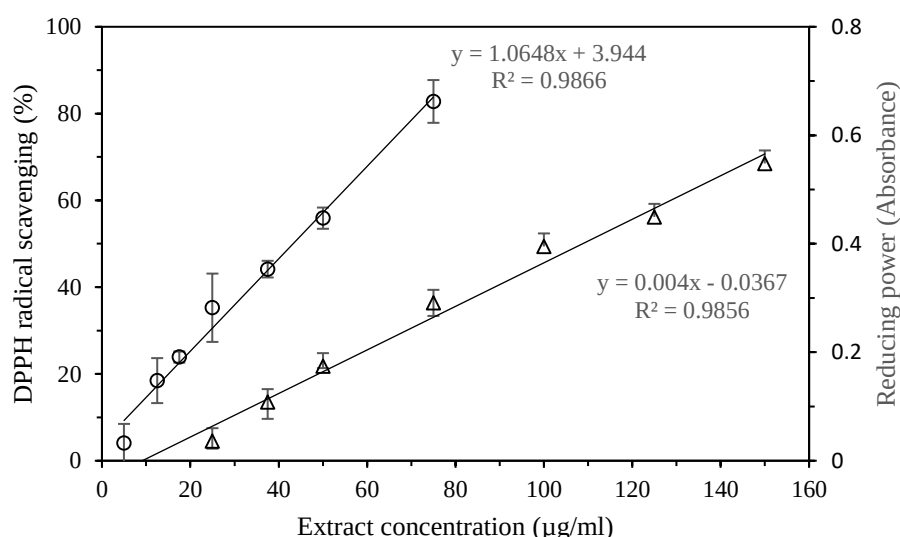


Figure 1: DPPH radical scavenging activity (○) and ferric reducing power effect (Δ) of the methanolic extract of *Rosmarinus officinalis*.

Furthermore, in previous works, to the best of our knowledge, it has not been observed literature data dealing with the effect of the ferric reducing power and the total antioxidant capacity of the methanolic extract of rosemary.

The third parameter tested, the total antioxidant capacity evaluated by the phosphomolybdenum method, estimating the capacity of the methanolic extract of rosemary displayed a calculated value of 97.43 mg AAE/g (Table 1). The phosphomolybdenum method is based on the reduction of molybdenum by the

antioxidants and the formation of green molybdenum (V) complex, which has absorption of 695 nm. It is established that in the phosphomolybdenum and DPPH test procedures, respectively, electron and hydrogen transfer from antioxidant analytes to Mo (VI) complex and DPPH- takes place. In these two tests, the transfer takes place at distinct redox potentials and is conditioned upon the antioxidant's chemical structure. While the DPPH scavenging assay detects typically antioxidants such as flavonoids and polyphenols the phosphomolybdenum assay detects antioxidants like ascorbic acid, certain phenolics, α -tocopherol, and carotenoids (Prieto *et al.*, 1999). With regard to this, there may occasionally be differences in antioxidant potential between these two kinds of tests (Harini *et al.*, 2012). Rather the phosphomolybdenum assay results show that rosemary has a strong reducing power against transition metal ions.

Moreover, despite that some flavonoids are potent antioxidants, it was noticed their limited contribution to the antioxidant activity. This could explain the fact that some extracts, even though containing relatively higher levels of flavonoids, displays lower antioxidant capacity compared to the rosemary extract bearing relatively less flavonoids. Although in most cases, no correlation has been found between the antioxidative and scavenging properties of plant flavonoids (Basaga *et al.*, 1997).

Furthermore, the boosted antioxidant effect of rosemary extract can probably be endorsed to the significantly higher content of carnosic acid, or to the synergistic effects of its varied components (Kontogianni *et al.*, 2013). Moreno *et al.* (2006) analyzed the separated fractions of rosemary methanol extract by HPLC chromatography; they found that fractions containing rosmarinic acid, carnosol and carnosic acid were associated with high antioxidant activity, where the peak of the main compound corresponding to carnosic acid exhibited the higher activity. Thus, rosemary phytochemical constituents were demonstrated endowed with a greater antioxidant activity than some synthetic antioxidants (Chen *et al.*, 1992; Inatani *et al.*, 1983). Indeed, both carnosol and carnosic acid had stronger antioxidant activities than BHT and BHA. (Chen *et al.*, 1992), therefore, the use of rosemary bioactive compounds in foods, cosmetics and drugs, requires further studies on their mechanism of action.

