ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Comparative Study on the Essential Oils Extracted from Tunisian Rosemary and Myrtle: Chemical Profiles, Quality, and Antimicrobial Activities

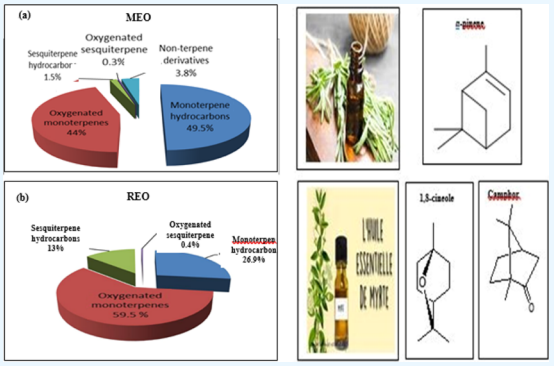
Ines Dhouibi, Guido Flamini, and Mohamed Bouaziz*

Cite This: *ACS Omega* 2023, 8, 6431−6438

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Rosemary (*Rosmarinus officinalis* L.) and myrtle (*Myrtus communis* L.) are perennial herbs, typical of the Tunisian flora, with an intense aromatic flavor. Their essential oils, obtained by hydro-distillation, were analyzed by gas chromatography coupled to mass spectrometry and by infrared Fourier transform spectrometry. In addition, these oils were assessed for their physicochemical properties as well as their antioxidant and antibacterial activities. The physicochemical characterization proved to be of good quality by analyzing pH, water content (%), density at 15 °C (g/cm^3), and iodine values according to standard test methods. The study of the chemical composition allowed for the identification of 1,8-cineole (30%) and α-pinene (40.4%) as the main constituents of myrtle essential oil, while 1,8-cineole (37%), camphor (12.5%), and α-pinene (11.6%) were identified as principal components in rosemary essential oil. The evaluation of their antioxidant activities permitted to obtain the IC_{50} values, which ranged between 22.3 and 44.7 μg/mL for DPPH and between 15.52 and 28.59 μg/mL for ferrous chelating assay, for rosemary and myrtle essential oils, respectively, thus indicating that rosemary essential oil is the most effective antioxidant. Furthermore, the antibacterial activity of the essential oils was tested in vitro against eight bacterial strains by the disc diffusion method. The essential oils showed antibacterial effects on both Gram-positive and Gram-negative bacteria.



1. INTRODUCTION

Aromatic and medicinal plants are known to play a considerable economic role in the industrial, agro-food, perfume, cosmetics, and pharmacy sectors.[1] Indeed, plants represent a limitless source of traditional and effective remedies, thanks to their active ingredients, namely, alkaloids, flavonoids, phenols, tannins, vitamins, and essential oils.

Among these plants, rosemary belongs to the Lamiaceae family, and it is native to the Mediterranean region. Although it grows spontaneously, it is widely cultivated throughout the world as an ornamental plant and small evergreen perennial that grows up to 2 m in height.[2]

The importance of rosemary essential oil (REO) lies in its uses in medicine and its powerful chemopreventive properties.[3] Moreover, REO stands out for its biological activities,[4] which obviously depend on its chemical composition.[5]

Myrtle (Myrtaceae family) is a wild aromatic diploid shrub. It is among the high drought tolerant evergreens 0.5−3 m in height and ovate leaves 3−5 cm long. This plant is not only native to North Africa, Southern Europe, and Western Asia but is also typical of the Mediterranean flora.[6] In Tunisia, the only species found is *Myrtus communis* L., which grows wild in the coastal areas, inland hills, and northern forest areas. Two varieties of myrtle have been described by the old local Tunisian flora: *M. communis* var. italica L. and *M. communis* var. baetica L.[7] Although they possess similar vegetative characters, they have different morphologies.

The myrtle essential oil (MEO) is essentially used for the treatment of bronchitis, tuberculosis, diarrhea, hemorrhoids, and prostatitis.[8] Furthermore, its anti-inflammatory, antibacterial, and wound-healing properties make it a potential candidate for reducing pain and ulcer size in cases of minor recurring aphthous stomatitis.[9] In addition, this plant has important applications in several fields, namely, culinary, pharmaceutical, therapeutic, and industrial. Indeed, MEO has been used by food industries as a flavoring for meats and sauces and by the cosmetic industry as a hair tonic.[10] Its chemical composition may vary according to many factors (organ type, harvesting time, and agricultural practices, among

Received: October 18, 2022
Accepted: January 25, 2023
Published: February 10, 2023

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

others) that are likely to be responsible for the observed diverse biological responses observed.[11]

Bearing in mind the properties of rosemary and myrtle, this study aims to explore the yield and quality of Tunisian rosemary and myrtle essential oils. It also aims to determine the physicochemical properties of these oils and to evaluate its antioxidant and antibacterial activities.

2. MATERIALS AND METHODS

2.1. Plant Material. The essential oils were extracted using the aerial part (leaves and twigs) of plants. Rosemary was collected in October 2020 in the Chebba region, Central-Eastern Tunisia, while myrtle was collected in June of the same year in the Haouaria region, North-East of Tunisia.

2.2. Extraction of the Essential Oils. A Clevenger-type apparatus was used for the extraction of the essential oil of each plant. Indeed, 1 kg of fresh aerial parts of each plant was hydrodistilled for 4 h.[12]

After extraction, the essential oils were recovered as such without the addition of any solvent and subsequently stored in a refrigerator at 4 °C in hermetically closed opaque-glass flasks.

The dry weight of samples was calculated based on the previously determined moisture content, which was used to calculate the yield of the essential oils as follows:

$$\text{essential oil yield (\%)} = \text{weight of collected oil/dry weight of sample} \times 100\%$$

2.3. Analyses of Volatile Compounds. The gas chromatography coupled to mass spectrometry analysis was performed with a Varian CP 3800 gas-chromatograph coupled with a Saturn 2000 mass spectrometer (both Varian, Palo Alto, CA). Analytical conditions: injector and transfer, line temperatures 220 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas was helium at 1 mL/min; injection volume was 0.2 μL (10% hexane solution); the split ratio was 1:30. The identification of the essential oil constituents was performed by comparing their retention time with those of the authentic samples and by means of their LRI relative to the *n*-hexane.

