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Essential Oil Composition, Free-Radical-Scavenging and Antibacterial effect from leaves of *Myrtus Communis* in Tunisia

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ABSTRACT

This study was designed to examine the chemical composition, the antioxidant activity as well as the antibacterial activity of the essential oil and various extracts of *Myrtus Communis*. Essential oil, obtained by steam distillation, was analyzed by gas chromatography - mass spectrometry technique (GC-MS). The antioxidant activity of the essential oil and *Myrtus communis* various extracts were determined by DPPH. GC-MS analysis of the essential oil resulted in the identification of 24 compounds; representing 98.7% of the oil, and α-pinene (44.1%), 1,8-cineole (36%) and limonene (5.5%), constituted the major compounds. The antioxidant activity (IC_{50}=70 µg/mL) of the methanolic extract was the highest in comparison with the other extracts. The antibacterial activity was tested against six bacteria. All extracts exhibited antibacterial activities against gram-positive bacteria, such as Bacillus subtilis. Based on these results, *Myrtus Communis* could be used as a new source of antioxidant and antibacterial compounds.

Keywords: *Myrtus Communis,* Essential oil, Chemical composition, Antioxidant activity, antibacterial activity.

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INTRODUCTION

Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine [1]. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species.

Aromatic and medicinal plants are the source of natural antioxidants thanks to their main secondary metabolites such as polyphenols and essential oils. In Tunisia, more than 25% of the spontaneous flora is recognised as having medicinal and aromatic properties [2]. Among this species, *Myrtus communis L*. commonly named myrtle is considered as the most representative one. It is considered as the most representative one from the Myrtaceae family. Myrtle has been used since ancient times as a spice, as well as for medicinal and food preparation purposes [2]. It is traditionally used as an antiseptic, disinfectant drug and hypoglycaemicagent [3]. *Myrtus communis L.* has been reported to possess hypoglycaemic, antimicrobic, antihemorrhagic proprieties [4,5]. Essential oils are used in the pharmaceutical, cosmetic and food industries [2,6]. Essential oil content and composition of plants may be highly affected by genetic and environmental factors [7]. There are several reports regarding composition of myrtle essential oils in different countries, including Algeria [8], Tunisia [9,10], Albania [11], Iran [12], Italy [13]; Turkey [14], and Greece [15]. High chemical variations have been observed in these researches [16].

The main goal of the present study is to evaluate the antioxidant properties and antibacterial activity of the essential oil and various extracts (hexane, ethyl acetate, and methanol) of *Myrtus communis L.* from Tunisia.

MATERIALS AND METHODS

Plant material

Fresh leaves from *Myrtus Communis* were collected in March (2014), from Kef (North-west of Tunisia). A voucher specimen (Number LCSN 141) has been deposited in the Herbarium Laboratory of Chemistry of Natural Products, Faculty of Sciences, Sfax University, Tunisia.

Plants extracts

The dried leaves (260 g) of *Myrtus Communis* were extracted by maceration with hexane, ethyl acetate, and methanol three times at room temperature. Following filtration of the suspension the crude extracts were concentrated under vacuum at 40°C.

Isolation of essential oils

The essential oils were extracted by hydrodistillation of the dried plant leaves using a modified Clevenger-type apparatus for 3 h. The essential oil obtained was separated from water and dried over anhydrous sodium sulfate and stored in a dark glass test tube at 4 °C until tested and analyzed.

Gas chromatography-mass spectrometry (GC-MS)

GC/EIMS (Electron Impact Ionization Mass Spectrometry) analyses were performed with a Varian CP-3800 gas chromatograph, equipped with a HP-5 capillary column (30 m × 0.25 mm, 0.25 mm film thickness) along with a Varian Saturn 2000 ion-trap mass detector. Concerning the analytical conditions they are: 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 1ml/min; injection volume was 0.2 ml (10% hexane solution); split ratio was 1:30. Identification of the constituents was based on comparing the retention times with those of authentic samples while comparing their linear retention indices (I.r.i.) relative to the series of n-hydrocarbons (C8-C25), and on computer matching against commercial and home-made library mass spectra built up from pure substances and components of known oils and MS literature data [17,18]. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization.

Determination of total flavonoids

The total flavonoids contents in the various extracts were determined according to Akrout *and al*. [19], using a method based on the formation of a flavonoid-aluminium complex, having the maximum absorbance at 430 nm. Quercetine was used to make the calibration curve. 1 mL of diluted sample was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu UV min 1240 UV–Vis spectrophotometer and the total flavonoid content was expressed in mg quercetine equivalent (QE) per g of extract.

