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Biological characteristics of the essential oils and methanol extracts from Rosmarinus Officinalis

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Abstract
In this work, we study the chemical composition and antioxidant activity of the essential oils and methanol extracts of Rosmarinus Officinalis growing in Tunisia. The composition of essential oils was characterized by GC/MS and GC. Antioxidant activities of the essential oils and methanol extracts from Rosmarinus Officinalis were evaluated by using DPPH radical scavenging. In all tests, methanol extracts showed better antioxidant activity than essential oils. But the essential oils showed better antimicrobial activities than methanol extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.*

Key words: Essential oil, Rosmarinus Officinalis, antibacterial activity, antioxidant activity, and methanol extracts.

Introduction
Rosmarinus Officinalis is an herbaceous with a strong characteristic aroma and green leaves, which grows in many Mediterranean countries [1-16]. The microbial contamination of food still poses important public health and economic concerns for the human society. For these reasons essential oils and plant extracts [10] are studied for their antimicrobial activities and most essential oils derived from plants are known to possess insecticidal, antifungal, a caricidal, antibacterial and cytotoxic activities [11]. Therefore, the essential oils and extracts from Rosmarinus Officinalis are applied in the fields of pharmacology, medical and clinical microbiology, and phytopathology and food preservation [12].
In this work, we carried out a study of the antimicrobial and antioxidant activities of the essential oils and methanol extracts.

2. Materials and methods
2.1. Distillation of essential oil
300 g ground Rosmarinus Officinalis were submitted to water distillation for 4 hours using a Clevenger apparatus. The essential oils obtained were dried over anhydrous sodium sulfate and filtered and stored at +4°C [17-20].

2.2. Preparation of methanolic extracts
The plant materials were macerated in methanol for 48 heures and filtered to obtain the methanol extracts from which the solvents were evaporated using a rotary vacuum evaporator and stored at +4°C. The plant materials were cut into small pieces and extracted in methanol by using a soxhlet apparatus for 8 hours (adapted from chang, ostrich-Matijasevic, Hsich and Huang 1977). The solvents were evaporated using a rotary vacuum evaporator and stored at +4°C.

2.3. Analysis of the essential oils
For GC/MS analysis, a Hewlett-Packard G 1800A GCD System, equipped with a HP-Innowax silica capillary column (60m * 0.25mm Ø, film thickness 0.25µm) was used. Then, we compare the relative retention times and mass spectra of the essential oils compounds with the data from the baser library, Mass-Finder and Adams GC/MS libraries.

2.4. Antimicrobial activity

The antibacterial activity of the essential oils and the methanol extracts were tested against the bacteria such as (*Escherichia coli, Klebsiella pneumonia, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus*) and against reference bacteria *Escherichia coli* (ATCC7625), *Pseudomonas aeruginosa* (ATCC7624), *Staphylococcus aureus* (ATCC 76110). This study was carried out in the laboratory of the urgent medical care center of Tunis via two methods. The bactericidal effect study destroys 99.99% of the inoculums bacteria [21].

2.4. a. Method of serial dilution

This method consists to mixing the equal volumes of every bacteria inoculums with the methanol extracts or/and the essential oils. After incubation during 24 hours in 37°C, we sow the surface of Muller-Hinton agar one streak of every tube contents [17, 18, 22, 23]. After incubation during 24 hours, we are counting the number of the colonies which develop at every streak. This is repeated three times [16].

2.4. b. Disc diffusion method

0.1 ml from bacterial suspension was spread on the Mueller Hinton agar (MHA). Then, we place on the inoculate plates, the filter paper discs (6 mm in diameter) [9]. At a very low concentration, we compare the antimicrobial activities discs with standards antibiotics [24].