2.4. Fourier-Transform Infrared Spectroscopy. A Perkin Elmer spectrometer was used to obtain the FT-IR spectra of the samples, each of which was scanned at a wave number range of 4000−400 cm^{-1} with a resolution of 2 cm^{-1}.[13]

2.5. Quality Evaluation. Density at 15 °C measurement was performed as recommended by the Brazilian Pharmacopeia V edition.[14] The iodine index was measured by simple titration under AOAC (2000).[15] In addition, the moisture content determination was carried out following the method of AOAC.[16] Furthermore, the determination of the pH was performed following the method recommended by the National Agency for Sanitary Surveillance.[17]

2.6. Antioxidant Activities. *2.6.1. Antiradical Activity against DPPH.* The determination of the DPPH free radical-scavenging activity of REO and MEO was conducted following the method of Rekik et al.[18] A volume of 500 μL of each sample at different concentrations (1−5 mg/mL) was added to 375 μL of 99% ethanol and 125 μL of DPPH solution (0.02% in ethanol) knowing that DPPH (1,1-diphenyl-2-picryl-hydrazyl; M = 394 g/mol) is a free radical source. The obtained mixtures were shaken and then incubated for 60 min in the dark at room temperature. The measurement of the scavenging capacity was carried out spectrophotometrically by controlling the decrease of absorbance at 517 nm. In its radical form, the DPPH has an absorption band at 517 nm, disappearing with the reduction by an antiradical compound. A low absorbance of the reaction mixture reveals high DPPH-free radical-scavenging activity. A reaction mixture with a low absorbance has a strong capacity to scavenge DPPH free radicals. The measurement of DPPH radical-scavenging activity involved the use of ascorbic acid as a positive control following the steps below:

$$\text{\% scavenging effect} = \text{ADPPH} - \text{AE/ADPPH} \times 100$$

with AE denoting the absorbance of the solution when the sample extract is added at a specific level, and ADPPH is the absorbance of the DPPH solution.

2.6.2. Iron (Fe^{2+}) Chelating Activity. The iron chelating activity of the different samples was estimated according to the method of Decker and Welch,[19] with slight modifications. Indeed, 50 μL of 2 mM $FeCl_2$, $4H_2O$ was added to 100 μL of each sample diluted in 450 μL of water (since oil does not dissolve in water, we used Tween 80 as a surfactant (5% oil; 2.5% Tween 80)). The obtained mixtures were incubated at room temperature for 5 min. The reactions were started by adding 200 μL of 5 mM of 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine). The mixtures were then strongly shaken and left to stand at room temperature for 10 min. Similarly, the control tube was prepared, replacing the sample with distilled water. Ascorbic acid was used as a positive control. The solutions absorbance was measured at 562 nm, and the inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated as follows:

$$\text{metal chelating activity \% Ab} = \left(\frac{\text{AC} + \text{AB} - \text{AS}}{\text{AC}}\right) \times 100$$

where AC, AB, and AS are the control absorbance, the blank, and the sample reaction tubes, respectively.

2.7. Antibacterial Activity. *2.7.1. Microbial Strains.* The antibacterial activities of REO and MEO were tested against four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698), *Bacillus cereus* (ATCC 14579), and *Listeria monocytogenes* (ATCC 19115) and four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 43972), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterobacter aerogenes* (ATCC 13048).

2.7.2. Agar Diffusion Method. For the antibacterial activity assay, the culture suspensions (200 μL) of the microorganisms (10^6 colony-forming units CFU/mL of bacteria cells anticipated by absorbance at 600 nm) were placed on Luria-Bertani (LB) agar, already casted in Petri dishes. Next, an amount of 60 μL of each extract (at a concentration of 25 and 50 mg/mL) was loaded into the wells (6 mm in diameter) perforated in the agar layer. Hence, the Petri dishes were incubated for 1 h at 4 °C and then for 24 h at 37 °C. Gentamicin was utilized as a positive standard. Antibacterial activity was assessed by the determination of the growth inhibition zone (whose diameter is expressed in millimeters) around the wells.[20]

2.8. Statistical Analysis. The obtained results were expressed as mean standard deviation (SD) of three measurements. The determination of the significant differences between the values of all parameters was carried out at $P < 0.05$ in compliance with the one-way ANOVA: Student

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

Table 1. Chemical Composition of MEO and REO[a]