Determination of Total Phenolics

The total phenolic content was determined using the Folin-Ciocalteu method, described by Oktay *and al.* [20] with some modifications. 0.5 mL of Folin–Ciocalteu reagent was added to a solution containing 1 mL of extract, with a known concentration (1 mg/mL) and 3 mL of distilled water. The mixture was kept for 6 min and then 0.5 mL of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/per g of extract) through the calibration curve of gallic acid.

Antioxidant activity tested by DPPH assay

Antioxidant scavenging activity was studied by using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Fki *and al.* [21] with some modifications. Briefly, 1mL of 0.1 Mm DPPH methanolic solution was added to a 1mL of either methanolic solution of extract (sample) or methanol (control). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of free radical, DPPH, in percent (I %) was calculated by using the following equation:

$$I\ (\%) = [1-(A\ sample/A\ control)] \times 100.$$

Where the $A\ Control$ is the absorbance of the methanol control and the A_{Sample} is the absorbance of the extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. Synthetic antioxidant, butylate hydroxytoluene (BHT) and Vitamin E, was used as positive control.

Antibacterial activity

The antibacterial activity of *Myrtus Communis* extracts were tested against 6 strains of bacteria: *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella entirica*, *Salmonella sp*. The agar diffusion method was employed for the purpose of determining the *Myrtus Communis* antibacterial activities, in accordance with the method described by Vlietinck and Vanden Berghe [22]. The fractions were dissolved in 100% DMSO up to a final concentration of 3 mg/ml and sterilized by filtration through 0.22 µm Nylon membrane filter. The bacterial strains were cultured in MH (Muller Hinton) broth for 24 hours. Then, 200 µl of each suspension bacteria (106 CFU estimated by absorbance at 600 nm) was spread on MH agar. Bores were made by using a sterile borer and were loaded with 50 µl of each sample extract along with 25 µl of essential oil. Penicillin (10 µg/well) was taken as positive reference standard. All the plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition in millimeters.

RESULTS AND DISCUSSION

Essential oil analysis

The yield of *Myrtus Communis* L. leaves essential oil was 0.14%. Table 1 shows the constituents of the essential oil and their percentage composition as well as their Kovats Index (KI) values listed in order of elution. In the essential oil extracted from *Myrtus Communis* leaves 24 compounds were identified, corresponding to 98.7% of the total essential oil. The chemical composition analysis showed that the essential

oil of *Myrtus Communis* contains a complex mixture of monoterpene hydrocarbons (52%), oxygenated monoterpenes (42.4%), sesquiterpene hydrocarbons (0.2%), phenylpropanoids (0.5%) and others (3.6%). α-pinene (44.1%), 1,8-cineole (36%) and limonene (5.5%) were the major constituents of the essential oil extracted from *Myrtus Communis* leaves from Kef-Tunisia. The chemical composition of essential oil of *Myrtus Communis* leaves grown in North East of Tunisia (Jbal Stara of Haouaria) was reported [23]. α-pinene (58.05%), 1,8-cineole (21.67%) and β-pinene (6.45%) were the major compounds of this essential oil. Comparing the different chemical compositions, we noted a variation. This can be explained by the region and period of plant harvest.

Table 1. Chemical components of *Myrtus communis L*. Essential Oil

NO	KI	Compound	(%) Relative peak area
1	856	(E)-2-Hexenal	0.1
2	898	Propyl butanoate	0.8
3	941	*α*-Pinene	44.1
4	955	Camphene	0.2
5	982	*β*-Pinene	0.3
6	1003	Isobutyl 2-methylbutanoate	0.8
7	1013	δ-3-Carene	0.2
8	1015	2-Methylbutyl isobutyrate	0.3
9	1028	*p*-Cymene	0.8
10	1032	Limonene	5.5
11	1034	1,8-Cineole	36.0
12	1063	γ-Terpinene	0.4
13	1076	Cis-linalool oxide	0.1
14	1090	Terpinolene	0.5
15	1101	Linalool	3.2
16	1141	Trans-pinocarveol	0.1
17	1167	Borneol	0.2
18	1179	4-Terpineol	0.3
19	1191	α-Terpineol	2.5
20	1259	Linalyl acetate	0.2
21	1352	α-Terpinyl acetate	0.4
22	1383	Geranyl acetate	1.0
23	1403	Methyl eugenol	0.5
24	1419	β-Caryophyllene	0.2
		Monoterpene hydrocarbons	52.0
		Oxygenated monoterpenes	42.4
		Sesquiterpene hydrocarbons	0.2
		Phenylpropanoids	0.5
		Others	3.6
Total			98.7

