



IN-VITRO ANTIBACTERIAL ACTIVITY AND CHEMICAL COMPOSITION OF SYRIAN *ERICA MANIPULIFLORA* ESSENTIAL OIL

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*In current research, we aimed to investigate the chemical composition of the EO of *Erica manipuliflora* extracted in two growth periods (before flowering and full flowering periods), to evaluate the differences between the two EOs in antibacterial activity against four pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis*). Results showed the existence of 17 and 23 volatile components in the EOs extracted from *E. manipuliflora* before flowering and in full flowering periods, respectively. The main components in EO extracted from the plant before flowering period were Germacrene-D, tau-cadinol, β -caryophellene, Trans-pinocarveol and β -pinene; which represented 35.02, 26.36, 14.78, 9.25 and 3.65%, respectively. The EO extracted from the plant collected in full flowering period were β -citril, β -pinene, Germacrene-D, tau-cadinol and Trans-pinocarveol, which represented 22.26, 18.50, 16.90, 11.89 and 5.58%, respectively. The EO extracted from *E. manipuliflora* in the full flowering period was more active than that extracted before flowering period towards the bacterial species mentioned above. The most sensitive bacterium towards *E. manipuliflora* EOs was *Bacillus subtilis*, while the most resistant one was *Salmonella enteritidis*.*

INTRODUCTION

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components^{1&2}.

In many countries, folk medicines widely resort to medicinal plants as primary tools to treat many diseases; the study of such traditional practices has uncovered many drugs of importance and is still a cornerstone of modern drug discovery³. In the last decades, herbal medicines have received much attention in Western and Eastern countries as sources of biologically active substances, notably for the

discovery of antioxidant, antimutagenic, anticarcinogenic and cytotoxic agents⁴.

The *Ericaceae*, a large cosmopolitan family represented by 4100 species regrouped in 124 genera, notably *Arbutus*, *Calluna* and *Erica*, present the highest diversity under the Mediterranean climates⁵. Medicinal properties have long been recognized for some *Ericaceae* which led to their inclusion in the list of species that may enter into the composition of herbal medicines⁶. The therapeutic functions of *Ericaceae* species are generally attributed to their abundant polyphenolic compounds⁷.

The genus *Erica* L. is a member of the family *Ericaceae* and more than 800 species naturally grow in the world, mainly in the coastal areas of the Mediterranean Sea⁸⁻¹¹.

Since ancient times, *Erica* species have been used as a traditional medicine for the treatment of burns and wounds. The studies

have been showed that *Erica* species possess antiulcer^{12&13}, antimicrobial^{14&15}, antibacterial, cytotoxic¹⁶⁻¹⁹, and antioxidant²⁰⁻²⁴ activities. It has also been reported that *Erica* species have polyphenolic, flavonoid, coumarin and triterpenoid, compounds²⁵⁻³².

According to our knowledge, there is no study in the literature on antibacterial activity and chemical composition of essential oil of *E. manipuliflora* growing in Syria. So this study aimed to evaluate the antibacterial activity *Erica manipuliflora* essential oil extracted from aerial parts of the plant in two different growth periods (before flowering and full flowering periods), against some pathogenic bacteria, namely: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* by estimating the value of minimum bactericidal concentration (MBC). The second aim was to investigate chemical composition of *Erica manipuliflora* essential oil, extracted in the two periods, by means of gas chromatography-mass spectrometry (GC-MS) technique.

MATERIALS AND METHODS

Plant materials

The current study was executed in the National Commission for Biotechnology (NCBT), Damascus, Syria. *Erica manipuliflora* was collected from Masyaf, Syria (850 m over sea level, Latitude: 34° 53' 20.47" North, Longitude: 35° 53' 11.72" East, climate: cold in winter and moderate in summer) in two growth periods: before flowering and full flowering. The aerial parts of plant were washed with tap water, wiped with clean cloth and dried at room temperature, then used immediately for essential oils extraction.

Extraction of EOs

The EOs of washed-air dried aerial parts of *E. manipuliflora* were extracted by hydrodistillation using Clevenger type apparatus. The upper oily layers were separated from the aqueous lower layers by centrifuging (6000 rpm for 20 min.). The oily layers were collected and dried over anhydrous sodium sulphate, and stored in amber air-tight glass vial at -20°C in the absence of light until they were used in antibacterial activity assay, or analyzed for chemical composition^{33&34}.

