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Original Article

ESSENTIAL OILS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF THE LEAVES OF *ROSMARINUS OFFICINALIS*, *SALVIA OFFICINALIS* AND *MENTHA PIPERITA* CULTIVATED IN AGADIR (MOROCCO)

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ABSTRACT

Objective: This study was designed to determine the chemical composition and the antibacterial activity of hydro-distillated essential oils (EOs) extracted from leaves of three aromatic and medicinal plants cultivated in Agadir (Morocco): *Rosmarinus officinalis, Mentha piperita* and *Salvia officinalis.*

Methods: EOs chemical composition was determined by GC and GC/MS. Disc diffusion method and agar dilution technique were used to evaluate their antibacterial activity against eight microbial strains. Moreover, a statistical study, consisting in a student test, was conducted.

Results: The most important constituents identified were α-pinene (34.83%), α-thujone (24.05%) and Menthol (41, 23%) respectively in the Rosemary, the Sage and Mint. All tested EOs exhibited an antibacterial activity at different levels against the studied strains. *Micrococcus luteus* was the most sensitive to the tested EOs with Minimum Inhibitory Concentration values of 5.8, 2.35 and 20 mg/ml for Rosemary, Sage and Mint EOs respectively. Thus, these EOs can be used as antibacterial supplement in the developing countries to develop new therapeutic agents.

Conclusions: Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate their potential.

Keywords: Antibacterial activity, Essential oils, *Rosmarinus officinalis, Salvia officinalis, Mentha piperita*.

INTRODUCTION

Multiple resistances to currently available antibiotics by pathogens, responsible of various human diseases, increase at an alarming rate involving the need to look for antimicrobial agents of natural origin deriving especially from numerous medicinal plants. For example, *Staphycoccus aureus*, the principal agent implied in nosocomial infections, has developed many mechanisms of resistance to antibiotics [1] inducing a prolongation of the pathological state and an increase in the mortality rate. This study is therefore one of the approaches to solve such problems by using aromatic and medicinal plant's proprieties.

Medicinal and aromatic plants have traditionally been used in folk medicine as well as in food conservation, through their inhibition potential against bacteria, fungi and yeast. Most of their properties are due to essential oils produced as secondary metabolites [2]. Moreover, it is now evident that biological activities of the essential oils/extracts are correlated to the presence of specific chemical compounds [3].

Among several aromatic plants with antimicrobial activity, those of the family Lamiaceae, such as *Rosmarinus officinalis* (Rosemary), *Mentha piperita* (mint) and *Salvia officinalis* (sage) are prominent. The genus Salvia contains about 900 species, cultivated for culinary, medicinal and ornamental purposes, mainly dispersed in the Mediterranean area, Southeast Africa, Central and South America and for which many pharmacological studies intended to identify the compounds responsible for their therapeutic effects [4]. Rosemary is a perennial evergreen herb, with fragrant needle-like leaves, widely used in traditional medicine and cosmetics. Rosemary species are also important for their medicinal uses and their powerful antibacterial, cytotoxic, antimutagenic, antioxidant, antiphlogistic and chemopreventive properties [5]. Finally, *M. piperita* is an important medicinal plant growing throughout North America, Asia and Europe, primarily cultivated for its Peppermint oil which is extracted from the flowering plant's leaves [6] and used for flavoring pharmaceuticals and oral preparations.

In this context, our study was investigated to determine the chemical composition and the antibacterial activity of essential oils extracted from three aromatic and medicinal plants (AMP) cultivated in Agadir (Morocco): *R. officinalis, M. piperita* and *S. officinalis*.

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *R. officinalis, S. officinalis* and *M. piperita* were collected in 2012, during their respective flowering stage, from a well-controlled biological cultivation in Jacky garden located in Biougra (31 °12'36" N et 8 °51'0" W), in the province of Chtouka Ait Baha, at 35 km from Agadir city. The authenticated voucher specimens of plant's species, identified botanically by Prof. A. Farah, were placed in the herbarium of phytochemistry laboratory of the National Institute of Medicinal and Aromatic Plants/University Sidi Mohamed Ben Abdellah, Fez, Morocco. The different samples have been adopted the following codes: *Rosmarinus officinalis* code: FA/RP/INPMA/121, *Salvia officinalis* code: FA/RP/INPMA/122, *Mentha piperita's* code: FA/RP/INPMA/126.