Determination of Total Phenolics and Flavonoids in Extracts

The obtained values for phenolic and flavonoid contents are summarized in (Table 2). The results of total phenols content were expressed in milligram of gallic acid equivalents per gram of extract and the results of the flavonoids content were expressed in milligram of quercetin equivalents per gram of extract. All extracts were found to be rich in flavonoids and polyphenols. Among the different extracts, the methanolic extract showed the highest amount of phenolic (176.75 mg GAE / g) followed by ethyl acetate (154.14 mg GAE/g extract) and hexane (141 mg GAE/g extract) extracts. Total flavonoids content varied from 31.88 to 19.01 mg QE /g. The order of flavonoid contents in the extracts is: Methanol > Ethyl acetate > Hexane. Furthermore, the polarity of solvent is also one of interest in the processing of phenolics and flavonoids extraction. Usually, the more polar solvents are considered to be suitable for the extraction of phenolic and flavonoid contents [24].

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Table 2. Total phenolic and flavonoid contents in the different extracts

Extracts	**Total phenolics** (mg gallic acid/per g of extract)	**Total flavonoids** (mg quercetin /per g of extract)
Hexane	141 ± 0.15	19.01 ± 0,24
Ethyl acetate	154.14 ± 1.1	26.94 ± 0.36
Methanol	176.75 ± 0.95	31.88 ± 0.75

Average ± Standard Deviation were obtained from three different experiments.

DPPH Free Radical Scavenging Activity

The free radical-scavenging activity of the *Myrtus communis* leaves extracts was determined by means of the DPPH test. The obtained results are represented in (Table 3). Accordingly, antioxidants were proved to reduce diphenyl picryl hydrazyl, having a violet color, to a yellow compound, diphenylpicrylhydrazine, whose color intensity is inversely proportional to the ability of antioxidants in the medium to give protons. In this study, the methanolic extract exhibited the strongest free radical-scavenging activity with an IC_{50} value of 70 µg/mL, followed by the ethyl acetate extract (IC_{50} =120 µg/mL) and hexane extract (IC_{50} =220 µg/mL). The essential oil of *Myrtus communis* exhibits moderated free radical-scavenging activity with an IC_{50} value of 600 µg/ml when compared to Butylate hydroxytoluene BHT (IC_{50} =17 µg /mL) and vitamin E (IC_{50}= 26 µg/mL). This activity would be due to the high content of phenolic compounds (176.75 mg GAE / g) in methanol extract from leaves of *Myrtus communis*. In earlier study, Hayder *and al.* [25] studied the effect of extraction solvent on antiradical activity of myrtle leaf extracts from Tunisia and they mentioned that polar extracts such as aqueous extract (IC_{50} = 1.90 µg/mL) and methanol extract (IC_{50} = 6.50 µg/mL) exhibited a higher antiradical scavenging activity than a polar extracts (hexane) and essential oil which presented an IC_{50} superior at 100 µg/mL. In fact, trying to correlate the observed activity of the chemical composition of essential oils, it is well known that myrtle essential oils contained especially monoterpenes (α-pinene, β-pinene, limonene, 1,8-cineole, linalool) and these compounds were all tested individually in earlier studies and did not exhibit strong antioxidative activities using the same procedure like DPPH assay [23].

Table 3. IC_{50} of different extracts from *Myrtus communis* leaves

Extracts	**IC_{50} (µg /mL)**
Hexane	220
Ethyl acetate	120
Methanol	70
Essential oil	600
Vitamin E	26
BHT	17

Antibacterial activity

The screening of the antimicrobial activities of the essential oil along with the various extracts of *Myrtus communis* against common Gram-positive (Enterococcus facealis, Baccilus cereus, Baccillus subtilus, Listeria monocytogenèse) and Gram-negative bacteria (Salmonella sp., Salmonella enterica) were evaluated through the inhibition diameter. The relevant results are summarized in (Table 4). The highest activity was observed against Enterococcus facealis with strongest inhibition zones (26 mm) recorded for the hexane extract this value is higher than penicillin with 14 mm. Generally, plant extracts were usually more active against Gram-positive bacteria than Gram-negative bacteria [26]. The *Myrtus communis* has demonstrated an interesting antibacterial activity, especially in respect of both gram-positive and negative bacteria. The methanolic and essential oil extracts have all had an important activity against different strains. *M. communis L.* extracts profile constitutes polyphenolic compounds, phenolic acids, tannins and flavonoids, whose antimicrobial activity varies. Some results have indicated that phenolic compounds significantly contributed to the antibacterial activity [27]. Randrianarivelo *and al.* [28] showed that major myrtle oil compounds oxygenated terpenes, such as 1,8-cineole and linalool exhibit potent antibacterial activity.