components	molecular formula	MW (g/mol)	LRI (compound)	RT (min)	myrtle EO	rosemary EO
monoterpene hydrocarbons					49.5	26.9
tricyclene	$C_{10}H_{16}$	136.23	928	4.46		0.2
α-thujene	$C_{10}H_{16}$	136.23	933	4.55	0.4	
α-pinene	$C_{10}H_{16}$	136.23	941	4.72	40.4	11.6
camphene	$C_{10}H_{16}$	136.23	955	5.07	0.2	4.5
β-pinene	$C_{10}H_{16}$	136.23	982	5.77	0.6	3.1
myrcene	$C_{10}H_{16}$	136.23	993	6.14	0.2	1.5
α-phellandrene	$C_{10}H_{16}$	136.23	1006	6.54		0.2
δ-3-carene	$C_{10}H_{16}$	136.23	1013	6.71	0.7	0.5
α-terpinene	$C_{10}H_{16}$	136.23	1019	6.92		0.4
p-cymene	$C_{10}H_{14}$	134.21	1028	7.18	2.0	2.1
limonene	$C_{10}H_{16}$	136.23	1032	7.31	4.3	2.1
γ-terpinene	$C_{10}H_{16}$	136.23	1062	8.30	0.3	0.3
terpinolene	$C_{10}H_{16}$	136.23	1090	9.36	0.4	0.4
oxygenated monoterpenes					44.0	59.5
1,8-*cineole*	$C_{10}H_{18}O$	154.24	1034	7.40	30.0	37.0
4-*terpineol*	$C_{10}H_{18}O$	154.25	1179	12.90	0.6	1.1
α-terpineol	$C_{10}H_{18}O$	154.25	1191	13.51	3.5	2.9
bornyl acetate	$C_{12}H_{20}O_2$	196.29	1287	17.49		0.3
linalyl acetate	$C_{12}H_{20}O_2$	196.29	1259	16.24	1.0	
linalool	$C_{10}H_{18}O$	154.25	1101	9.83	2.0	0.7
endo-fenchol	$C_{10}H_{18}O$	154.25	1112	10.35		0.1
trans-pinocarveol	$C_{10}H_{16}O$	152.23	1141	11.31	0.4	
camphor	$C_{10}H_{16}O$	152.23	1145	11.50	0.4	12.5
exo-2-hydroxy cineol acetate	$C_{12}H_{20}O_3$	212.29	1345	19.95	0.2	
α-terpinyl acetate	$C_{12}H_{20}O_2$	196.29	1352	20.27	1.7	
geranyl acetate	$C_{12}H_{20}O_2$	196.29	1383	21.85	4.2	
sesquiterpene hydrocarbons					1.5	13.0
α-copaene	$C_{15}H_{24}$	204.35	1377	21.35		0.8
β-caryophyllene	$C_{15}H_{24}$	204.36	1419	23.18	1.2	8.0
α-ylangene	$C_{15}H_{24}$	204.36	1373	21.15		0.2
α-humulene	$C_{15}H_{24}$	204.36	1455	24.62	0.2	1.0
germacrene B	$C_{15}H_{24}$	204.36	1557	28.88	0.1	
oxygenated sesquiterpene					0.3	0.4
caryophyllene oxide	$C_{15}H_{24}O$	220.35	1582	29.95	0.3	0.4
nonterpene derivatives					3.8	
2-methylbutyl 2-methylbutyrate	$C_{10}H_{20}O_2$	172.26	1103	9.93	1.9	
2-methylbutyl isobutyrate	$C_9H_{18}O_2$	158.24	1015	6.87	0.3	
isobutyl 2-methylbutyrate	$C_9H_{18}O_2$	158.24	1002	6.46	0.9	
propyl butyrate	$C_7H_{14}O_2$	130.18	898	4.24	0.7	

[a]Linear retention index (LRI).

Newman−Keuls test, using SPSS Statistics 17.0 for Windows (SPSS Inc., 2008).

3. RESULTS AND DISCUSSION

3.1. Yield and Chemical Composition of Essential Oils. While the EO yield obtained for rosemary (0.91%) was in good agreement with those reported in the literature by Hcini et al. and Hosni et al. for Tunisian plants,[21,22] that of myrtle was lower (0.75%). However, this is not surprising as the yield depends on numerous biotic and abiotic factors, such as the harvest period, the harvested organs type, soil, and rainfall, to cite a few.

The oils compositions summarized in Table 1 were colorless and with a strong fragrant odor. In myrtle essential oil, 28 constituents were characterized, accounting for 99.1% of the whole oil, while in rosemary, 24 volatiles were identified with 91.9% of the oil.

Monoterpenes were the main chemical class of both oils (Figure 1), even if hydrocarbon derivatives prevailed in myrtle (49.5%) and oxygenated ones in rosemary (54.6%).

In the case of myrtle, α-pinene (40.4%) and 1,8-cineole were the main volatiles, followed by limonene and geranyl acetate (4.3 and 4.2%, respectively). REO was mainly composed of 1,8-cineole (37.0%), camphor (12.5%), and α-pinene (11.6%), together with β-caryophyllene and camphene (8.0 and 4.5%, respectively).

3.2. Infrared Analysis. Figure 2A,B shows the FT-IR spectra (4000−400 cm^{-1}) for MEO and REO and the specific band positions and intensities, respectively. They reveal some key feature bands that are likely to be used for the differentiation between the major volatile substances found in MEO and REO, such as 1,8-cineole, camphor, and α-pinene. The identification of the functional groups was based on the FT-IR peaks ascribed to stretching and bending vibrations.

