IJMS
Vol 39, No 1, January 2014

Original Article

In Vitro Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria

Ayman Al-Mariri, PhD; Mazen Safi, PhD

Department of Molecular Biology and Biotechnology, Atomic Energy Commission, Damascus Syria

Correspondence:
Ayman Al-Mariri, PhD;
Department of Molecular Biology and Biotechnology,
Atomic Energy Commission,
Kafer Sousa, 17th of April Ave.,
P.O. Box 6091, Damascus, Syria.
Tel: +963 11 213580
Fax: +963 11 6112289
Email: ascientific1@aec.org.sy
Received: 31 October 2012
Revised: 10 December 2012
Accepted: 24 February 2013

Abstract

Background: Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria. The aim of this study was to evaluate the antibacterial activity of 28 plant extracts and oils against four Gram-negative bacterial species.

Methods: Experimental, in vitro, evaluation of the activities of 28 plant extracts and oils as well as some antibiotics against *E. coli* O157:H7, *Yersinia enterocolitica* O9, *Proteus* spp., and *Klebsiella pneumoniae* was performed. The activity against 15 isolates of each bacterium was determined by disc diffusion method at a concentration of 5%. Microdilution susceptibility assay was used in order to determine the minimal inhibitory concentrations (MICs) of the plant extracts, oils, and antibiotics.

Results: Among the evaluated herbs, only *Origanum syriacum* L., *Thymus syriacus* Boiss., *Syzygium aromaticum* L., *Juniperus foetidissima Wild*, *Allium sativum* L., *Myristica fragrans* Houtt, and *Cinnamomum zeylanicum* L. essential oils and *Laurus nobilis* L. plant extract showed anti-bacterial activity. The MIC_{50} values of these products against the Gram-negative organisms varied from 1.5 (*Proteus* spp. and *K. pneumoniae*) and 6.25 µl/ml (*Yersinia enterocolitica* O9) to 12.5 µl/ml (*E. coli* O:157).

Conclusion: Among the studied essential oils, *O. syriacum* L., *T. syriacus* Boiss., *C. zeylanicum* L., and *S. aromaticum* L. essential oils were the most effective. Moreover, Cephalosporin and Ciprofloxacin were the most effective antibiotics against almost all the studied bacteria. Therefore, *O. syriacum* L., *T. syriacus* Boiss., *C. zeylanicum* L., and *S. aromaticum* L. could act as bactericidal agents against Gram-negative bacteria.

Please cite this article as: Al-Mariri A, Safi M. In Vitro Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria. Iran J Med Sci. 2014;39(1):36-43.

Keywords ● Gram-negative bacteria ● Antibiotic resistance ● *Cinnamomum zeylanicum* ● *Syzygium aromaticum*

Introduction

Medicinal and aromatic plants are used on a large scale in medicine against drug-resistant bacteria, which are considered one of the most important reasons for the lack of success of treatment in infectious diseases. Medicinal plants are the major sources of new medicines and may constitute an alternative to the usual drugs.[1]

Aromatic oils are used in many industries, including food

preservation,[2] pharmacy, and medicine.[3,4] They are expected to form new sources of antimicrobial drugs, especially against bacteria.[5] The antibacterial effectiveness of aromatic oils has been divided into a good, medium, or bad.[6,7] These oils can also produce some defense products against several natural enemies.[8] In addition, and in order to continue their natural growth and development, aromatic oils may produce some secondary metabolites in response to some external stress.[9]

The extracts and oils of 28 plants used in this work have been traditionally employed by people for various purposes in different parts of the world. *Cinnamomum zeylanicum* essential oil has antibacterial and antifungal activities[10] as well as anti-diabetic properties;[11] *Citrus limon* and *Rosmarinus officinalis* L. essential oils possess antioxidant properties;[12,13] *Citrus aurantium* has immunological effects in humans;[14] *Eucalyptus globulus* oil has good antimicrobial activities;[15,16] *Thymus pannonicus* essential oil has an excellent effect against *E. coli* O157:H7;[17] light thyme essential oil inhibits the growth of *E. coli* O157:H7 in foods;[18] *Brillantaisia lamium* extract exhibits antibacterial and antifungal effects against *Staphylococcus aureus, Enterococcus faecalis, Candida tropicalis*, and *Cryptococcus neoformans*;[19] and finally *Crinum purpurascens* herb extract has antimicrobial activities against *Salmonella paratyphi* A and B.[20] Traditionally, many plant extracts and oils are used as medicinal plants in Syria for many purposes, particularly for respiratory and gastrointestinal disorders.

The aim of this study was to screen the in vitro antibacterial activity of 28 plant extracts and oils against some Gram-negative bacteria, including: *E. coli* O157:H7, *Yersinia enterocolitica* O9, *Proteus* spp., and *Klebsiella pneumoniae*.

Materials and Methods

Microorganisms and Growth Conditions

Fifteen local isolates of *E. coli* O157:H7, *Y. enterocolitica* O9, *Proteus* spp., and *K. pneumoniae* were grown for 24-48 h in 2YT agar (peptone, 16 g/liter; yeast extract, 10 g/liter; NaCl, 5 g/liter; agar, 13 g/liter [Difco, BD, Spars, MD]). The bacteria were suspended in a sterile phosphate-buffered saline (PBS). Bacteria abundance in the PBS was monitored by recording the optical density (OD) at 590 nm.[21] The exact doses were assessed retrospectively by viable counts on 2YT agar plates.

Plant Samples Collection

Rosmarinus officinalis L., *Origanum syriacum* L., *Thymus syriacus* Boiss., *Salvia palaestina* Benth., *Mentha piperita* L., and *Lavandula stoechas* L. (*Lamiaceae*); *Citrus aurantium* L. and *Citrus medica* L. (*Rutaceae*); *Syzygium aromaticum* L., *Myrtus communis* L., and *Eucalyptus camaldulensis Dehnh*. (*Myrtaceae*); *Cinnamomum zeylanicum* L. and *Laurus nobilis* L. (*Lauraceae*); *Juniperus foetidissima Wild* (*Cupressaceae*); *Pelargonium roseum* L. (*Geraniaceae*); *Scilla maritima Squill* and *Allium sativum* L. (*Liliaceae*); *Pinus halepensis* Miller. (*Pinaceae*); *Artemisia herba-alba* Asso. (*Compositae*); *Anabasis haussknechtii* