ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM *Myrtus communis* L. ORGANS (LEAVES, BERRIES, FLOWERS AND BUDS) HARVESTED AT THREE DEVELOPMENT STAGES

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Abstract. The seasonal variability of yield, chemical composition and antibacterial activity of *Myrtus communis* L. essential oils was investigated. The essential oils were extracted by hydro-distillation technique from leaves, berries, flowers and floral buds collected at three stages of development from northeast Algeria. The essential oil yield values ranged from 0.09 to 0.94 % (w/w). They depend significantly on the phenological stage and the plant part used for the extraction of essential oils. Myrtle leaf is the valuable organ for the essential oil production with a yield varied from 0.44 to 0.94 % (w/w); the highest yield was obtained at the flowering stage. The GC-MS analysis indicated that all essential oils of myrtle were dominated by monoterpenes, α-pinene (37.16-49.39 %) and 1,8-cineole (25.87-49.11 %), which were the main compounds found in essential oils at each harvest period. The antibacterial activity was evaluated using the disk-diffusion method against *Escherichia coli,* Extended- Spectrum- β-Lactamase, Carbapenem-Sensitive *Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis*, and *Salmonella* spp. The essential oil of Myrtle berries and leaves extracted before flowering stage showed the best inhibitory effect against all tested bacterial strains, with inhibition zones diameter rangingfrom 11.24-26.52 mm and 12.84-29.84 mm, respectively. *Myrtus communis* L. essential oils displayed however a variable degree of antimicrobial activity with an absolute action against *Klebsiella pneumonia*.

Key words: *Myrtus communis* L.; vegetative cycle; phenological stage; essential oil composition; antibacterial activity.

INTRODUCTION

The genus Myrtus comprises two species: *Myrtus communis* L. (common myrtle) is native to the Mediterranean littoral; and *Myrtus nivellei* Batt. and Trab. (Saharan myrtle) isendemic tothe Central Sahara [26, 27, 40, 45]. Both species grow over a wide range in Algeria; *Myrtus communis* L., where better known under the name of “Rihane”, grows wild throughout Tell Atlas, Coastal Regions and Constantine [46], and *Myrtus nivellei* widespread in Central Regions (Tefedest, Hoggar, Tassili N'immidir and Tassili N’ajjer) [30, 40].

In this study, we are interested by *Myrtus communis* L., it is an evergreen shrub 1.8-2.4 m high with white fragrant flowers and purple-black berries [42]. Myrtle is one of the important aromatic and medicinal species of the Myrtaceae family. In folk medicine, different parts of this plant are used in the preparation of extracts with many pharmacological activities, including; antiseptic, anti-ulcer, anti-diarrheal, anti-diabetic and anti-inflammatory activities [29, 53], which are commonly ascribed to essential oils, polyphenols, hydrolysable tannins and flavonoids [3, 38]. In Algeria, decoction or infusion of aerial parts (leaves, flowers and buds) of myrtle are traditionally claimed to cure diabetes, hypertension, respiratory disorder, otitis, diarrhea and hemorrhoids [11, 15, 36, 43]. *Myrtus communis* L. essential oils extracted from different organs: leaves, stems, berries and flowers, are employed for their antimicrobial, tonic and balsamic properties [21]. They are also extensively used in perfume and cosmetic industries [20].

The chemical composition of myrtle essential oils collected from different Mediterranean regions has been studied. These studies demonstrate that *Myrtus communis* L. essential oils can be separated into two groups depending on their myrtenyl-acetate content; one with a high content of myrtenyl-acetate and a low content of α-pinene, and another with a lack of myrtenyl-acetate and high contents of α-pinene and 1,8-cineole as the main constituents [4, 7, 19, 24, 27, 40, 50]. Also, a series of correlated experimental studies have been carried out to determine the biochemical activities of myrtle essential oils [5, 12, 16, 49].

Extensive phytochemical researches have been conducted to investigate the essential oil composition and biological activities of myrtle leaves and fruits. Comparatively, very few studies have been undertaken with the objective to investigate the chemical composition and antibacterial activity of essential oils isolated from flowers and buds [33]. In
Algeria, there is no research on the aerial parts of this plant; as well as on the effects of seasonal variability on the yield, chemical composition and antibacterial activity of myrtle essential oils.

Hence, the aim of the present study is to investigate the variability of chemical composition and to evaluate the antimicrobial activity of essential oils obtained from different parts of *Myrtus communis* L. (leaves, berries, flowers and buds) growing spontaneously in northeastern Algeria during the principal stages of its vegetative cycle: pre-flowering, flowering and fruiting.