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

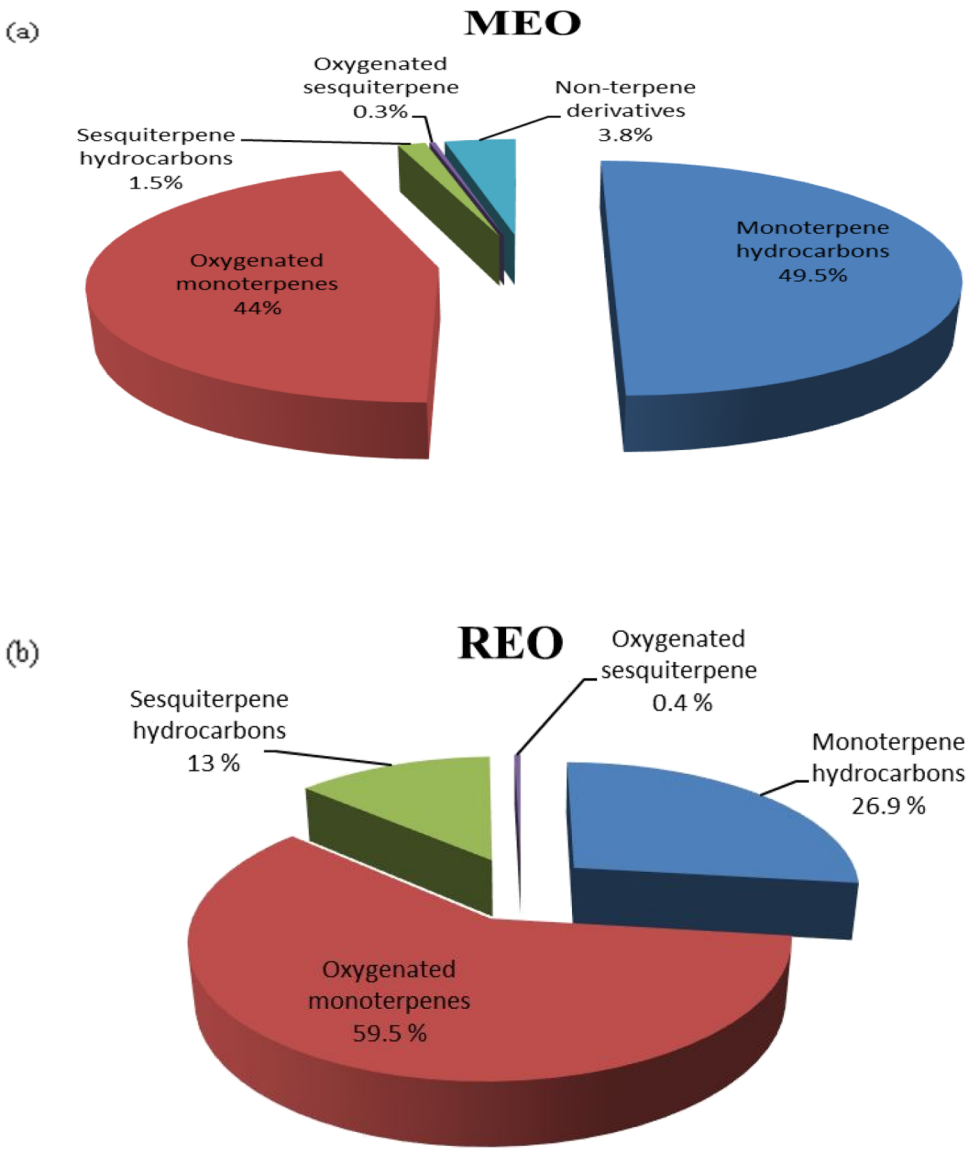


Figure 1. Different classes of compounds in (a) MEO and (b) REO.

The vibrational spectra of MEO presented in Figure 2A were dominated by the bands of its major components, namely α-pinene (at 843 and 787 cm^{-1}) and 1,8-cineole (at 1375; 1234; 1080; 996 and 843 cm^{-1}). These bands corroborated distinctive signals in the FT-IR spectrum due to the wagging vibrations of CH and CH_2 groups (996 cm^{-1} for 1,8-cineole and 843 cm^{-1} for α-pinene). Thus, less intensive bands of 1,8-cineole located in the FT-IR spectrum were accredited to C−O−C symmetrical (1080 cm^{-1}) and asymmetrical (1234 cm^{-1}) stretching vibrations. An extra band was noticed at 3384 cm^{-1}, which is likely to be ascribed to a stretching vibration of hydroxyl group. The aforementioned characteristic key absorption bands are in good agreement with those previously reported in the literature for essential oils from Myrtaceae species.[23] As can be seen in Figure 2B, the REO FT-IR spectrum displays diverse distinctive peaks. All the above-mentioned components contribute to C−H stretching bands not only from 2967 to 2881 cm^{-1}, but also 1447 and 1375 cm^{-1}. The peak observed at 1741 cm^{-1} is essentially ascribed to the carbonyl group of camphor, while the peaks at 1215 and 992 cm^{-1} revealed the presence of an ether function, present on 1,8-cineole. Eventually, the peaks at 1080 and 1053 cm^{-1} are closely related to C−O bond asymmetric stretching. As for the peak at 3472 cm^{-1}, it is linked to the principal IR band, and particularly to the O−H stretching of the O−H group of α-terpineol, 4-terpineol, borneol, linalool, and trans pinocarveol, as reported in a previously published study.[24]

3.3. Physicochemical Properties. Table 2 shows the physicochemical parameters of REO and MEO. The iodine value of oil is indicative of the oil unsaturation degree. The higher the iodine values are, the higher the degree of unsaturation (carbon to carbon double bonds) of the oil is.[25]

A greater iodine value implies a high susceptibility of the oil to oxidation,[26] and the iodine values of the essential oils were found to be 145 mg I_2/100g oil for REO and 155 mg I_2/100g oil for MEO. In this context, MEO has the highest iodine value.

Both oils had densities lower than water, 0.98 and 0.87 g/cm^3 for REO and MEO, respectively. This parameter is associated with each oil chemical composition, which is affected by many factors such as phenotype, harvest time, soil type, storage, process, and extraction conditions.[27] The

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

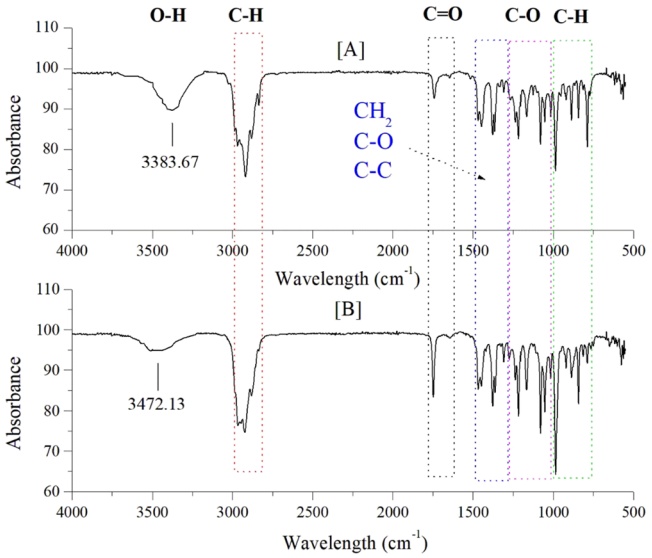


Figure 2. FT-IR spectra (4000−400 cm^{-1}) of (A) MEO and (B) rosemary EO.