Essential oils extraction

Leaves of the three studied plants were separated from aerial parts and finely ground, then 100 g of each one was subjected to hydrodistillation for three hours by using Clevenger-type [7]. The extracted EOs were treated with anhydrous sodium sulfate (Na_2SO_4) to eliminate all water, then filtered and kept in the dark at 4 °C until tested and analyzed.

Gas Chromatography-Mass spectrometry

Separating and analyzing the EOs samples was done by a system with electronic pressure control: Hewlett Packard (HP6890 series)

coupled with a mass spectrometer (HP 5973 series). The GC was equipped with a fused silica capillary column (5% phenyl methyl polysiloxane), (30 m × 0.25 mm), coated with 0.25 µm film Rtx-5MS. The injection and detection temperatures were set at 250 and 280 °C, respectively. The applied oven temperature program was: 40 °C for 5 min, rising at 19 °C/min to 280 °C and held for 5 min. The control mode was split and split ratio 5:10. Carrier gas was helium with flow-rate of 1.4 ml/min. 1 µl of oil was introduced directly into the source of the MS via a transfer line with a split ratio of 1:50.

The mass spectra were recorded over a range of 30 to 1000 atomic mass unit at 0.5 s/scan. Solvent cut time was 3 min. Ionization energy was 70eV. The inlet and ionization source temperature was 280 °C.

Essential oil components were identified based on their retention indices (determined with reference to a homologous series of normal alkanes) and by comparison of their mass spectral fragmentation patterns with those reported in the literature [8] and with authentic compounds.

Antimicrobial activities

Microbial strains

The bacterial cultures used in this study were *Escherichia coli ATCC 25922*, *Salmonella* sp.*, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumouniae*, *Enterococus aerogenes ATCC13048, Micrococcus luteus ATCC14452, Bacillus subtilis ATCC6633* and *Staphylococcus aureus* ATCC29213.

Antimicrobial screening

The essentials oil's antimicrobial activities were determined by two different methods:

Disc diffusion method

The agar disc diffusion method was used to determine the antimicrobial activity of the studied essential oils according to Clinical and Laboratory Standards Institute (CLSI).

After spreading 2 ml of the bacterial suspension (10^8CFU/ml) on Mueller Hinton Agar (MHA) plates and incubating briefly (20 min), the culture's excess was eliminated. Filter paper discs (6 mm in diameter) were impregnated by 5 µl of different dilutions of the EOs (1, 1/2, 1/5, 1/10, 1/20 and 1/100) and were placed on the inoculated plates. These plates were maintained at 4 °C for 3h then incubated at 37ºC for 24h. All tests were performed in triplicate. The inhibition zone diameters (I. Z. Φ) were measured in millimeters. The EOs antibacterial activity is classified into three levels: low activity (inhibition zone Φ ≤12 mm), moderate activity (12 mm<inhibition zone Φ <20 mm) and strong activity (inhibition zone Φ ≥20 mm).

The minimal inhibitory concentration (MIC)

The essential oil's MIC was determined by the agar dilution technique against a panel of bacterial strains. This method is recommended by the National Committee for Clinical Laboratory Standards [9].

The emulsification was performed with a 0.2% agar solution. A serial dilution of each oil, ranging from 1/100 to 1/5000, was prepared in Trypticase Soy Agar medium (TSA) at 37 °C. Plates were dried at room temperature for 30 min prior to spot inoculation with 2 µl of the exponential culture of each organism (10^8CFU/ml), then incubated at 37 °C for 24 h. MIC was determined as the lowest oil concentration inhibiting visible growth of each organism on the agar plate [10]. Experiments were carried out in triplicate.

Statistical analysis

All the experimental results were subject to an analysis of variance (ANOVA) and means comparison was performed according to the Student test (α=5%) [11].

RESULTS

Oil yield and chemical constituents

Hydrodistillation of the studied plants (*Salvia officinalis, Rosmarinus officinalis* and *Mentha piperita*) yielded 1.97 %, 1.9 % and 1.29 % w/w respectively.

Chemical composition of the essential oils

The main EOs components obtained by GC and GC–MS analyses are summarized in table 1. A total of 18, 19 and 23 compounds were identified respectively for *M. piperita, S. officinalis* and *R. officinalis* essential oils.