MATERIAL AND METHODS

1. Plant material

Myrtle aerial parts were collected at the pre-flowering (March), flowering (June) and fruiting (October) stages, from El-Machroha region, Souk-Ahras province, northeastern Algeria (latitude 36° 25' 0.60" N longitude 7° 55' 36, 4" E, altitude 748 m). The plant material was identified by professor Azzedine Chefrour Mohamed Cherif Messaadia University-Souk Ahras, Algeria.

Leaves, flowers, buds and berries were manually isolated from the aerial parts and dried at room temperature for two weeks.

2. Essential oils extraction

At every phenological stage, different plant parts were separately hydrodistilled for three hours using a Clevenger-type apparatus. The extracted oils were stored in sealed vials in the dark at 4 °C until further analysis. All extractions were performed in triplicate and oil yields were expressed on a dry weight (% w/w) basis.

3. Chemical analysis

The chemical analysis of the extracted essential oils was carried out using Agilent Technologies (USA) HP 6890 Gas Chromatograph (GC) coupled with HP5973 Mass Spectrometer (GC/MS). GC/MS analysis of the compounds was performed on an HP-5MS capillary column (30x0.25 mm) coated with 5 % phenyl, 95 % dimethyl-polysiloxane, and 0.25 µm film thickness. Samples of 0.2 µL were injected in split mode (split ratio 50:1). Helium carrier gas at a flow rate of 0.5 mL/min was used. Oven temperature was held at 60 °C, and then programmed to 250 °C at a rate of 2 °C/min.

The mass spectrometer was operated in electron impact ionization (70 ev) and scan range of 30-550 m/z. For RI calculation, a mixture of homologues *n*-alkanes (C_8-C_{30}) was co-injected under the same chromatographic conditions. Oil compounds were identified by comparison of their retention indices (RI) with those reported in the literature and by comparison of their mass spectral with the mass spectradata published by Adams (2007) [1]. Determination of the percentage composition was based on peak area.

4 Antimicrobial activities

4.1 Microbial strains

Antibacterial activity of *Myrtus communis* L. essential oils was tested against *Escherichia coli*; Extended-Spectrum β-Lactamases (ESBL), Carba-penem-sensitive *Klebsiella pneumoniae, Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), and *Salmonella* spp. Microorganisms were provided from the Clinical Microbiology Laboratory, Faculty of Medicine, University of Badji Mokhtar Annaba (Algeria).

4.2 Antimicrobial screening

Based on a defined standard process, agar disc-diffusion assay was performed *in vitro* to determine the antimicrobial activity of the essential oils. Microbial inoculums were prepared in physiological solution with a fresh culture. Relative optical density was adjusted to a 0.5 Mc Farland Standard (10^8 CFU/mL).

Muller-Hinton (MH) agar was solidified. After solidification, the inoculums were streaked onto the surfaces of agar plates using sterile swabs. Sterile paper discs of 6 min diameter were impregnated with 10 µL of the essential oil and placed onto the surface of the agar. All the tests were performed in triplicates. Petri dishes were incubated at 37 °C for 24 hours. The antimicrobial activity of myrtle essential oilswas assessed by measuring (in mm) the inhibition zone diameters.

5. Statistical analysis

All extractions and inhibition zone measures were carried out in triplicate. The results were expressed as means ± standard deviation and analyzed by MNITAB 16. Analysis of variance was performed by ANOVA procedures and the significant differences between means were determined by Tukey test, therefore, *p* values < 0.05 were regarded as significant.

RESULTS

1. Essential oil yield

The essential oils yield of different *Myrtus communis* L. organs studied during three development stages are presented in Table 1, showing that the yield values varied from 0.44 - 0.94%, 0.61%, 0.32% and 0.09% for leaves, buds, flowers and berries; respectively.

2. Essential oil chemical composition:

Variations of chemical composition of *Myrtus communis* L. essential oils extracted from four different plant parts are studied. The results (Table 2) revealed that α-pinene (monoterpene hydrocarbon) and 1,8-cineole (oxygenated monoterpene) were the major constituents and represented together approximately 85 % of the total oils components in all myrtle organs collected during the three harvesting periods taking into account the presence of other component at appreciable amounts as linalool (1.53-2.61 %), α-terpineol (1.91-3.29 %), eugenol (trace 4.21 %) and geranyl acetate (0.99-2.79 %). The percentage of α-

Table 1. Essential oil yields of different *Myrtus communis* L. parts during three harvesting periods (means ± SD with Tukey test)

Vegetative stage	Yields (% w/w) ± SD *			
	Leaves	flowers	Floral buds	Berries
Pre-flowering	0.44 ± 0.02^{C}	-	-	-
Flowering	0.94 ± 0.02^{Aa}	0.32 ± 0.02^{c}	0.61 ± 0.04^{b}	-
Fruiting	0.79 ± 0.02^{Ba}	-	-	0.09 ± 0.01^{b}

*Yields are expressedon the basis of dry matter weight. Values are given as means ±SD of three measures. Means followed by different capital letters in the columns and small letters in the rows are significantly different at p=0.05 (Tukey test)

pinene was varied from 37.16 to 49.39 %, while the concentrations of 1,8-cineole were varied from 25.87 % in buds to 49.11 % in leaves (Figure 1).