Table 2. Physical and Chemical Properties of REO and MEO[a]

properties	units	REO	MEO
density	g/cm^3	0.98 ± 0.00[a]	0.87 ± 0.00[b]
pH		3.42 ± 0.01[a]	3.056 ± 0.01[b]
iodine value	mg I_2/100g	145 ± 0.30[b]	155 ± 0.32[a]
water content	%	0.98 ± 0.00[b]	1.22 ± 0.00[a]

[a]Values are means ± SD (standard deviation). Values with different superscript letters [a,b] within each row are significantly different at $P < 0.05$.

obtained values are in good agreement with those found in a research work from South Africa about essential oils for cosmetic use.[28] On the other hand, values comprised between 1.206 and 1.228 $g{\cdot}cm^{-3}$ were found for the density of the essential oils from nine medicinal herbs grown in Egypt, which were obtained through hydrodistillation.[29]

Humidity or moisture is an important factor that influences the extraction and yield of essential oil. Furthermore, volatile problems represent the weight loss undergone by the product after heating to 105 °C in the operating conditions.[30] In fact, obtained the results of REO and MEO are 0.98 and 1.22%, respectively. In addition, these essential oils are clear liquids with a pH value of around 3.42 (REO) and 3.05 (MEO).

According to the parameters found in the literature and reported by the official AFNOR (2002) rules,[31] both essential oils are fresh and of good quality. The results have proven that the MEO and REO are stable and do not cause worrying oxidation with proof of good storage.

3.4. Antioxidant Activity. Table 3 lists the results of the REO and MEO antioxidant activities. Both essentials were able to decrease the stable, purple-colored radical DPPH into the yellow-colored DPPH−H.

Indeed, the IC_{50} values were 22.3 μg/mL for REO and 44.7 μg/mL for MEO (Table 3). The oxygenated monoterpenes and mixtures of mono- and sesquiterpene hydrocarbons have been reported to be the main responsible for the DPPH radical neutralization.[32] Camphor, one of the main constituents of REO, is well recognized to have high antioxidant activity levels.[33] This can explain the higher antioxidant activity of

Table 3. Antioxidant Activities of REO and MEO: DPPH Radical Scavenging Activity and Ferrous Chelating Effect[a]

	IC_{50} (μg/mL) for REO	IC_{50} (μg/mL) for MEO	[b]IC_{50} (μg/mL) for standard
DPPH radical scavenging activity	44.7 ± 0.09[c]	52.5 ± 0.11[b]	75 ± 0.15[a]
ferrous chelating effect	13.52 ± 0.03[b]	28.59 ± 0.06[a]	14.26 ± 0.03[c]

[a]Data are expressed in mm and given as means ± SD; [a,b,c] Different letters in different samples within the same concentration indicate significant differences ($P < 005$). [b]IC_{50} value for standard: ascorbic acid in DPPH scavenging activity and EDTA in ferrous chelating effect.

REO with respect to MEO (IC_{50} = 22.3 vs IC_{50} = 44.7 μg/mL). Besides, 1,8-cineole, the main constituent of REO (Table 1), may play an important role.

The results about the antioxidant activity of REO are in very good agreement with those of the literature. Indeed, Jedidi et al.[34] have reported that the REO radical scavenging capacity, expressed as IC_{50} in the DPPH assay, is 100.6 μg/mL. This difference in activity has been related to the different chemical compositions of REOs. In another study, Adel et al.[35] found that the antioxidant activity of REO, which was collected from Gafsa (south-west of Tunisia), measured by the DPPH assay is 61% at 300 μg/mg.

Moreover, the findings of the MEO activity in the present study are higher than those previously reported[36] for another Tunisian one, although 1,8-cineole and α-pinene were the major components for both oils. The same was also true in the case of another Tunisian essential oil.[37]

Table 4 also illustrates the ferrous chelating effect of REO and MEO. This test confirmed the higher activity of REO, whose results are even more effective than that reported by Raeisi et al.[38] (81.23 mg/mL). On the contrary, its activity result was lower than found in another study (0.4−2 μg/mL),[39] even if the major constituent of REO was still 1,8-cineole (49.7%). According to the results reported in Table 3, the IC_{50} = value (15.52 μg/mL for REO and 28.59 μg/mL for MEO) reveals the capacity of the two oils to interfere with the Fe^{2+}-ferrozine complex formation, suggesting their ability to capture ferrous ions before ferrozine. A similar activity has been reported by Wannes et al.[40] for the essential oils of Tunisian myrtle flower (IC_{50} = 5 mg/mL), having α-pinene, 1,8-cineole, and limonene as major constituents.

The DPPH scavenging ability and ferrous chelating effect extent confirmed a positive relationship. However, the assessment of the metal chelating effect revealed that REO and MEO were more active than those were observed in the DPPH free radical scavenging activity.

Both antioxidant tests showed that REO and MEO were less effective as an antioxidant than the reference compound ascorbic acid.

3.5. Antibacterial Activity. The antibacterial activity of rosemary and myrtle essential oils against four strains of Gram-positive and four strains of Gram-negative bacteria is listed in Table 4.

REO and MEO proved a varying degree of antibacterial activity at both 25 and 50 mg/mL. However, MEO exhibited the highest activity as the diameters of inhibition were larger than those observed for REO, which is in agreement with the findings of Fadil et al.[41] Furthermore, both responses should be considered as "sensitive" (between 9 and 14 mm) for *S.*

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

Table 4. Antibacterial Activities of REO and MEO against Different Gram+ and Gram− Strains[a]

	gentamicine	REO		MEO	
concentration (mg/mL)	30 mg/mL (mm)	25 mg/mL (mm)	50 mg/mL (mm)	25 mg/mL (mm)	50 mg/mL (mm)
Escherichia coli	15.0 ± 0.03[b]	09.0 ± 0.02[e]	10.0 ± 0.02[d]	11.0 ± 0.02[c]	16.0 ± 0.03[a]
Salmonelle enteric	15.0 ± 0.03[c]	11.0 ± 0.02[d]	20.0 ± 0.04[a]	15.0 ± 0.03[c]	17.0 ± 0.03[b]
Pseudomonasaeruginosa	22.0 ± 0.05[a]	14.0 ± 0.03[d]	20.0 ± 0.04[b]	14.0 ± 0.03[d]	19.0 ± 0.04[c]
Enterobacter aerogenes	19.0 ± 0.04[c]	18.0 ± 0.04[d]	27.0 ± 0.05[a]	17.0 ± 0.03[e]	24.0 ± 0.05[b]
Listeria monocytogenes	18.0 ± 0.04[a]	14.0 ± 0.03[d]	14.0 ± 0.03[d]	15.0 ± 0.03[c]	16.0 ± 0.03[b]
Staphylococcus aureus	36.0 ± 0.07[a]	12.0 ± 0.02[c]	14.0 ± 0.03[b]	12.0 ± 0.02[c]	14.0 ± 0.03[b]
Bacillus cereus	22.0 ± 0.05[a]	12.0 ± 0.02[d]	14.0 ± 0.03[c]	12.0 ± 0.02[d]	21.0 ± 0.04[b]
Micrococcus luteus	18.0 ± 0.04[b]	13.0 ± 0.03[c]	21.0 ± 0.04[a]	12.0 ± 0.02[d]	18.0 ± 0.04[b]