Bacterial strains source, subculture and maintenance

Bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis*) were obtained from the National Commission for Biotechnology (Damascus-Syria). The strains were preserved in a mixture consists of Nutrient Broth: glycerol; 80: 20 (V/V), and stored at -80°C. Before antibacterial activity tests, the stored cultures were streaking on Nutrient agar plates and incubation at 37°C for 24 hrs. Good isolated colonies were selected to prepare bacterial suspensions used for determination of MBC values.

Antibacterial activity assay

Antibacterial activity assay (expressed as MBC value) was performed according to the method described by Sheeladevi and Ramanathan³⁵ with some modifications. Sterile Nutrient broth was distributed aseptically in sterile 2 mL tubes. Well isolated colonies of each bacterial strain grown on Nutrient agar for 24 hrs. were used to prepare cell suspensions in the concentration of 10⁶ cfu/ml. *E. manipuliflora* EOs were used to prepared concentrations of 2, 4, 8, 16 and 32 mg.ml⁻¹ in Nutrient Broth, using Tween 80 (0.5% by volume) as emulsifier. Ten µl of each bacterial suspension (10⁶ cfu/ml) was inoculated into each tube containing the mixture of essential oil, tween 80 and Nutrient Broth. The tubes were inoculation with 10 µl of each bacterial suspension and vortexed, then incubated at 37°C for 24 hrs. Nutrient Broth plus the emulsifier (tween 80) without the addition of any EOs, was used as positive control. After incubation period passed, 10 µl of each tube was transferred into a Petri dish containing Nutrient agar. The inoculated Petri dishes were incubated at 37°C for 24 hrs. and the presence/absence of growth was recorded (+: growth present; -: growth absent). The lowest EO concentration at which every bacterium did not demonstrate any visible growth on the Petri dish after incubation for 24 hrs. at 37°C was determined as MBC.

Gas chromatography-mass spectrometry analysis

The chemical composition of EOs extracted from *E. manipuliflora* were identified

by means of gas chromatograph (Agilent 7890A, autosampler Agilent 7683B) equipped with mass spectrometry (Agilent 5975C) using HB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). GC/MS spectrum was obtained according to the following conditions: carrier gas, Helium at 104 ml/min; split ratio, 1: 100; injection volume, 1 µl; temperature of injector 250°C; column oven temperature program as following: 40°C for 2 min.; then 40→70°C at 2.5°C/min.; 70→130°C at 2°C/min. for 2 min.; 130→160°C at 2.5°C/min.; 160→200°C at 5°C; 200→220°C at 10°C; total run time 68 min.; post run 240°C for 3 min.

The ion source temperature was adjusted at 230°C, while the quadrupole temperature was 150°C. The ionization mode used was electronic impact at 70 electron volt and scanning range m/z was 50-650. The relative percentage of the individual components was

calculated from GC/MS peak areas. Identification was performed by comparison of the component mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries).

RESULTS AND DISCUSSION

Identification of volatile components in *E. manipuliflora* EOs

GC/MS technique was used to identify the chemical composition of *E. manipuliflora* EOs extracted from the plants collected in two periods of growth: before flowering and full flowering. The results demonstrated overall 26 different compounds. Qualitative and quantitative analyses of these EOs is listed in table 1.

Table 1: Chemical composition of *Erica manipuliflora* essential oils extracted from the aerial parts, as identified by GC/MS technique.

Peak No.	Component	Before flowering %	Full flowering %
1	α-pinene	0.35	0.36
2	Camphene	0.25	0.00
3	β-pinene	3.65	18.50
4	β-myrcene	0.00	0.17
5	Isoterpinolene	0.25	0.15
6	D-limonene	0.00	0.82
7	Trans-pinocarveol	9.25	5.58
8	Germacrene-D	35.02	16.90
9	α-terpineol	5.59	2.17
10	tau-cadinol	26.36	11.89
11	β-linalool	0.40	0.15
12	Isopulegol	0.28	0.00
13	Berbenol	0.72	0.16
14	Terpinene-4-ol	1.08	0.00
15	2-pinene-4-ol	0.00	0.61
16	β-caryophyllene	14.78	0.00
17	Linalool (mono)	0.00	1.20
18	β-phenylethanol	0.29	2.61
19	Verbenol	0.00	2.19
20	Decanal	0.95	1.75
21	β-citral	0.00	22.26
22	Trans-geraniol	0.00	1.27
23	β-bisabolene	0.00	2.15
24	Trans-caryophyllene	0.17	7.61
25	Tricosane	0.60	0.24
26	Heptacosane	0.00	1.26

Table 1 revealed the existence of 26 volatile compounds in *E. multipliflora*. The major compound was Germacrene-D, which represented 35.02 and 16.90% in the EOs extracted from aerial parts of this plant in the two growth periods (before flowering and full flowering, respectively). These values were higher than that recorded by Kuş *et al.*³⁶ (14.76 and 15.55%, in aerial parts and flower part, respectively); followed by *tau*-cadinol (26.36 and 11.89% respectively in the two growth periods mentioned above). These values were higher than that recorded by Kuş *et al.*³⁶ which were 7.53 and 4.11% in the aerial parts and flower part, respectively.