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Table 4. Inhibition zone diameter produced by the extracts of *Myrtus communis*

Bacterial strains		Inhibition zone diameter (mm)				
		Hexane	ACOEt	MeOH	EO	Pinicillin
Gram+	*Enterococcus facealis*	26±0.70	-	18±1.41	11±1.41	14±0.0
	Baccilus cereus	-	18±0.35	19±1.41	19±0.70	13±0.35
	Baccillus subtilus	23±0.70	10±1.41	11±1.41	8±0.70	14±0.0
	Listeria monocytogenèse	-	-	11±0.35	23±0.0	20±0.0
Gram-	*Salmonella sp.*	-	8±1.41	12±0.70	-	R
	Salmonella enterica	-	8±0.0	11±0.17	10±1.41	15±0.0

The values are average of 3 assays ± Standard deviation (SD). (R): resistant

CONCLUSIONS

The results of this study revealed the importance of comparing and exploring the variance of essential oil and various extracts from *Myrtus Communis* leaves from Kef-Tunisia. The antioxidant activity varied between extracts from leaves of *Myrtus communis.* The methanolic extracts exhibited the highest antioxidant activity. Furthermore, the antimicrobial activity showed that all the extracts from leaves of *Myrtus communis* have a high activity against common Gram-positive and Gram-negative bacteria.

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REFERENCES

[1] Pezzuto JM. Biochem Pharmacol 1997; 2: 121-133.
[2] Messaoud C, Zaouali Y, Ben Salah A, Khoudja ML, Boussaid M. Flavour Fragr J 2005; 20: 577-582.
[3] Messaoud C, Béjaoui A, Boussaid M. Biochem Syst Ecol 2011; 39: 570-580.
[4] Özek T, Demirci B, Baser KHC. J Essent Oil Res 2000 ; 12: 541-544.
[5] Sepici A, Gurbuz I, Cevik C, Yesilada E. J Ethnopharmacol 2004; 93: 311-318.
[6] Mulas M, Cani MR, Brigagli N. Acta Hortic 1998; 457: 271-278.
[7] Rahimmalek M, Tabatabaei S, et al. Ind. Crops Prod 2009; 29: 348-355.
[8] Brada M, Tabti N, Boutoumi H, Watheletc JP, Lognayd G. J Essent Oil Res 2012; 24 (1): 1-3.
[9] Wannes WA, Mhamdi B, Kchouk ME, Marzouk B. Riv. Ital. Sostanze Gr 2011; 88 (2): 128-134.
[10] Snoussi A, Chaabouni MM, Bouzouita N, Kachouri F. J Essent Oil Res 2011; 23 (2): 10-14.
[11] Asllani U, J. Essent Oil Res 2000; 12 (2) : 140-142.
[12] Pezhmanmehr M, Dastan D, Ebrahimi SN, Hadian J. J. Essent Oil Bear 2010; 13 (1): 123-129.
[13] Flamini G, Pier Luigi C, Morelli I, Maccioni S, Baldini R. Food Chem 2004; 85: 599-604.
[14] Cakir A, Biochem. Syst Ecol 2004; 32: 809-816.
[15] Chryssavgi G, Vassiliki P, Athanasios M, Kibouris T, Michael K. Food Chem 2008; 107: 1120-1130.
[16] Rahimmalek M, Mirzakhani M, Ghasemi PA. Ind Crops Prod 2013; 51: 328- 333.
[17] Adams RP., *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*., Allured Publ. Corp, Carol Stream, IL 2007.
[18] Davies NW. J Chromatogr 1990; 503: 1-24.
[19] Akrout A, Alarcon GL, El Jani H, Madrid PC. J Food Chem Toxicol 2011; 49: 342-347.
[20] Oktay M, Gulcin I, Kufrevioglu OI. Food Sci Technol 2003; 36: 263-271.
[21] Fki I, Allouche N, Sayadi S. Food Chem 2005; 93: 197-204.
[22] Vlietinck AJ, Vanden Berghe DA. J. Ethnopharmacol 1991; 32: 141-153.
[23] Wannes WA, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B. Food Chem Toxicol 2010; 48 (5): 1362-1370.
[24] Khlif I, Jellali K, Thomas M, Halabalaki M, Skaltsounis LA, Allouche N. Journal of Chemistry 2015.
[25] Hayder N, Abdelwahed A, Kilani S, et al. Mutat Res 2004; 564:89-95.
[26] Negi PS, Jayaprakasha GK. Journal of Food Sci 2003; 68: 1473-1477.
[27] Shan B, Cai YZ, Brooks JD, Corke H. Int J Food Microbiol 2007; 117: 119.
[28] Randrianarivelo R, Sarter S, Odoux E, Brat P, Lebrun M, Romestand B, Menut C, Andrianoeliso HS, Raherimandimby M, Danthu P. Food Chem 2009; 114: 680-684.