[a]Data are expressed in mm and given as means ± SD; [a,b,c,d,e] Different letters in different samples within the same concentration indicate significant differences ($P < 005$).

aureus. The comparison of the essential oil activities with the control antibiotic gentamicin demonstrated that both essential oils exhibited an important activity against *E. coli* (16.0 for MEO), *S. enterica* (20.0 mm for REO and 17.0 mm for MEO), *Enterobacter aerogenes* (27.0 and 24.0 mm), and *M. luteus* (21.0 and 18.0 mm) at 50 mg/mL. Besides, the used solvent (ethanol) did not convey any activity on the strains under investigation, which supports the adequate choice of this solvent.

REO and MEO were found to be rich in monoterpene compounds (86.4 and 93.5%, respectively), which is in good accordance with the results reported in the literature for their antibacterial activities.[42] Interestingly, the richness of the oils in monoterpenes such as 1,8-cineole confirms their strong antibacterial activities against several bacteria.[43] On the other hand, the antibacterial effects of minor compounds, such as caryophyllene oxide and terpinene-4-ol, were also known.[43]

4. CONCLUSIONS

In the current investigation, the essential oils of rosemary and myrtle were extracted and their biological activity and chemical composition were described. Out of the obtained 32 components, the composition of REO showed that 1,8-cineole (37%), camphor (12.5%), and pinene (11.6%) were the main constituents. Out of the identified 28 components, pinene (40.4%) and 1,8-cineole (30%) were the predominant substances in MEO. The presence of these compounds was totally supported by IR spectroscopy thanks to noticeable bands. The findings of the physicochemical investigation, on the other hand, showed that both essential oils were of high grade.

Chelating power and DPPH tests on the antioxidant activity revealed that REO had the highest level of activity. Finally, the studied pathogenic bacteria were susceptible to the essential oils' in vitro antibacterial activity at modest doses, between 25 and 50 mg/mL. The bacteria against which an important activity was discovered by REO's antibacterial test were *S. enterica*, *P. aeruginosa*, *E. aerogenes*, and *M. luteus*. As for MEO, it exhibited an important activity against *E. coli*, *S. enterica*, *P. aeruginosa*, *E. aerogenes*, *L. monocytogenes*, *B. cereus*, and *M. luteus*. In view of these results, MEO was proven to be the most effective. Furthermore, REO and MEO can be reliably used in commercial applications as an antioxidant and antibacterial agent alone or in combination with conventional preservatives to prevent harmful microbial deterioration in some food modules or as a treatment for wounds.

This research work has confirmed that these two aromatic and medicinal plants represent a very interesting reservoir, whose essential oils are characterized by specific therapeutic and pharmacological properties that need to be exploited by future research.

AUTHOR INFORMATION

Corresponding Author

Mohamed Bouaziz − *Laboratory of Electrochemistry and Environment, National School of Engineers of Sfax, University of Sfax, 3038 Sfax, Tunisia; Higher Institute of Biotechnology of Sfax, University of Sfax, 3038 Sfax, Tunisia;* orcid.org/0000-0001-9107-7027; Email: mohamed.bouaziz@fsg.rnu.tn

Authors

Ines Dhouibi − *Laboratory of Electrochemistry and Environment, National School of Engineers of Sfax, University of Sfax, 3038 Sfax, Tunisia*

Guido Flamini − *Dipartimento di Farmacia, Università di Pisa, 56126 Pisa, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c06713

Funding

The authors thank the Ministry of Higher Education and Scientific Research (Laboratory LR14ES08), Tunisia, and MedOOmics Project—"Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting"—"Arimnet2/0001/2015", for its financial support to this research work.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

REO: rosemary essential oil; MEO: myrtle essential oil; GC−MS: gas chromatography/mass spectrometry

REFERENCES

(1) Boussaada, O.; Ammar, S.; Saidana, D.; Chriaa, J.; Chraif, I.; Daami, M.; Helal, A. N.; Mighri, Z. Chemical Composition and Antimicrobial Activity of Volatile Components from Capitula and Aerial Parts of Rhaponticum Acaule DC Growing Wild in Tunisia. *Microbiol. Res.* **2008**, *163*, 87−95.

(2) Akrout, A.; Hajlaoui, H.; Mighri, H.; Najjaa, H.; Jani, H. E.; Zaidi, S.; Neffati, M. Chemical and Biological Characteristics of Essential Oil of Rosmarinus Officinalis Cultivated in Djerba. *J. Essent. Oil Bear. Plants* **2010**, *13*, 398−411.