The EO extracted from plant collected before flowering period characterized by containing β -caryophyllene (14.78%); while this component was absent in the EO extracted from the plant collected in the full flowering period. Besides, the percent of β -caryophyllene were higher than that recorded in other research, which were 7.24 and 5.97%, in aerial parts and flower part, respectively (Kuş *et al.*³⁶).

The EO of *Erica multipliflora* collected before flowering period contained high amounts of Trans-pinocarveol, α -terpineol, β -pinene (9.25, 5.59 and 3.65%, respectively). However, Trans-pinocarveol and α -terpineol were higher than that recorded by Kuş *et al.*³⁶ (5.78 and 6.85%, respectively).

β -citral was the most abundant component in the EO of *E. manipuliflora* collected in full flowering period, which represented 22.26% and was absent in the EO extracted from the plant collected before flowering period; followed by β -pinene, which represented 18% in the EO extracted from the plant collected in the full flowering period, and only 3.65% in the EO extracted from the plant collected before flowering period. The other main distinct components in EO of *E. manipuliflora* collected in full flowering period were Trans-caryophyllene, Verbenol, β -bisabolene, which represented 7.61, 2.19, 2.15%, respectively, and were absent in the EO of *E. manipuliflora* collected before flowering period. The percent of verbenol was higher than that recorded by Kuş *et al.*³⁶, who found that the verbenol represents 1.17% in the EO extracted from *E. manipuliflora* flower part.

The differences in the chemical composition between EOs extracted from the plant in the two growth periods (before flowering and full flowering stages) can be illustrated by the fact that Sesquiterpene hydrocarbons are the most abundant compounds in aerial parts of essential oils; while monoterpenoids are found to be most abundant compounds in flower part of essential oil (Kuş *et al.*³⁶). So major components of the EO extracted from the plant collected before flowering stage were: Germacrene-D, *tau*-cadinol and β -caryophyllene (35.02, 26.36 and 14.78%, respectively), and all of them are classified as Sesquiterpenes; while the major components in the EO extracted from the plant collected in the full flowering period were β -citral and β -pinene (22.26 and 18.50%, respectively) and they both classified as monoterpenoids.

Erica manipuliflora EO antibacterial activity

The essential oils of *E. manipuliflora* extracted in two growth periods namely: before flowering and full flowering, were tested *in vitro* against 2 gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and 2 gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*), using MBC method. Each EO was prepared in 5 concentrations (2, 4, 8, 16, and 32 mg.ml⁻¹) by adding the suitable amounts of EO, tween 80 (0.5% V/V) and NB to complete the volume to 1 ml. Each test was done in triplicates. The values of MBCs are shown in table 2.

Table 2 showed that Gram-positive bacteria were less resistant to *E. manipuliflora* EOs than Gram-negative bacteria, and this result was in agreement with that of Smith-Palmer *et al.*³⁷, who found that Gram-positive bacteria are more sensitive and usually less resistant to the plant-origin antimicrobials, because of the lipopolysaccharide outer membrane of Gram-negative bacteria, which restricts diffusion of hydrophobic compounds, compared to Gram-positive bacteria^{38&39}.

Bacillus subtilis was the most sensitive bacterium to *E. manipuliflora* EOs, with MBC values equal to 16 and 8 mg.ml⁻¹ in the EOs extracted from the plant before flowering and full flowering periods, respectively. *Salmonella enteritidis* was the most resistant bacterium to the EOs (MBC values were 32 mg.ml⁻¹ for each of the two EOs).

Table 2: Minimum bactericidal concentration (MBC) values of EOs extracted from *Erica manipuliflora* in two growth periods against Gram-positive and Gram-negative bacteria.