For leaves essential oils, 53, 45 and 50 components representing respectively 99.16 %, 99.45 %, and 99.47 % of the total essential oil composition were identified during pre-flowering, flowering, and fruiting stages. From the above data, it appears that the major compounds of α-pinene and 1,8-cineole were present in higher concentration during the all stages of myrtle vegetative cycle. The percentage of α-pinene reached a maximum level at the flowering stage in flower buds (49.32 %) followed by leaves of the same development stage (45.80 %), while the values decreased during the pre-flowering as well as fruiting within the leaves witch are respectively (40.71 and 40.75%). Relatively speaking, what seems obvious is that 1,8-cineole reached the highest level (49.11 %) at the fruiting stage. However, the lowest concentration was found in the leaves in the fruiting stage (25.87%).

In berries essential oil 69 compounds were found representing 97,72 % of the total essential oil. This analysis revealed that α-pinene (38.17 %) and 1,8-cineole (39.21 %) present almost at the same percentage.

The chemical composition of essential oils obtained from flowers and floral buds are composed of 65 and 48 components respectively in 95.47 % and 97.42 % of the total volume. The main constituents were equal; α-pinene and 1,8-cineole for flowers and floral buds essential oils.

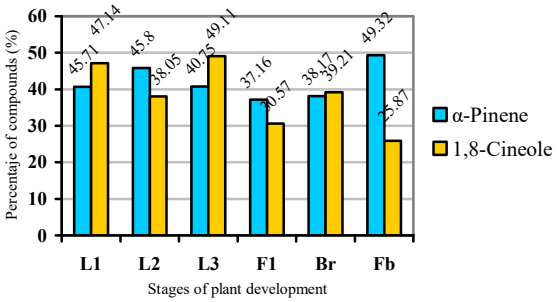

Figure 1. Variations of the major components in *Myrtus communis* L. oils at three stages of development. L1: Leaves collected at pre-flowering stage; L2: Leaves collected at flowering stage; L3: Leaves collected at fruiting stage; FL: Flowers; Br: Berries; Fb: Floral buds.

Table 2. Essential oil composition (%) of different *M. communis* L. parts at three stages of development

		Pre-flowering stage	Flowering stage			Fruiting stage	
Volatile compound*	**RI**	**Leaves**	**Leaves**	**Flowers**	**Floral buds**	**Leaves**	**Berries**
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2,4-Dimethyl-3-pentanone	-	-	-	-	-	0.027	-
(E)-2-Hexanal	845	0.015	0.037	-	-	0.078	-
(E)-3-Hexen-1-ol	851	0.011	-	-	-	-	-
Isobutylisobutyrate	913	0.119	0.086	0.013	-	0.097	-
α- Thujene	925	0.097	0.079	0.488	0.235	0.015	0.357
α- Pinene	937	**40.71**	**45.80**	**37.16**	**49.32**	**40.75**	**38.178**
α- Fenchene	944	0.130	-	0.055	-	0.049	0.057
Camphene	946	-	0.151	0.082	0.115	0.078	0.075
Thuja-2,4(10)-diene	951	0.030	0.022	0.033	-	-	0.056
Verbenene	968	-	-	-	-	-	0.029
Sabinene	971	-	-	0.016	-	0.039	-
β- Pinene	973	0.375	0.286	0.445	0.378	0.279	0.542
Myrcene	991	0.075	0.066	0.083	0.097	0.070	0.114
δ-2-Carene	1000	-	-	-	-	-	0.014
Isobutyl isovalerate	1003	0.496	0.449	-	0.066	0.395	0.077
α-Phellandrene	1004	-	-	0.517	0.386	-	-
δ-3- Carene	1009	0.081	0.125	0.705	0.673	0.107	1.029
α-Terpinene	1016	-	-	0.253	0.312	-	-
2-Methylbutyl isobutyrate	1017	0.081	0.152	-	-	0.105	0.015
p-Cymene	1027	-	-	-	-	0.052	-
1,8- Cineole	1032	**47.14**	**38.05**	**30.57**	**25.87**	**49.11**	**39.21**
(Z)-β-Ocimene	1039	-	-	0.088	-	-	-
(E)-β-Ocimene	1049	-	0.032	0.102	0.097	-	-
γ- Terpinene	1058	-	0.196	0.703	0.595	0.042	-
Cis-Linaooloxide	1072	0.025	-	0.011	-	-	-
α-Terpinolene	1087	0.025	-	1.017	1.004	0.054	-
Trans-Linalooloxide	1088	-	0.303	-	-	-	-
p-Cymenene	1089	0.036	-	-	0.032	-	0.161
O-Guaiacol	1091	-	-	-	0.158	-	-
Linaool	1104	1.855	2.613	1.96	1.556	2.374	1.533
2-Methylbutyl isovalerate	1106	0.604	0.409	0.367	-	0.490	0.367
Endo-frenchol	1114	0.086	0.055	0.042	0.032	0.056	-
Phenylethylalcohol	1115	-	-	-	0.153	-	0.055
Cis -p-Menth-2-en-1ol	1121	0.020	0.017	0.055	-	-	0.024
α- Campholenal	1125	0.042	0.017	0.025		0.035	0.066
Trans-Pinocarveol	1137	0.260	0.035	0.032	-	0.159	0.141
Trans-p-Menth-2-en-1-ol	1140	0.028	-	0.021	-	-	-
Trans-Verbenol	1144	0.160	0.048	-	-	-	0.391
Veratrol	1149	-	-	-	0.266	-	-
Trans-Pinocamphone	1158	-	-	-	-	-	0.016