R. officinalis EO contained mainly α-pinene (34.83%), 1.8-cineole (28.30%) and other components at relatively low levels: Camphor (10.54%) and Camphene (6.21%).

The *Salvia officinalis* E. O's major components were □-thujone (24.05%), Camphor (17.16 %) and 1.8-Cineole (16.77%). *M. piperita* E. O contains mainly Menthol (41.23%), Menthofuran (13.18%) and Menthyl acetate (10.22 %).

Table 1: Volatile compounds identified in the studied plant's essential oils

RI	Compounds	*M. Piperita*	*R. officinalis*	*S. officinalis*
931	α-Thujene	0.20	0.18	3.19
939	α-Pinene	0.22	34.83	6.55
953	Camphene	-	6.21	3.26
967	Verbenene	-	0.59	-
975	Sabinene	0.36	1.04	0.55
979	β-Pinene	0.53	2.56	3.27
1005	α-Phellandrene	-	0.58	-
1011	3-Carene	-	0.42	-
1026	P-cymene	-	0.67	0.5
1031	Limonene	3.02	1.43	-
1033	1,8-Cineole	7.06	28.30	16.77
1070	cis-Sabinenehydrate	0.24	-	-
1098	Linalool	0.05	1.69	-
1102	α-Thujone	-	-	24.05
1123	Chrysanthenone	0.42	-	-
1143	Camphor	-	10.54	17.16
1152	Menthone	4.46	-	-
1156	isoborneol	-	0.49	2.34
1164	Menthofuran	13.18	-	-
1165	Neomenthol	2.79	-	-
1166	Borneol	-	1.64	1.62
1171	Menthol	41.23	-	-
1177	terpinen-4-ol	-	0.32	0.12
1189	α-terpineol	-	0.21	0.07

1204	verbenone	-	0.08	-
1243	Carvone	-	0.06	0.12
1273	Neomenthylacetate	0.45	-	-
1285	Bornylacetate		1.43	0.62
1295	Menthylacetate	13.10	-	-
1305	Isomenthylacetate	0.87	-	-
1352	α-terpinylacetate		0.06	0.05
1388	β-Bourbonene	0.37	-	-
1409	α-Gurjunene	-	-	11.04
1418	β-Caryophyllene	2.55	1.05	3.32
1460	α-humulene	-	0.12	0.11
Total		91.1	94.5	94.71

Antibacterial activity

Disc diffusion results

Pure EOs of *R. officinalis* and *S. officinalis* showed a strong antibacterial effect against *M. luteus* with respective I. Z. Φs of 30±2 mm and 28.5±1.8 mm, and low antibacterial action for *P. aeruginosa* (10.6±0.86 and 10.6±2.5 mm respectively) (fig. 1, tables 2, 3).

The antibacterial activity on *B. subtilus* and *E. aerogenes* revealed a significant difference for the dilution 1/2 and the pure EO at α=5%. But, no significant differences have been recorded between the inhibition zone diameters for the dilutions 1/5, 1/10, 1/20 and the other dilutions neither for *P. aeruginosa* nor for *E. aerogenes.*

S. officinalis oil showed a strong activity against *M. luteus* (28.5±1.8 mm) and a moderate one against *E. aerogenes, B. subtilis* and *E. coli* (18.5±1.5, 16±1.75 and 13.5±2.5 respectively) (fig. 1, table 4). Significant differences were observed for *M. luteus* and *E. aeroginosa* only with pure *S. officinalis* EO, and also, for *B. subtilis* and *P. aeruginosa* at (α=5%), with different concentrations of this EO except for dilutions 1/5 and 1/100.

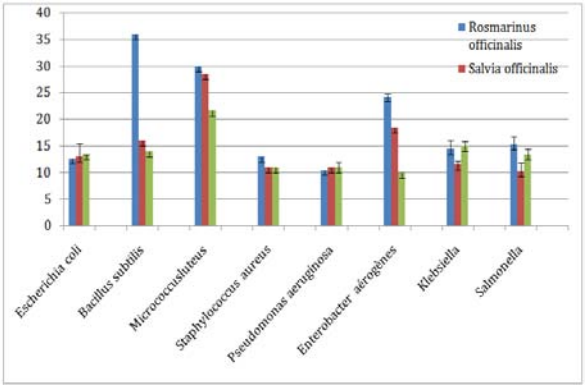

Fig. 1: The inhibition zone's diameters obtained with the different pure studied Eos
The values of I. Z. Φ (in mm) represent the means of three replicates (n=3±SD)