(3) Aouadi, M.; Sebai, E.; Saratsis, A.; Kantzoura, V.; Saratsi, K.; Msaada, K.; Sotiraki, S.; Akkari, H. Essential oil of Rosmarinus officinalis induces in vitro anthelmintic and anticoccidial effects

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438

against Haemonchus contortus and Eimeria spp. in small ruminants. *Vet. Med.* **2021**, *66*, 146−155.
(4) Ben Jemia, M.; Tundis, R.; Pugliese, A.; Menichini, F.; Senatore, F.; Bruno, M.; Kchouk, M. E.; Loizzo, M. R. Effect of Bioclimatic Area on the Composition and Bioactivity of Tunisian Rosmarinus Officinalis Essential Oils. *Nat. Prod. Res.* **2015**, *29*, 213−222.
(5) Farhat, A.; Benmoussa, H.; Bachoual, R.; Nasfi, Z.; Elfalleh, W.; Romdhane, M.; Bouajila, J. Efficiency of the optimized microwave assisted extractions on the yield, chemical composition and biological activities of Tunisian Rosmarinus officinalis L. essential oil. *Food Bioprod. Process.* **2017**, *105*, 224−233.
(6) Baytop, T. *Türkce Bitki Adlari Sözlügü*; Türk tarih kurumu, 1997.
(7) Kanoun, K.; Belyagoubi-Benhammou, N.; Ghembaza, N.; Bekkara, F. A. Comparative Studies on Antioxidant Activities of Extracts from the Leaf, Stem and Berry of Myrtus Communis L. *Int. Food Res. J.* **2014**, *21*, 1957.
(8) Mahboubi, M. Effectiveness of Myrtus Communis in the Treatment of Hemorrhoids. *J. Integr. Med.* **2017**, *15*, 351−358.
(9) Dhifi, W.; Jazi, S.; El Beyrouthy, M.; Sadaka, C.; Mnif, W. Assessing the Potential and Safety of Myrtus Communis Flower Essential Oils as Efficient Natural Preservatives against Listeria Monocytogenes Growth in Minced Beef under Refrigeration. *Food Sci. Nutr.* **2020**, *8*, 2076−2087.
(10) Snoussi, A.; Chaabouni, M. M.; Bouzouita, N.; Kachouri, F. Chemical Composition and Antioxidant Activity of Myrtus Communis L. Floral Buds Essential Oil. *J. Essent. Oil Res.* **2011**, *23*, 10−14.
(11) Gortzi, O.; Lalas, S.; Chinou, I.; Tsaknis, J. Reevaluation of Bioactivity and Antioxidant Activity of Myrtus Communis Extract before and after Encapsulation in Liposomes. *Eur. Food Res. Technol.* **2008**, *226*, 583−590.
(12) Bouaziz, M.; Yangui, T.; Sayadi, S.; Dhouib, A. Disinfectant Properties of Essential Oils from Salvia Officinalis L. Cultivated in Tunisia. *Food Chem. Toxicol.* **2009**, *47*, 2755−2760.
(13) Hedayati, S.; Niakousari, M.; Mohsenpour, Z. Production of Tapioca Starch Nanoparticles by Nanoprecipitation-Sonication Treatment. *Int. J. Biol. Macromol.* **2020**, *143*, 136−142.
(14) Cavalcanti, N. C. T.; Sousa, G. D.; Tabosa, M. A. M.; Soares Sobrinho, J. L.; Leal, L. B.; Santana, D. P. . Assay and Physicochemical Characterization of the Antiparasitic Albendazole. *Braz. J. Pharm. Sci.* **2012**, *48*, 281−290.
(15) Aladekoyi, G.; Orungbemi, O. O. Comparative Studies of Physico-Chemical Composition and Antibacterial Activities of Essential Oil Extracted from Medicinal Plants of Scent Leaves (Ocimum Basilicum Lamiaceae and Ocimum Gratissimum Lamiaceae). *Res. J. Food Sci. Nutr.* **2016**, *1*, 28−34.
(16) Association of Official Analytical Chemists; Horwitz, W. *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, 1975; Vol. *222*.
(17) Muniz, M. A. P.; dos Santos, M. N. F.; da Costa, C. E. F.; Morais, L.; Lamarão, M. L. N.; Ribeiro-Costa, R. M.; Silva-Júnior, J. O. C. Physicochemical Characterization, Fatty Acid Composition, and Thermal Analysis of Bertholletia Excelsa HBK Oil. *Pharmacogn. Mag.* **2015**, *11*, 147.
(18) Rekik, O.; Ben Mansour, A.; Jabeur, H.; Rodriguez Gutierrez, G.; Bouaziz, M. Effect of Pollution on the Quality of Olive Oils from Trees Grown Near a Phosphoric Acid Factory. *Eur. J. Lipid Sci. Technol.* **2019**, *121*, No. 1800490.
(19) Decker, E. A.; Welch, B. Role of Ferritin as a Lipid Oxidation Catalyst in Muscle Food. *J. Agric. Food Chem.* **1990**, *38*, 674−677.
(20) Yakoub, A. R. B.; Abdehedi, O.; Jridi, M.; Elfalleh, W.; Nasri, M.; Ferchichi, A. Flavonoids, Phenols, Antioxidant, and Antimicrobial Activities in Various Extracts from Tossa Jute Leave (Corchorus Olitorus L.). *Ind. Crops Prod.* **2018**, *118*, 206−213.
(21) Hcini, K.; Sotomayor, J. A.; Jordan, M. J.; Bouzid, S. Chemical Composition of the Essential Oil of Rosemary (Rosmarinus Officinalis L.) of Tunisian Origin. *Asian J. Chem.* **2013**, *25*, 2601−2603.
(22) Hosni, K.; Hassen, I.; Chaâbane, H.; Jemli, M.; Dallali, S.; Sebei, H.; Casabianca, H. Enzyme-Assisted Extraction of Essential Oils from Thyme (Thymus Capitatus L.) and Rosemary (Rosmarinus Officinalis L.): Impact on Yield, Chemical Composition and Antimicrobial Activity. *Ind. Crops Prod.* **2013**, *47*, 291−299.
(23) Wang, L.-H.; Sung, W.-C. Rapid Evaluation and Quantitative Analysis of Eugenol Derivatives in Essential Oils and Cosmetic Formulations on Human Skin Using Attenuated Total Reflectance−Infrared Spectroscopy. *Spectroscopy* **2011**, *26*, 43−52.