2.5. Antioxidant activity

Basing in the method described by tepe et al [25], we study the antioxidant activity of the methanol extracts and essential oils of Rosmarinus Officinalis. 5 ml of a methanol solution of 2,2'-diphenylpicrylhydrazyle (DPPH) 0.002% in methanol add to 50 µl of essential oil and to methanol extracts. The mixture was incubated at room temperature for 30 min. We used the stable antioxidant Trolox (1mM) (sigma-Aldrich) as a synthetic reference [22, 26]. At λ = 520 nm, we measured the absorbance against corresponding blank inhibition. The inhibition percentage of free radical DPPH (I%) was calculated in the following way:

$$I(\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

A_{blank}: the absorbance of the control reaction.
A_{sample}: the absorbance of the test extract.
Tests are repeated three times.

3. Results and discussions

3.1. Chemical composition and antimicrobial activities

We study the composition of essential oils of Rosmarinus Officinalis in the different locations in Tunisia (Table 1). Results show that the content of 1,8-cineole (56.2 %) of the essential oils from SidiBouzid were the highest among other constituents and the other oils of Zaghouan.

Many study [1, 3, 6] reported that two major types of Rosmarinus oil can be distinguished. The oils with over 40% of 1,8-cineole was characteristically from Morocco (52.1%), Turkey, Tunisia, Greece, Yugoslavia, Italy and France.

But in Zaghouan, we found 1,8-cineole contents oils (12.6%) which is lower than those of Sidi Bouzid. We can explain that by the climate. In Sidi Bouzid, there is a very hot climate but a moderately hot in Zaghouan. We found a moderately high contents in Zaghoun oils like (camphor; 8.7%) and (verbanone; 36.7%). Where as in Sidi Bouzid oils the camphor compound is remarkably low (3.2%) and no verbanone was detected with trace amount. Wheras no verbanone was detected with a trace amount. The α-pinene contents were moderately high in the two regions: Sidi Bouzid oils (9.8%) and Zaghouan oils (5.3%) (Table1).

3.2. Antioxidant activities

We determine the DPPH radical-scavenging activities of the essential oils and methanol extracts of R.Officinalis. Then, we compare our results with references (Table 2).

Table 1: Composition of the essential oils of Rosmarinus Officinalis

Compounds	Sidi Bouzid (%)	Zaghouan (%)
Tricyclene	tr	tr
The **α-pinène**	**9.8**	**5.3**
The α-Fenchene	0.2	0.1
Camphène	5.7	5.8
L'β-pinene	1.9	0.5
Thuja-2,4(10)-diene	0.1	0.1
Myrcene	0.8	0.2
l'α-Terpinene	0.5	0.3
1,8-Cineole	**56.2**	**12.6**
(Z)-β-Ocimene	0.8	0.2
L'γ-terpinene	0.5	0.1
(E)-β-Ocimene	----	----
p-Cymene	0.8	1.1
Terpinolene	0.1	0.1
(Z)-3-Hexenol	-----	-----
Nonanal	tr	tr
Filifolone	tr	-----
Linalool oxide (Furanoid)	0.1	0.9
Camphor	**3.2**	**8.7**
Linalool	0.2	0.2
Bornyl acetate	2.1	1.1
Terpinen-4-ol	6.6	11.3
The β-Caryophyllene	0.1	0.4
Myrtenol	0.6	Tr
The α -Terpineol	1.1	3.9
Cis-Verbenol	0.7	0.7
Borneol	0.1	0.6
Verbanone	**3.1**	**36.7**
Caryophyllene oxide	0.8	0.6
Methyl eugenol	Tr	0.1
Eugenol	0.3	0.3
Geranyl acetate	1.5	1.4
Linalyl acetate	Tr	Tr
Total	97.9%	93.3%

Tr, trace.

Table 2: Antioxydant activity of methanol extracts and essential oils of R.Officinalis, measured by the DPPH test and by the inhibitory effect.

echantillon	Concentration (mg/ml)	% inhibition
Trolox	1	93.4 %
Essential oil (Sidi bouzid)	1	21.1 %
Essential oil (Zaghouan)	1	18.5 %
Methanol extract (Sidi bouzid)	1	38.7 %
Methanol extract (Zaghouan)	1	35.6 %

Results show that methanol extracts of R.Officinalis from the two different regions in Tunisia (Sidi bouzid and Zaghouan) reduced the concentration of DPPH free radical with higher efficacy than the two essential oils. We can note that methanol extracts from the two regions of Tunisia are more performance than the two essential oils. So results show that essential oils and methanol extracts reduced the concentration of DPPH. Other study shows that oils with a higher monoterpenic have poor performance antioxidant activities [25].