Chemical Composition of the Essential Oil

The essential oil yield of the distilled dry leaves and branches of rosemary collected in “*Taoura*” was 1.52%. The percentage chemical composition of the rosemary essential oil corresponding to the chromatographic profile presented in

Figure 2 was summarized in Table 2. The total number of chemical constituents identified in the essential oil was 47, representing 99.37% of the total oil content.

The distilled sample from *R. officinalis* was constituted mainly of oxygenated monoterpenes hydrocarbons (63.32%). Hydrocarbons 1,8-cineole (31.75%) and camphor (18.94%) and monoterpene hydrocarbon α -pinene (11.13%) were identified as the main constituents of *Taoura*'s rosemary essential oil. Besides, in a considerable amount, monoterpene β -pinene (5.89%), camphene (4.89%), and oxygenated

monoterpene borneol (4.65%), α -terpineol (3.85%), bornyl acetate (2.03%) and sesquiterpene trans-caryophyllene (3.17%) were also detected (Table 2).

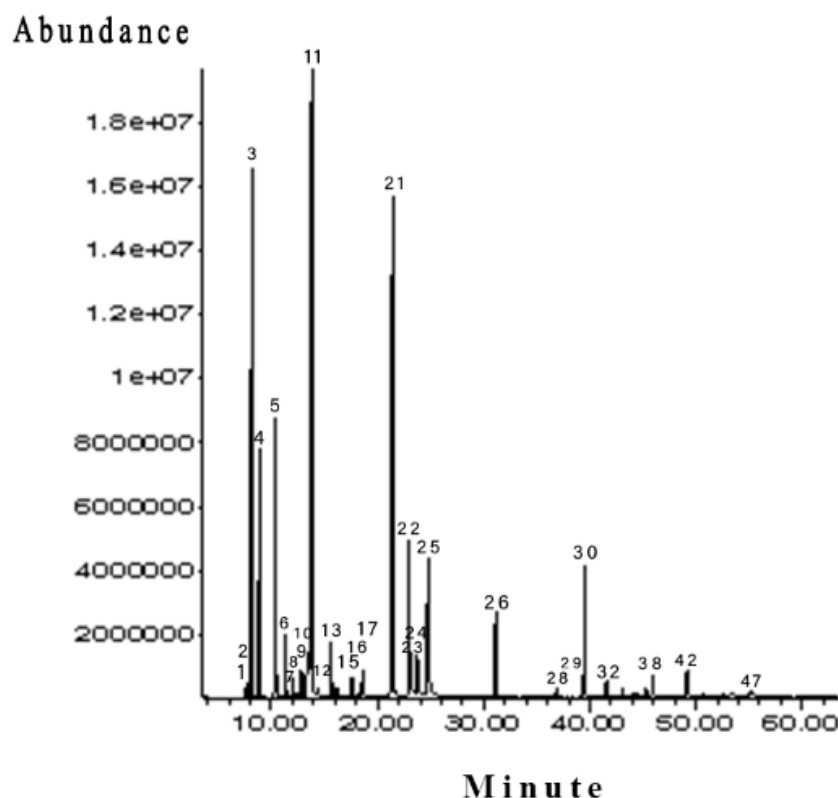


Figure 2: GC-MS profile of Taoura's wild *Rosmarinus officinalis* essential oil.

These results confirm that Taoura's wild *Rosmarinus officinalis* EO belongs to 1,8-cineole chemotype which agrees with the work done by Hendel *et al.* (2019) reporting that the Algerian rosemary essential oil of the humid or sub-humid areas contain 1,8-cineole as the main component together with lower amount of α -pinene and camphor. This chemical profile is also, to some extent, close to the Italian chemotype (Sharma *et al.*, 2020) and to that of Serbia (Rašković *et al.*, 2014).

Thus, the essential oil composition may be influenced by several factors including methods of extraction, genetic, environment and the nutritional status of the plants as well as other factors that can influence the oil composition (Özcan & Chalchat, 2008).