0	1	2	3	4	5	6	7
Pinocarvone	1160	0.077	-	-	0.050	-	0.071
Borneol	1165	0.040	0.024	-	0.025	0.020	0.020
δ- Terpineol	1168	0.033	0.046	0.097	0.031	0.032	0.031
Terpinen-4-ol	1176	0.338	0.217	0.357	0.334	0.214	0.502
p-Methyl-acetophenone	1185	-	-	-	-	-	0.096
α- Terpineol	1193	2.366	3.294	3.522	3.200	1.916	2.596
Estragol	1198	0.157	0.285	0.170	-	0.204	0.138
Verbenone	1207	0.082	0.011	0.044	-	0.044	0.136
Endo-Fenchylacetate	1218	0.012	0.019	0.019	-	-	0.018
Cis-Carveol	1222	0.069	0.023	0.024	-	-	0.097
(z)-3-Hexenyl isovalerate	1233	0.055	0.048	0.067	0.048	0.034	0.110
Carvone	1243	0.046	-	-	-	-	0.061
Linalylacetate	1258	0.242	0.750	0.986	0.172	0.257	0.408
Geranial	1272	0.032	-	-	0.422	0.015	0.063
Bornylacetate	1283	0.013	0.014	0.022	-	-	0.027
Benzylisobutanoate	1298	0.012	-	0.036	-	0.011	0.020
Trans-Pinocarvylacetate	1301	-	0.019	-	-	-	0.283
Carvacrol	1313	-	-	-	-	-	0.112
Cis-pinocarvylacetate	1319	-	-	-	-	-	0.173
Myrtenylacetate	1326	-	-	-	-	-	0.203
Exo-2-Hydroxycineol acetate	1341	0.039	0.066	0.123	0.131	0.035	0.128
α-Terpinylacetate	1349	0.472	0.250	0.598	0.474	0.125	0.395
Citronellylacetate	1358	0.026	0.027	-	-	0.017	-
Eugenol	1362	-	0.026	0.797	4.218	-	-
Nerylacetate	1366	0.061	0.099	0.261	-	0.046	-
Geranylacetate	1387	1.525	1.936	2.552	1.578	0.999	2.794
Methyleugenol	1409	0.634	1.438	3.110	2.863	0.554	1.289
(E) Caryophyllene	1416	0.164	0.355	1.325	-	0.123	1.162
γ-Elemene	1432	-	-	0.125	0.105	-	0.117
Aromdendrene	1435	-	-	-	-	-	0.041
α- Humulene	1450	0.053	0.150	0.507	0.278	0.036	0.368
Geranylpropanoate	1476	-	0.017	0.024	0.049	0.014	0.102
α-Amorphene	1483	-	-	0.036	-	-	0.060
Phenyl ethyl-2-methylbutanoate	1487	-	0.017	0.056	0.035	-	-
α-Selinene	1492	-	-	0.050	-	-	0.053
Tridecanonoe	1497	-	-	-	-	-	0.077
(E)-Methylisoeugenol	1501	-	-	0.079	0.062	-	0.010
Geranylisobutanoate	1514	-	0.047	-	0.035	-	0.117
Tetramethylhydroquinone	1521	0.117	0.513	1.465	0.512	0.060	0.214
Trans-cadina-1(2),4-diene	1532	-	-	0.115	-	-	0.062
α-Cadinene	1535	-	-	0.031	0.098	-	-
Selina-3,7(11)-dienne	1538	-	-	0.121	0.071	-	0.059
Flavesone	1543	-	-	-	0.042	-	-
Geramacreneβ	1554	-	0.054	0.750	0.604	-	0.244
Elemicin	1555	-	0.022	-	-	-	0.048
Caryophyleneoxide	1579	0.250	0.145	0.542	0.186	0.127	1.428
Geranylisovalerate	1603	-	0.024	0.236	0.122		0.475
Humuleneepoxide II	1605	-	0.046	0.183	0.048	0.042	0.278
Iso-Leptospermone	1619	-	-	0.133	0.040	-	0.077
2-Hydroxy-4-methoxy-3,6dimethylbenzaldehyde	1626	0.057	0.152	1.027	0.231	0.036	0.309
4,6-Dimethoxy-5-vinyl-1,2-benzodioxole	1655	-	-	0.122	-	-	0.044
Eudesm-7(11)-en-4-ol	1693	-	-	0.135	-	-	0.016
Amorpha-4,9-dien-2-ol	1717	-	-	0.379	-	-	0.079
Nonadecane	1903	-	-	0.374	-	-	-
		97.42	**99.16**	**99.45**	**99.47**	**95.47**	**97.72**