Table 2: Antibacterial activity of *Rosmarinus officinalis* essential oil

Bacteria	Inhibition zone's diameters (mm)					
	pure	1/2	1/5	1/10	1/20	1/100
Escherichia coli	12.66±0.79	9.66±2.08	8.5±0.5	8±0.6	7±0.7	6±0
Bacillus subtilis	36±2	27.3±3.21	10±1	7±1.32	6.83±0.76	6.66±0.76
Micrococcus luteus	30±2	12±2	11±1.73	10.33±1.039	9.83±1.039	9±0.5
S. aureus	13±1.5	11.5±0.74	10.5±0.5	9±0.5	8.75±0.66	8±0.5
Pseudomonas aeruginosa	10.5±0.86	10±0.86	9.5±0.86	9±1	8.36±0.7	7.83±0.76
Enterobacter aerogenes	24.3±1.12	12.16±0.46	9.1±0.36	8.1±0.96	7.6±0.69	6.6±0.58
Klebsiella	14.5±1.32	12±1	9.5±0.5	9±1	8.5±0.5	8±1
Salmonella	15.33±1.9	13.33±1.52	9.6±0.57	9.3±0.28	8.5±0.5	6.83±0.95

Table 3: Antibacterial activity of *Mentha piperita* essential oil

Bacteria	Inhibition zone's diameters (mm)					
	pure	1/2	1/5	1/10	1/20	1/100
Escherichia coli	13±4.06	11.6±4.04	10.5±3.27	9±2.64	8.5±2.14	7±1
Bacillus subtilis	14±3.74	13±3.6	10.83±3.29	10±3.16	9.63±3.1	8±2.67
Micrococcus luteus	21.6±1.52	19±1	17.6±1.75	16.33±1.75	12.6±1.15	9.6±0.76
S. aureus	11±1	9±0.5	8±1	7.16±0.76	6.6±0.76	6±0
Pseudomonas aeruginosa	11±0.86	10±1	9.5±1	9±1	8.5±1	8±1
Enterobacter aerogenes	10±1	9±1	8.5±0.5	7.83±0.76	7.5±0.62	7±0.5
Klebsiella	15±2	14±2	12±1.5	9±1	8±0.86	-
Salmonella	13.5±1.32	10±2	9.16±1.75	7.6±1.34	6.6±1.34	6.6±1.34

The values of (I. Z. ϕ) are given as means of three replicates and their standard deviation.

M. luteus was also the most sensitive bacteria to Peppermint oil (I. Z. Φ = 21.1±1.52 mm).

However, *E. aerogenes, P. aeruginosa* and *S. aureus* remains the most resistant ones with I. Z. Φ of 10±1 mm, 11±0.86 mm and 11±1 mm respectively. *K. pneumoniae* revealed an important sensitivity, followed by *Bacillus subtilis*, *Salmonella sp.* & *E. coli* (fig. 1, table 3).

The inhibition zone diameters recorded for *M. luteus* were significantly different from those of *B. subtilus* for the dilutions 1/2 and 1/100.

However, other dilutions didn't reveal any significant difference (α=5%), compared to those of *P. aeruginosa* for all dilutions in comparison with the pure essential oil.

The same observation was made for *M. luteus* and *E. aerogenes*, with a significant difference showed for dilutions 1/2 and 1/100 compared to the other tested dilutions. In the case of *B. subtilis* and *P. aeruginosa*, only the dilution 1/2 showed a significant difference for the inhibition zone diameters.

Minimum inhibitory concentration results

MIC values determined by the broth microdilution method, showed values ranging from 2.35 to 100 mg/ml for *R. officinalis, M. piperita* and *S. officinalis* EOs (tables 5, 6 and 7).