Table 2. Chemical profile of *Taoura's* wild *Rosmarinus officinalis* essential oil.

N°	RT	K _{exp.}	K _{lit.}	Compound	(%)
Monoterpene hydrocarbons					
1	7,55	925	923	Tricyclene	0.187
2	7,77	929	928	α -Thujene	0.284
3	8,14	937	936	α -Pinene	11.129
4	8,86	951	950	Camphene	4.889
5	10,39	981	978	β -Pinene	5.887
6	11,30	998	992	β -Myrcene	1.295
7	12,05	1010	1004	α -Phellandrene	0.432
8	12,35	1015	1011	δ -3-Carene	0.119
9	12,84	1022	1017	α -Terpinene	0.689
10	13,38	1030	1025	<i>p</i> -cymene	1.516
12	14,36	1045	1048	(E)- β -Ocimene	0.186
13	15,62	1064	1060	γ -Terpinene	1.217
15	17,55	1093	1087	Terpinolene	0.515
Oxygenated monoterpene hydrocarbons					
11	13,80	1036	1032	1,8-Cineole	31.746
14	16,15	1072	1067	<i>Cis</i> -sabinene hydrate	0.220
16	18,29	1104	1098	<i>Trans</i> -sabinene hydrate	0.136
17	18,58	1108	1099	Linalool	0.663
18	19,35	1119	1116	endo-fenchol	0.046
19	19,90	1127	1121	<i>Cis-p</i> -Menth-2-en-1-ol	0.041
20	20,19	1131	1125	α -Campholenal	0.061
21	21,34	1147	1143	Camphor	18.939
22	22,90	1169	1166	Borneol	4.647
23	23,00	1171	1171	neoiso-Isopulegol	0.049
24	23,73	1181	1177	Terpinen-4-ol	0.893
25	24,74	1196	1190	α -Terpineol	3.846
26	31,13	1290	1285	Bornyl acetate	2.033
Sesquiterpene hydrocarbons					
27	35,20	1353	1352	α -Cubebene	0.058
28	36,58	1374	1370	α -Ylangene	0.197
29	36,87	1379	1376	α -Copaene	0.207
30	39,50	1421	1420	<i>Trans</i> -Caryophyllene	3.165
31	40,70	1440	1441	Aromadendrene	0.083
32	41,50	1454	1453	α -Humulene	0.416
33	43,10	1480	1476	γ -Muurolene	0.218
35	44,56	1504	1500	α -muurolene	0.095
36	44,95	1511	1513	γ -Cadinene	0.508
37	45,32	1517	1524	δ -Cadinene	0.214
38	45,93	1528	1533	α -Cadinene	0.508
40	46,90	1545	1541	α -Calacorene	0.054
Oxygenated sesquiterpene hydrocarbons					
34	44,10	1496	1494	epi-Cubebol	0.037
39	46,70	1541	1544	<i>Cis</i> -sesquisabinene hydrate	0.047
41	47,45	1554	1551	<i>Cis</i> -Muuro-5-en-4- β -ol	0.012
42	49,15	1584	1581	Caryophyllene oxide	0.673
43	50,67	1610	1605	Humulene epoxide II	0.115

44	51,84	1632	1627	1-epi-cubenol	0.108
45	52,60	1646	1642	Cubenol	0.186
46	53,36	1660	1654	α -Cadinol	0.395
47	55,14	1692	1700	Amorpha-4,9-dien-2-ol	0.414
Monoterpene hydrocarbons					28.345
Oxygenated monoterpene hydrocarbon					63.320
Sesquiterpene hydrocarbons					5.723
Oxygenated sesquiterpene hydrocarbons					1.987
Total					99.375

The GC-MS analysis was carried-out by CRAPC-Expertise SPA.

RT: retention time, **K_{exp}** : experimental Kovats index, **K_{lit}** : Literature Kovats index.