*Components are listed in order of elution on HP-5 MS; RI: retention indices relative to C8-C30 *n*-alkanes on HP-5 MS column.

The chemical class characterization (Table 3) showed that myrtle essential oils predominately composed of monoterpene hydrocarbons (40.61 % – 53.24 %) and oxygenated monoterpenes (33.76 – 55.30 %); followed by sesquiterpenes and phenylpropanoids as minor chemical classes. Analysis of variance showed that these classes' distributions differed significantly during the phenological stages.

3 Antibacterial activity

The antibacterial activity of essential oils obtained from different organs of *Myrtus communis* L. was qualitatively evaluated by disk diffusion method against three gram-negative (*Escherichia coli, Klebsiella pneumoniae* and *Salmonella* spp) and two gram-positive bacteria (*Staphylococcus aureus and Enterococcus faecalis)*. The diameters of growth inhibition zones are presented in Table 4.

Table 3. Percentage of volatile compound classes from different *M.communis* parts at three phenological stages

	Pre-flowering stage	Flowering stage			Fruiting stage	
Chemical classes	**Leaves**	**Leaves**	**flowers**	**Floral buds**	**Leaves**	**Berries**
Monoterpene hydrocarbons	53.24^{A}	46.75^{B}	41.54^{D}	41.55^{D}	41.74^{C}	40.61^{E}
Oxygenated monoterpenes	33.76^{F}	47.69^{D}	55.30A	54.90^{B}	40.89^{E}	48.71^{C}
Sesquiterpene hydrocarbons	1.271^{C}	0.700^{D}	0.286^{F}	0.467^{E}	3.366^{B}	3.470^{A}
Oxygenated sesquiterpene	0.048^{C}	0.046^{D}	0.042^{E}	0.000^{F}	0.183^{B}	0.278^{A}
Phenylpropanoids	7.143^{A}	1.770^{C}	0.758^{F}	0.791^{E}	4.156^{B}	1.485^{D}
Others	1.940^{D}	2.190^{C}	1.487^{F}	1.750^{E}	5.096^{A}	3.158^{B}

Values followed by the same capital letter did not share significant differences at p=0.05 (Tukey test)

Table 4. Antibacterial activity of essential oils extracted from different *Myrtus communis* L. parts

Strains tested	Pre-flowering stage	Flowering stage			Fruiting stage	
	Leaves	**Leaves**	**flowers**	**Floral buds**	**Leaves**	**Berries**
Escherichia coli	12.84 ± 0.90^{Da}	10.44 ± 0.44^{Cc}	$< 6^{De}$	9.13 ± 0.81^{Dcd}	9.03 ± 0.53^{Dd}	11.24 ± 1.19^{Cb}
Staphylococcus aureus	19.92 ± 2.00^{Ca}	10.53 ± 0.07^{Cd}	9.83 ± 0.47^{Bd}	10.84 ± 0.05^{Ce}	11.27 ± 0.25^{Cb}	21.96 ± 1.47^{Ba}
Enterococcus faecalis	22.07 ± 1.21^{BCa}	11.49 ± 0.26^{Bd}	9.76 ± 0.62^{Be}	11.40 ± 0.19^{Bd}	13.36 ± 0.83^{Bc}	26.52 ± 1.37^{Aa}
Klebsiella pneumoniae	29.84 ± 3.29^{Aa}	15.65 ± 0.45^{Ab}	15.92 ± 0.31^{Ab}	14.57 ± 0.17^{Ac}	15.86 ± 0.05^{Ab}	25.05 ± 0.36^{Aa}
Salmonella spp	22.47 ± 1.02^{Ba}	7.94 ± 0.07^{Dc}	7.74 ± 0.30^{Cc}	9.72 ± 0.21^{Db}	8.00 ± 0.37^{Ec}	21.69 ± 0.26^{Ba}