Table 4: Antibacterial activity of *Salvia officinalis* Essential oil

Bacteria	Inhibition zone's diameters (mm)					
	pure	1/2	1/5	1/10	1/20	1/100
Escherichia coli	13.5±2.5	12.3±1.52	7.83±1.03	6.6±1.01	6.2±0.72	-
Bacillus subtilis	16±1.75	14.1±1.89	12±1	11±1.32	10±1	9±0.86
Micrococcus luteus	28.5±1.8	16.6±3.05	14.25±0.74	11±1	9.6±0.58	7±1
S. aureus	11±1	10.5±1	10±1	9.6±1.15	8.83±95	7.5±0.5
Pseudomonas aeruginosa	11±0.5	10±1	9.16±2.35	8.5±0.5	8±0.5	7.33±0.57
Enterobacter aerogenes	18.5±1.5	15.6±2.08	12.5±1.32	10±1	9.5±1	8±1
Klebsiella	11.6±1.52	11±1	9.5±0.5	8±1	7.5±1	7±1
Salmonella	10.3±2.51	9.6±2.56	9.06±2.4	8.73±2.19	7.16±1.07	6.33±0.57

The values of (I. Z. ϕ) are given as means of three replicates and their standard deviation.

Table 5: Antimicrobial activity of *R. officinalis* EO assayed by the method of Minimum Inhibitory Concentration (MIC) (mg/ml) (n=3)

Concentration mg/ml	*Rosmarinus officinalis*							
	1.1	1.4	2.35	2.9	5.8	11	23	58
E. coli	+	+	+	+	-	-	-	-
Bacillus subtilis	+	+	+	+	+	-	-	-
Enterobacter aerogenes	+	+	+	+	+	+	-	-
Staphylococcus aureus	-	+	+	+	-	-	-	-
Micrococcus luteus	-	+	+	+	-	-	-	-
Salmonella spp	+	+	+	+	+	+	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	-
Klebsiella pneumoniae	+	+	+	+	+	-	-	-

Table 6: Antimicrobial activity of *S. officinalis* EO assayed by the method of Minimum Inhibitory Concentration (MIC) (mg/ml) (n=3)

Concentration mg/ml	*Salvia officinalis*							
	1.1	1.4	2.35	2.9	5.8	11	23	58
E. coli	+	+	+	+	+	+	-	-
Bacillus subtilis	+	+	+	+	-	-	-	-
Enterobacter aerogenes	+	+	+	+	+	-	-	-
Staphylococcus aureus	+	+	+	+	-	-	-	-
Micrococcus luteus	+	+	-	-	-	-	-	-
Salmonella spp	+	+	+	+	+	+	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	-
Klebsiella pneumoniae	+	+	+	+	+	+	-	-

R. officinalis EO had variable inhibition levels, with the lowest MIC values (5.8 to 11 mg/ml) observed for *M. luteus, S. aureus, E. coli* and *B. subtilis* and the highest value recorded for *P. aeruginosa* (58 mg/ml). In addition, the highest MIC values of both *M. piperita* and *S. officinalis* EOs were also observed for *P. aeruginosa* (100 and 58 mg/ml respectively). The lowest MIC values of *S. officinalis* and *M. piperita* EOs were observed for *M. luteus* (2.35 mg/ml) and for *Salmonella sp*. (20 mg/ml) respectively.

Table 7: Antimicrobial activity of *Mentha piperita* EO assayed by the method of Minimum Inhibitory Concentration (MIC) (mg/ml) (n=3)

Concentration mg/ml	*Mentha piperita*							
	2	2.5	4	5	10	20	40	100
E. coli	+	+	+	+	+	+	-	-
Bacillus subtilis	+	+	+	+	+	+	-	-
Enterobacter aerogenes	+	+	+	+	+	+	-	-
Staphylococcus aureus	+	+	+	+	+	+	-	-
Micrococcus luteus	+	+	+	+	+	-	-	-
Salmonella spp	+	+	+	+	+	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	+	-
Klebsiella pneumoniae	+	+	+	+	+	+	-	-

DISCUSSION

The essential oil's yield for *R. officinalis* in this study was higher than for those collected from different locations in previous studies. Indeed, E. O yields of 1.35% and 1.25% were found for *R. officinalis* collected respectively from Sidi Bouzid and Bizerte (Tunisia), while 0.8% and 0.6%, were obtained for those collected from Honaine and Tlemcen (Algeria) [12] and 1.27% in Zerhoun (Morocco) [13].

But, this yield was lower than those obtained for *Rosmarinus* collected from the regions of Kerman and Lalehzarin in Iran (2.1% and 2.6% respectively) [14].