In another experiment, the effect of rosemary essential oil on the free radicals scavenging activity was launched. Figure 3 shows the effect of rosemary essential oils on the DPPH radical scavenging activity. Only 10 μ l/ml of rosemary oil induced 80% inhibition of DPPH free radicals. The SC₅₀ value of rosemary oil was determined as 6.14 μ g/ml, while lavender oil displayed an SC₅₀ value equal to 37.77 μ g/ml (data not shown). In another way, the radical scavenging capacity of rosemary oil was six folds greater than that of lavender oil. Moreover, the SC₅₀ of rosemary essential oil was more efficient than the methanolic extract. The former being 7.6 folds stronger than the latter.

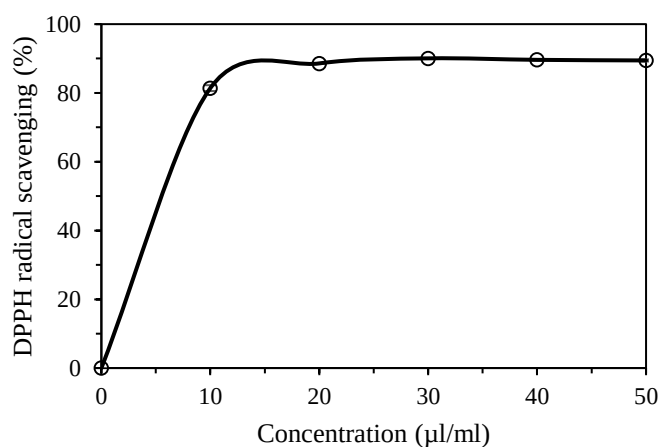


Figure 3. DPPH radical scavenging activity of Taoura's wild *Rosmarinus officinalis* essential oil.

In terms of comparison with vitamin C (Mazari et al., 2018), rosemary oil is 1.5 times more active towards DPPH free radicals scavenging in methanol. In their demonstration of the radical scavenging activity of rosemary essential oil, Rašković et al., (2014) reported an IC₅₀ value of 77.6 μ l/ml. In attempt to relate this activity to the phenolic content of the essential oil, they observed that the oil contained 153.35

mg of GAE/L. Moreover, Bozin *et al.* (2007) reported that the oxygenated monoterpenes (α - and β -thujone, bornyl acetate, camphor, and menthone) and the mixture of mono- and sesquiterpene hydrocarbons were the most implicated compounds in DPPH radicals deactivation.

In their prominent study on different chromatography fractions of rosemary essential oils of flowering, post flowering and vegetative stages, Beretta *et al.* (2011) concluded that the difference in the radical scavenging and anti-lipoperoxidant activity in EOs was straight-forward correlated to their different concentrations of hydroxylated derivatives (i.e. linalool, terpineol, terpinen-4-ol, 6-camphenol, borneol, isopulegol, geraniol, phenols, and 1,8 cineole).

Furthermore, (Ojeda-Sana *et al.*, 2013) evaluated the radical scavenging activity of the essential oil of two phenotypes of Argentina rosemary. They outlined IC₅₀ values ranging from 11 μ l/ml to 25 μ l/ml for the narrow and wide phenotype, respectively. According to their results, myrcene-rich essential oils showed twice the activity than the α -pinene-rich chemotype. The authors evaluated also the antioxidant activity of the three major compounds (α -pinene, myrcene and 1,8-cineole). The results showed a significant antioxidant activity of myrcene with an IC₅₀ of 4.5 μ l ml⁻¹, followed by the α -pinene with an IC₅₀ of 18 μ l ml⁻¹, whereas 1,8-cineole showed no activity.

Conclusions

The phytochemical composition and the antioxidant activity of rosemary extracts collected from a local natural site in Algeria, were investigated. The results revealed that methanolic extract of rosemary had a good content of phenolics and a great antioxidant activity. The chemical composition of the rosemary essential oil of “*Taoura*” showed that it was mainly constituted of monoterpene hydrocarbons, where the biggest fraction included (63%) of oxygenated monoterpene hydrocarbons, with 1,8-cineole and camphor (31.75 and 18.94%, respectively) as a key components. The second fraction included α -pinene hydrocarbons (11.13%) as main component. This led to consider that the essential oil of the site of “*Taoura*” belongs to the 1,8-cineole chemotype.

The collected data of a local wild plant could be interesting for economic operators wishing to valorize this resource, as raw material (rosemary leaves powder) or as more refined product (essential oil) to be used in the food, cosmetic or pharmaceutical industries.

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