*Diameter of the growth inhibition zone in mm (including disc diameter of 6mm).Values are given as means ±SD of three measures. Means followed by the different capital letters in the columns and small letters in the rows are significantly different at p=0.05 (Tukey test)

According to the results obtained, most of the essential oils of this aromatic plant showed a moderate inhibitory effect with the diameters of inhibition zone ranging from > 6 to 29.84 mm (Figure 2). The inhibition diameters of the bacterial strains varied considerably depending on the type of essential oil used. Our findings clearly revealed that berries and leaves essential oils, extracted before flowering stage, exhibited the best antibacterial activity against all strains tested with a mean diameter of inhibition zone varied from 11.24-26.52 mm and 12.84-29.84 mm, respectively. Flowers and floral buds essential oils showed a low antibacterial activity, which might explain the reason behind using predominantly the leaves and berries in traditional medicine [33].

As clearly shown in table 4, the highest antibacterial activity of myrtle essential oils was observed against Carbapenem-Sensitive *Klebsiella pneumonia* with a strongest inhibition zone of 29.84 mm recorded by essential oil extracted from leaves harvested before the flowering stage. The results may be attributed to the sensitivity of this strain to some antibiotics. Moreover, the results indicate that *Escherichia coli* Extended-Spectrum β-Lactamases (ESBL), known for their resistance to ß-lactamase, is the least sensitive strain to myrtle essential oils. Further, with the exception of leaves essential oils extracted before the flowering stage and berries essential oils, which showed slightly activity against *Salmonella* sp., the essential oils extracted from the other plant parts were found to be inactive on this strain.

DISCUSSION

The aim of this study is to investigate the impat of seasonal variations on yield, chimical composition and antibacterial activity of essential oils from Algerian *Myrtus communis*. The statistical analyzes revealed the existence of a significant difference in oil yields extracted from different plant parts collected at the flowering (leaves, flowers and floral buds) and fruiting (leaves and berries) stages. The results show that the leaf yield was higher than those obtained from the other parts at each stage. These results are in agreement with those published by Snoussi *et al.* (2011) [47]; Aidi *et al.* (2010) [2] and Jerkovic *et al.* (2002) [35] who showed that myrtle leaf is the most important organ for the essential oil production. Also, the results revealed that leaf oil yield is linked to harvest time with a maximum yield recorded in flowering stage (0.94 %) and a minimum in pre-flowering stage (0.44 %). Similar results were found by Jamoussi *et al.* (2005) [34]; Aidi *et al.* (2009) [4] and Barhouchi *et al.* (2016) [8] works who showed that the highest leaf yield obtained at flowering stage. For the Portuguese myrtle, Pereira *et al.* (2009) [44] reported that the maximum leaf oil yield is obtained at the fruiting stage the while Gardeli (2008) [27] reported that myrtle leaf oil yield did not vary significantly at the vegetative stages.

The chromatographic analysis indicated that α-pinene and 1,8-cineole were the major constituents in all essential oils sampls. The highest concentration of α-pinene was observed in floral buds and the lowest in flowers while the highest concentrations of 1,8-cineole was observed in leaves collected at fruiting stage. These results are similar of those obtained by Snoussi *et al.* (2011) [47] who studied the chemical composition of myrtle organs (leaves, berries, and floral buds) and reported that the maximum percentage of α-pinene was observed in floral buds essential oil (48.9%), and the highest content of 1,8-cineole was found in leaves essential oil (61.0 %). However, the values obtained show that the proportions of the two compounds follow a cyclic evolution in leaves, flowers and floral buds essential oils. The results are in agreement with those found by Snoussi *et al.* (2011) [47] and Jamoussi *et al.* (2005) [34].

According to Bradesi *et al.* (1997) [19] and Chalchat *et al.* (1998) [20], *Myrtus communis* L. essential oils separated into two groups (chemotypes) based on their content of myrtenyl acetate. Each

chemotype can be further divided in two sub-chemotypes, according to their relative ratio of α-pinene and 1,8-cineole. In the present study, our findings indicate that the investigated essential oils belong to the second group (α-pinene /1,8-cineole chemotype). This is in agreement with the results published by Bouzabata *et al.* (2013) [17] who reported that the composition of Algerian myrtle essential oils, collected from 16 localities, is dominated by α-pinene (27.4-59.2 %) and 1,8-cineole (6.1-34.3 %) and characterized by the lack of myrtenyl acetate. Similar results were found by Foudil-Cherif *et al.* (2013) [26]; Ben Ghnaya *et al.* (2013) [12] and Bekhechi *et al.* (2019) [9]. Touaibia *et al.* (2016) [49] and Mohamadi