3.3. Antimicrobial activities

We study the antimicrobial activities of the essential oils and methanol extracts of Rosmarinus Officinalis from the two different regions of Tunisia. Results indicate that the essential oils and the methanol extracts have moderate antibacterial activities. We note that *Staphylococcus aureus* (ATCC 76110), *Staphylococcus aureus* and *Escherichia coli* (ATCC7625) were the most sensitive microorganisms to the essential oils and methanol extracts (Table 3).

Table 3: Antimicrobial activity of the essential oils of Rosmarinus Officinalis

Microorganisms	HE (Sidi Bouzid)	HE (Zaghouan)	Methanol extract (Sidi Bouzid)	Methanol extract (Zaghouan)
Serratia marcescens	1/8	1/16	1/8	1/8
Acinetobacter baumanni	1/8	1/8	1/8	1/8
Staphylococcus aureus	1/128	1/128	1/64	1/32
pseudomonas aeruginosa	1/16	1/8	1/8	1/8
Klebsiella pneumonia	1/32	1/16	1/8	1/8
Escherichia coli	1/64	1/64	1/16	1/16
Staphylococcus aureus (ATCC 76110)	1/256	1/256	1/128	1/128
Pseudomonas aeruginosa (ATCC7624)	1/16	1/16	1/8	1/8
Escherichia coli (ATCC7625)	1/64	1/128	1/32	1/64

Table 3 shows that the Gram-positive stains are more sensitive than the Gram-negative bacteria examined *pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumanni* and *Serratia marcescens* are resistant to both oils from Sidi Bouzid and Zaghouan.

Results from the disc diffusion method (table 4) show that, *Staphylococcus aureus* (ATCC 76110) is the most sensitive microorganism.

Table 4: Antimicrobial activity of the essential oils of Rosmarinus Officinalis

Microorganisms	HE Sidi Bouzid	HE Zaghouan	Methanol extract (Sidi Bouzid)	Methanol extract (Zaghouan)
Serratia marcescens	9.7	10.5	8.5	9.0
Acinetobacter baumanni	8.5	8.1	8.0	7.5
Staphylococcus aureus	16.5	16.6	13.5	12.0
pseudomonas aeruginosa	7.5	8.5	7.0	7.0
Klebsiella pneumonia	9.5	10.0	7.5	7.1
Escherichia coli	14.0	14.5	9.5	8.5
Staphylococcus aureus (ATCC 76110)	17.0	17.1	14.5	13.5
pseudomonas aeruginosa (ATCC7624)	9.5	9.1	8.0	8.5
Escherichia coli (ATCC7625)	15.1	15.2	12.1	12.0

So the inhibition zones from *Staphylococcus aureus* and *Escherichia coli* (ATCC7625), on exposure to R.Officinalis oil-rich fractions from Zaghouan in March were respectively about 16.60 mm and 15.20 mm. this is indicate that our results are very close with the study conducted by (Santoyo et al, 2005) [24]. So, we can say that the antimicrobial property of the essential oils was basing in the presence compounds such as α-pinene; 1,8-cineole; camphor; verbanone and borneol. We can note that essential oils content borneol, camphor, and verbanone, have the most effective antimicrobial activities. In general, we note that methanol extracts exhibit very low the bacteria compared to the essential oils. So the antimicrobial screening showed low activity against *Staphylococcus aureus* but the rest of the extracts were inactive against other microorganisms (Table 3 and Table 4).

Conclusion

Results show that the essential oils of R.Officinalis from the two regions of Tunisia have antimicrobial activities. So the Gram-positive stains are more sensitive than the Gram-negative bacteria. We note that *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumanni* and *Serratia marcescens* are resistant to both oils from Sidi Bouzid and Zaghouan.

Finally, our study shows that in all tests, the essential oils have better antimicrobial activities than methanol extracts.

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