Bacterial strains	Concentration of <i>E. manipuliflora</i> EOs (mg.ml ⁻¹)									
	Before flowering					Full flowering				
	2	4	8	16	32	2	4	8	16	32
<i>Bacillus subtilis</i>	+	+	+	-	**	-	+	+	-	-
<i>Staphylococcus aureus</i>	+	+	+	-	-	-	+	+	-	-
<i>Escherichia coli</i>	+	+	+	+	-	+	+	+	-	-
<i>Salmonella enteritidis</i>	+	+	+	+	-	+	+	+	+	-

* + growth present

** - growth absent



Fig. 1: Antibacterial activity of the EO of *Erica manipuliflora* collected in full flowering period; from right to left *Staphylococcus aureus* and *Escherichia coli*.

According to our knowledge there are not any study in the literatures about the antimicrobial activity of the EOs extracted from *E. manipuliflora* against *B. subtilis*, *S. aureus*, *E. coli* and *S. enteritidis*, although literatures studied the antimicrobial activity of EOs extracted from other species of the genus *Erica*, or the antimicrobial activity of solvent extracts of *E. manipuliflora*^{10,14&15}. So this is the first study about the antibacterial activity of *E. manipuliflora* EOs against these four bacterial species.

The EO of *E. manipuliflora* collected in full flowering period was more active against some studied species namely: *B. subtilis* and *E. coli* than EO extracted from the same plant collected before flowering period (Fig. 1). This result may be due the presence of some active

components in the EO extracted from the plant in full flowering period such as β -citral, trans-geraniol, which are active against *S. aureus*, *E. coli*, and *S. enteritidis* subsp. *Typhimurium*⁴⁰.

Conclusion

Erica manipuliflora is a wild plant native to Mediterranean regions, including Syria. However, the composition of the essential oil of this plant are not investigated in Syria, and rare researches have been conducted about this EO around the world. Moreover, the antimicrobial activity of *E. manipuliflora* EO against bacteria is not evaluated. Results revealed differences between the EOs extracted from *E. manipuliflora* before flowering and in full flowering periods. The antibacterial study showed that these EO had a moderate activity

against some pathogenic bacteria, with minimum bactericidal concentration (MBC) values ranged from 8 to 32 mg.ml⁻¹. Also results revealed the differences in the EOs extracted from plant before flowering and in full flowering periods. We suggest studying the composition of volatile components of other species of the genus *Erica* native to Syria, and investigating the antimicrobial activity of these essential oils against other pathogenic bacteria.

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الفعالية المضادة للبكتيريا في الزجاج والتركيب الكيميائي للزيت العطري لنبات العجم (Erica manipuliflora) السوري

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هدفنا في البحث الحالي إلى دراسة التركيب الكيميائي للزيت العطري المستخلص من نبات العجم (*Erica manipuliflora*) المستخلص في مرحلتين مختلفتين من مراحل النمو (مرحلة ما قبل الإزهار ومرحلة الإزهار الكامل)، لتقدير الاختلافات بين الفعالية المضادة للبكتيريا للزيتين العطريين تجاه أربعة أنواع من البكتيريا الممرضة (*Staphylococcus aureus* ، *Bacillus subtilis* ، *Salmonella enteritidis* و *Escherichia coli*) في الزيوت العطرية المستخلصة من النوع *E. manipuliflora* في مرحلة ما قبل الإزهار ومرحلة الإزهار الكامل على التبالي. كانت المركبات الأساسية في الزيت العطري المستخلص من النبات في مرحلة ما قبل الإزهار هي Trans- β -caryophellene ، Germacrene-D ، τ -cadinol ، Germacrene-D ، β -pinene و terpinocarveol و β -pinene، التي مثلت ٣٠,٠٢٪ ، ٢٦,٣٦٪ ، ١٤,٧٨٪ و ٩,٢٥٪ على التبالي. كانت المركبات العطرية المستخلصة من النبات الذي تم جمعه في مرحلة الإزهار الكامل هي β -citrinal ، trans-terpinocarveol و τ -cadinol ، Germacrene-D ، Germacrene-D ، β -pinene و ١١,٨٩٪ ، ١٦,٩٠٪ و ١٨,٥٪ على التبالي. كان الزيت العطري المستخلص من نبات *E. manipuliflora* في مرحلة الإزهار الكامل أكثر فعالية من ذلك المستخلص في مرحلة ما قبل الإزهار تجاه أنواع البكتيريا المذكورة سابقاً. كان النوع الأكثر حساسية تجاه الزيت العطري المستخلص من نبات *Erica manipuliflora* هو *Bacillus subtilis* ، بينما كان النوع *Salmonella enteritidis* هو الأقل فعالية.