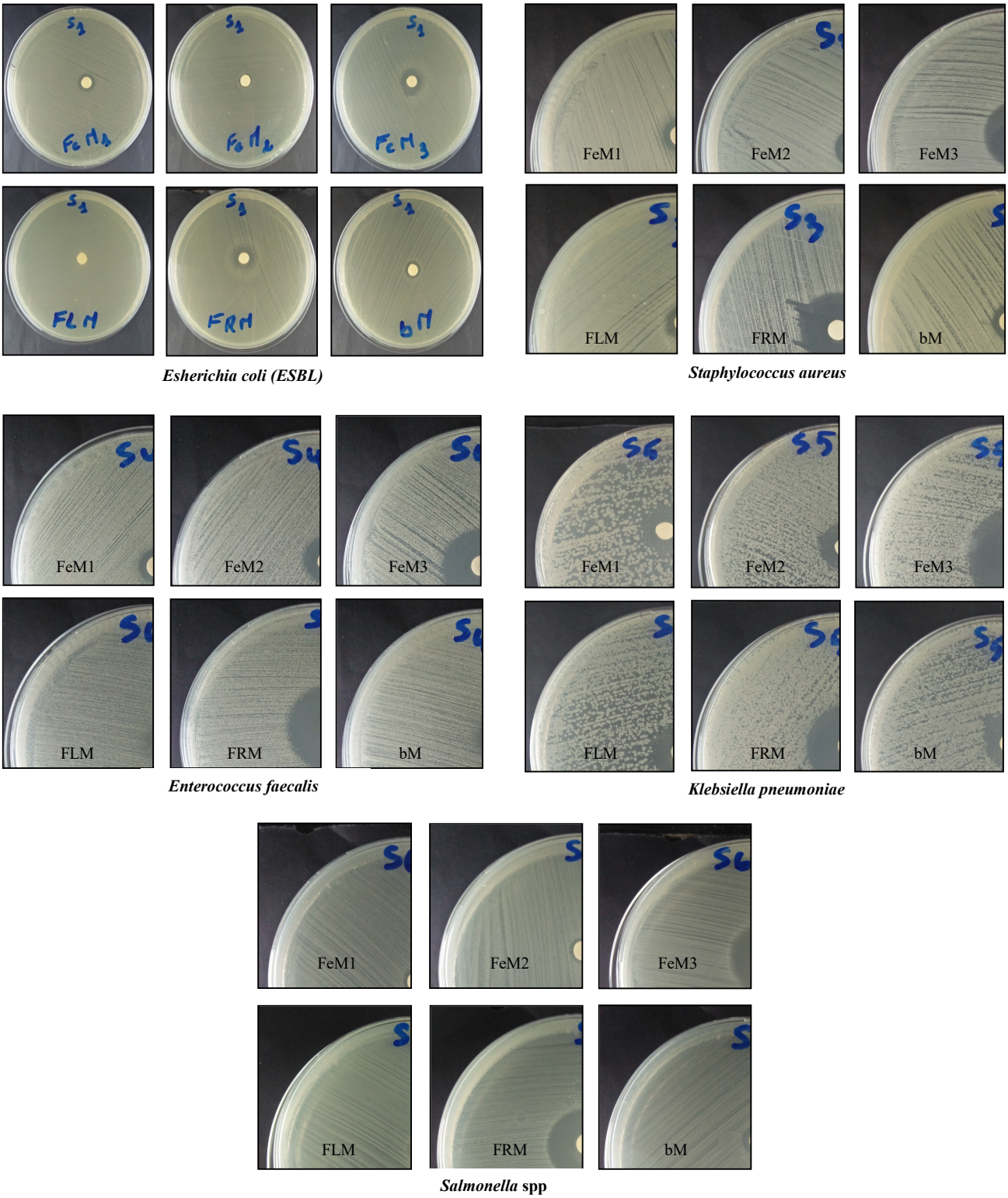

Figure 2. Antibacterial activity of essential oils from different *Myrtus communis* parts against five human pathogenic bacteria. FeM1: Leaves collected at flowering stage; FeM2: Leaves collected at fruiting stage; FeM3: Leaves collected at pre-flowering stage; FLM: Flowers; FRM: Berries; bM: Floral buds.

et al. (2021) [41], reported the presence of myrtenyl acetate in some populations of the Algerian myrtle essential oils. The α-pinene /1,8-cineole chemotype was the same chemotype in Corsica [19] and Tunisia [4, 35]. In contrast, essential oil from Spain is classified in the first group [13, 14] and characterized by a high content of myrtenyl acetate and low content of α-pinene [19].