The yield of *S. officinalis* essential oil was similar to that obtained in Tunisia [15] but higher than that obtained in other studies that recorded EO yields of 0.58% [16], 0.72% [17]; 1.02% for sage collected in the region of Marsa (Serbia) [18] and 1.63% for that collected in Djel west region (Algeria) [19]. Furthermore, Gupta *et al.* [20] found variable yields depending on the extraction method, corresponding to a rate of 1.42% for steam distillation, 1.39% for pentane vapor extraction and 1.40% for steam stripping of ether. Another study showed a variation of EOs yields from 0.90% to 0.20% depending on the crop growth stage [21].

The EO content of *M. piperita* (1.2%) was consistent with the results reported by Miladinović *et al.* [18] but higher than those obtained in other studies and ranging from 0.1 to 1% [22].

The variation in performance of E. O from different medicinal and aromatic plants is due to many factors, such as the plant origin [6], the extraction technique [23], the collection period [24], the plant material and also the ecological and geographical conditions [25].

Concerning the chemical composition of the tested essential oils, the identification of α-pinene (34.83%) and 1.8-cineole (28.30%) as major chemical compounds for *R. officinalis* EO presents some differences with previous investigations for the same species. Indeed, the major components found in the spontaneous rosemary in the Honaine region (Algeria) were α-pinene (23.1%), followed by camphor (15.3%) and β-pinene (12.2%), while the principal compounds were camphor (13.8%), α-pinene (12.6%) and cineole (11.8%) for grown rosemary, in the same region [25]. However, a sample from Bordj Bou Arreridj (Algeria) contained only 7.5% of cineole, camphor (12.1%), borneol (10.1%) and (E)-β-caryophyllene (13.9%) [26]. In Egypt, two rosemary samples revealed two different compositions with one typically dominated by camphor, α-pinene and cineole and the other rich in verbenone and camphor [27]. Whereas in Italy, the main components of this plant's essential oil were α-thujone (39.32%), α-humulene (12.42%), 1.8-cineole (7.73%), β–pinene (7.22%), β–thujone (3.07%) and camphre (2.12%).

The EO composition found for *S. officinalis* (α-thujone (24.05%) and Camphor (17.16%)) was partially consistent with the composition found for Tunisien *S. officinalis* (25.02% of α-thujone), but this EO also contains viridiflorol (18.96%), β–thujone (13.09%), 1.8-cineole (8.58%) and limonene (6.56%) [28]. However, the main compounds found in the essential oil of sage growing in Serbia were: bornyl acetate (4.91%) and α-humulene (3.97%) [29].

Menthol was the principal compound (41.23%) of *M. piperita*'s EO, followed by menthofuran (13.18%), α-thujone (24.05%) and Camphor (17.16%). Another study reported that the main components of this essential oil are menthol (19.1%), isomenthone (14.8%), limonene (10.6%), iso-menthanol (8.8%) and menthyle acetate (6.6%) [30].

The observed variations in the essential oil's chemical composition could be due to the ecological factors, to the plant's intrinsic and genotypic factors and to the extraction methodologies used. Hence, previous studies showed the effect of ecological factors [31] and also the importance of plant's used part, its age, its vegetative cycle's period and also the extraction and drying methods or even the genetic factors [32].

Results of the disk diffusion method showed that the studied EOs have antimicrobial activity against all tested microorganisms with a variation of the inhibition zone diameters (I. Z. Φ) according to the bacterial strain (fig. 1) and to the tested dilution (tables 2, 3 and 4).

The results obtained for *R. officinalis* EO are contradictory with those of Marino *et al.* [33], which recorded inhibition aureoles largely lower than those obtained in our study.

The slight resistance of *E. coli* to this EO (I. Z. Φ = 12.66±0.79 mm) is concordant with previous finding in Brazil, Sardinia and Iran [34].

Moreover, the resistance potential observed for *P. aeruginosa* against all tested EOs (I. Z. Φ ranging from 10.5+0.86 mm to 11±1 mm) is different from the results reported by Marino *et al.* [33] who recorded a very low inhibiting activity of *R. officinalis* against this bacterial species (I. Z. Φ = 7.5 mm).

The antibacterial activity of Rosemary's EOs would be due primarily to their principal components: α-pinene (23%), carvacrol (22.05%) and 1.8-cineole (14.33%) which have been reported to have a great antibacterial and antifungal power [35, 36].

The results obtained for *M. piperita* are in agreement with those reported by Tiwari *et al.* [37]. However, an important inhibiting power of this EO on *S. aureus* strains have been previously recorded [38] whereas our study showed its lower inhibitory activity (I. Z. Φ = 11±1 mm) which is in concordance with Delamare *et al.* results [32], in addition to Salvia oils.