(24) Dhouibi, I.; Masmoudi, F.; Bouaziz, M.; Masmoudi, M. A Study of the Anti-Corrosive Effects of Essential Oils of Rosemary and Myrtle for Copper Corrosion in Chloride Media. *Arab. J. Chem.* **2021**, *14*, No. 102961.
(25) Francilia, O. A.; Oluwasanmi, L. I.; Abosede, F. T.; Adenike, S. R.; Benedicta, A. O.; Solomon, A. I. Physicochemical Properties and Inhibitory Effects of Essential Oils from Selected Local Spices. *World J. Adv. Res. Rev.* **2020**, *5*, 058−066.
(26) Amos-Tautua, B. M. W.; Onigbinde, A. O. Physicochemical Properties and Fatty Acid Profiles of Crude Oil Extracts from Three Vegetable Seeds. *Pak. J. Nutr.* **2013**, *12*, 647.
(27) Teuscher, E.; Anton, R.; Lobstein, A. *Plantes Aromatiques: Épices, Aromates, Condiments et Huiles Essentielles*; Tec & Doc, 2005.
(28) Vermaak, I.; Kamatou, G. P. P.; Komane-Mofokeng, B.; Viljoen, A. M.; Beckett, K. African Seed Oils of Commercial Importance—Cosmetic Applications. *South Afr. J. Bot.* **2011**, *77*, 920−933.
(29) Stanciu, G.; Oancea, A.-I.; Oancea, E.; Chirila, E. Analytical Characterisation of Original Emulsion for Cosmetic Use. *Rev. Chim.* **2019**, *70*, 749−752.
(30) Ainane, T.; Gharby, S.; Talbi, M.; Abourriche, A.; Bennamara, A.; Oukkache, N.; Lamdini, H.; Elkouali, M. Moroccan Formulation of Oils for the Care of Hair: Chemical Composition and Antibacterial Activity. *SOJ Biochem.* **2016**, DOI: 10.15226/2376-4589/2/2/00108.
(31) Vilcot, C.; Leclet, H. *Les Indicateurs Qualité En Santé*; Afnor, 2002.
(32) Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Jovin, E. Antimicrobial and Antioxidant Properties of Rosemary and Sage (Rosmarinus Officinalis L. and Salvia Officinalis L., Lamiaceae) Essential Oils. *J. Agric. Food Chem.* **2007**, *55*, 7879−7885.
(33) Shukla, R.; Singh, P.; Prakash, B.; Dubey, N. K. Antifungal, Aflatoxin Inhibition and Antioxidant Activity of Callistemon Lanceolatus (Sm.) Sweet Essential Oil and Its Major Component 1, 8-Cineole against Fungal Isolates from Chickpea Seeds. *Food Control* **2012**, *25*, 27−33.
(34) Jedidi, S.; Aloui, F.; Selmi, H.; Jdaidi, N.; Ghribi, S.; Sebai, H. Phytochemical Profile and Antioxidant Properties of Essential Oils Isolated from Rosmarinus Officinalis Cultivated in Tunisia. *Mor. J. Agric. Sci.* **2022**, *3*, 229.
(35) Adel, K.; Zied, Z.; Ines, B. C.; Ahmed, B.; Neji, G.; Mohamed, D.; Radhouane, G. Chemical Constituents and Antioxidant Properties of Rosmarinus Officinalis L. Essential Oil Cultivated from the South-Western Tunisia. *J. Med. Plants Res.* **2011**, *5*, 6502−6508.
(36) Jerbi, A.; Abdennabi, R.; Gharsallah, N.; Flamini, G.; Kammoun, M. Essential Oil Composition, Free-Radical-Scavenging and Antibacterial Effect from Leaves of *Myrtus Communis* in Tunisia. *J. Essent. Oil Bear Plants* **2016**, *19*, 1503.
(37) Snoussi, A.; Essaidi, I.; Ben Haj Koubaier, H.; Zrelli, H.; Alsafari, I.; Živoslav, T.; Mihailovic, J.; Khan, M.; El Omri, A.; Ćirković Veličković, T. Drying Methodology Effect on the Phenolic Content, Antioxidant Activity of Myrtus Communis L. Leaves Ethanol Extracts and Soybean Oil Oxidative Stability. *BMC Chem.* **2021**, *15*, 31.
(38) Raeisi, M.; Hashemi, M.; Afshari, A.; Tabarraei, A.; Aminzare, M.; Jannat, B. Cinnamon and Rosemary Essential Oils Incorporated into Alginate Coating Improve Chemical and Sensorial Quality of Chicken Meat. *Available SSRN 3627606*, 2019.
(39) Soylu, E. M.; Soylu, S.; Kurt, S. Antimicrobial Activities of the Essential Oils of Various Plants against Tomato Late Blight Disease Agent Phytophthora Infestans. *Mycopathologia* **2006**, *161*, 119−128.
(40) Wannes, W. A.; Mhamdi, B.; Sriti, J.; Ben Jemia, M.; Ouchikh, O.; Hamdaoui, G.; Kchouk, M. E.; Marzouk, B. Antioxidant Activities of the Essential Oils and Methanol Extracts from Myrtle (Myrtus

Communis Var. Italica L.) Leaf, Stem and Flower. *Food Chem. Toxicol.* **2010**, *48*, 1362−1370.
(41) Fadil, M.; Fikri-Benbrahim, K.; Rachiq, S.; Ihssane, B.; Lebrazi, S.; Chraibi, M.; Haloui, T.; Farah, A. Combined Treatment of Thymus Vulgaris L., Rosmarinus Officinalis L. and Myrtus Communis L. Essential Oils against Salmonella Typhimurium: Optimization of Antibacterial Activity by Mixture Design Methodology. *Eur. J. Pharm. Biopharm.* **2018**, *126*, 211−220.
(42) Pirbalouti, A. G.; Mirbagheri, H.; Hamedi, B.; Rahimi, E. Antibacterial Activity of the Essential Oils of Myrtle Leaves against Erysipelothrix Rhusiopathiae. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, S505−S509.
(43) Lang, G.; Buchbauer, G. A Review on Recent Research Results (2008−2010) on Essential Oils as Antimicrobials and Antifungals. A Review. *Flavour Fragr. J.* **2012**, *27*, 13−39.

https://doi.org/10.1021/acsomega.2c06713
ACS Omega 2023, 8, 6431−6438