The chemical variations of myrtle leaves essential oil has been studied by several authors. Our findings are all in all compatible with those of Aidi *et al.* (2009) [4] which can be interpreted as a generalization result of his comparative study on chemical compositions of two Tunisian varieties. The authors reported that α-pinene reached a maximum level at the flowering stage (58.05 %) and a minimum level at the fruiting stage (29 %) for Italica variety, while 1,8-cineole reached a maximum level at the fruiting stage (25.25 - 30.77 %); respectively for the two *baetica* and *italica* varieties. Further, Gauthier *et al.* (1988) [28]; Jerkovic *et al.* (2002) [35] and Gardeli *et al.* (2008) [27] also studied the variations in chemical composition of *Myrtus communis* L. essential oils. The authors reported that significant seasonal variations were observed in the major essential oil compounds.

From our results, α-pinene and 1,8-cineole were the major constituents in berries essential oil. These two compounds reported also as the major components of the essential oils of myrtle berries collected in Tunisia [47] and Italy [25], but with different amounts. However, our results are not compatible with the findings reported by Asllani *et al.* (2000) [7] and Jerkovic *et al.* (2002) [35]. The authors showed that the most representing components in berries essential oil were myrtenyl acetate, limonene, linalool and α-pinene. Hennia *et al.* (2016) [32], in his research analyzed the chemical composition of the essential oil of seven populations of myrtle growing in northwestern of Algeria and divided them into three chemotypes: α-pinene/1,8-cineole (from three stations), α-pinene/limonene (from three stations) and limonene/α-pinene. Likewise, Brada *et al.* (2012) [18] reported that berries essential oil from Miliana (Algeria) characterized by greater amounts of linalool (36,2 %), estragole (18,4 %) and 1,8-cineole (11,4 %).

Concerning flowers and floral buds, very few verifiable details in the literature about the chemical compositions of essential oils extracted from these parts [33]. One further important consideration that should be taken into account here is that the results obtained herein are in conformity with those of Aidi *et al.* (2010) [5] who reported that α-pinene and 1,8-cineole were the major constituents in flowers essential oil extracted from the Tunisian *Myrtus communis* L., but with different percentages. Asllani *et al.* (2000) [7] and Jerkovic *et al.* (2002) [35] showed in the same vein that myrtenyl acetate, 1,8-cineole, limonene, and linalool were the main compounds in myrtle flowers essential oil.

Regarding floral buds oil, our results are similar with those reported in the solely study available about the chemical compositions of myrtle essential oil extracted from buds [47], where the authors reported that the main components in buds essential oil extracted from these plant collected from Tunisia were α-pinene (48.9 %) and 1,8-cineole (15.3 %).

The high diversity in the chemical composition of *Myrtus communis* L. essential oils may be attributed to changes in the plant physiological behavior facing to different environmental (soils, geographical location, temperature) and genetic factors [5, 7, 25, 32, 35, 37, 41].

The antibacterial activity of myrtle essential oils collected and studied in different countries has been reported by many researchers [8, 10, 22, 31, 41, 48, 51, 52, 54]. Due to the mismatch of the techniques applied at different vegetative stages, it was slightly inappropriate for us to compare the obtained results with those available in literature.

In several studies, the authors distilled the myrtle leaves and berries together at the fruiting stage, and leaves, flowers and floral buds at the flowering stage. Also, diverse variables affect the study results; such as, the disparity in chemical composition of myrtle essential oils, the antibiotic resistance of microorganisms and the methodology followed to assay the antibacterial activity.

To our knowledge, this is the first study that compares the inhibitory effect of essential oils obtained from four myrtle organs and analyzes the effect of harvesting time in this bio activity. Some studies however claim that the inhibition effect of myrtle essential oils might be attributed to the high level of monoterpenes, particularly α-pinene and 1,8-cineole [23]. On the contrary, our findings, which are consistent with those found by Mhamdi *et al.* (2014) [39], reveal that there is no correlation between the high percentages of the two above mentioned compounds and the antibacterial activity. Clearly, Zanetti *et al.* (2010) [52] demonstrated that the essential oils have a better antibacterial activity than each separate compound. The authors indicated that the minor constituents contribute to this activity and have a synergetic effect. As a matter of fact, the mechanisms of antibacterial activity of essential oils against microorganisms are not well understood. Some researchers have hypothesized that the chemical constituents of essential oils affect the structure and the functional properties of the cell wall. These compounds would thus increase the permeability of the cytoplasmic membrane to protons, reduced the absorption of nutrients and inhibited the cellular respiration via the disruption of membrane enzymes and proteins [6].

In conclusion, it can be said that *Myrtus communis* L. essential oils may be useful as an alternative source of bioactive molecules. It can be incorporated in chemical formulation of some commercial hygiene products and combined with the current used

antibiotics for the purpose of treating many infectious diseases.

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