The antibacterial activity of *Mentha piperita* EO can be attributed to the presence of active monoterpenes components, such as Menthol and Menthofuran, which have been proved to cause lesions in the micro-organisms cellular membrane inducing disturbance of cellular permeability and interruption of cellular proliferation [39]. Its low antibacterial activity on *S. aureus* with an inhibition zone diameter of 11±1 mm agrees with previous results [32].

In our study, *S. officinalis* oil exhibited a moderated activity against *B. subtilis* and *E. coli* which is consistent with other studies results [40, 41]. In addition, a low inhibiting effect was observed on *Salmonella sp. and P. aeruginosa*.

This variation in antibacterial activity of the tested EOs can be attributed both to the bacterial strains and the composition of these essential oils [42].

The strong antimicrobial activity of the tested essential oils against sensitive microorganisms can be attributed to the presence of high concentrations of 1.8-cineole, Camphen, camphor, α, β-thuyone and borneol having antibacterial and antifungal potential [43]. Moreover, the essential oil's minor constituents have also a strong antimicrobial activity [44] and the synergistic effects between all of the different major and minor constituents present in the essential oils should be taken into consideration to explain their biological activity [33].

The essential oil of *M. piperita* showed MIC values of 20 mg/ml for *Salmonella* sp. and *M. luteus*. These values were lower than those found for the same microorganisms by Mossi *et al.* [45] (>100 mg/ml) and higher than 10 mg/ml found for *P. aeruginosa* [45].

Concerning *P. aeruginosa*, neither *R. officinalis* E. O nor the other studied EOs did reveal an important inhibiting activity; whereas, this essential oil didn't show any growth inhibition of this bacterial species in other studies [46]. In addition, the moderate sensitivity showed for *K. pneumonia* and *Salmonella sp* (MIC values ranging from 11 to 23 mg. ml^{-1}) is concordant with the results of a previous study [45].

The observed MIC for *B. subtillis* with *S. officinalis* EO (5.72 mg. ml^{-1}) is greater than that reported by Delamare *et al.* [32]. But, 2.35 mg. ml^{-1} found for *M. luteus* in this work is lower than 6.93 mg. ml^{-1} reported in the literature [47]. *S. aureus* showed some resistance in comparison with other $Gram^{+}$bacteria which is similar to the results reported by Miladinović *et al.* [18].

The comparison of antimicrobial activities between Gram-negatives and Gram-positives show that all bacteria were susceptible to the essential oils of the investigated species. The highest MIC values were observed in Gram-negative bacteria treated with the essential oil of *M. piperita* (100 mg. ml^{-1}), while the lowest MIC values were obtained for Gram-positive bacteria treated with the E. O of *S. officinalis* (2.35 mg. ml^{-1}).

High MIC values were observed for *P. aeruginosa, Salmonella, K. pneumonie* and *S. aureus* treated with *S. officinalis* and *M. piperita* essential oils. The lowest MIC was observed for *M. luteus* treated with *M. piperita* essential oil (2.35 mg. ml^{-1}).

The results obtained in this work demonstrate that Gram-positive bacteria seem to be more sensitive to the essential oils than Gram-negative ones. Some authors reported that this is common for

essential oils of the Lamiaceae family plants [48]. However, this relation should not be used to define the antimicrobial activity [46] and thus each case should be carefully evaluated. From the results of *R. officinalis* we can note that *E. coli* presented the lowest MIC value (5.8 mg. ml-1) although being a Gram-negative microorganism. The lower susceptibility of Gram-negative bacteria to the essential oils may be explained in terms of diffusion limitations of essential compounds through their external membrane caused by the presence of a hydrophilic barrier. Although this barrier is not totally impermeable, it hinders the transport of macromolecules and hydrophobic components [49, 50].

The probable action mechanisms of the EOs mainly in the Gram-positive bacteria are based on the direct contact of their hydrophobic compounds with the cell membrane's phospholipids. This might cause structural damage or complete rupture of the cellular membranes, losses of nutrients, homeostatic control and interference in the respiratory system. They can also prevent the contact of human cells or food surfaces with the hydrophilic cells of growing microorganisms [49-51].

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CONFLICT OF INTERESTS

Declared None

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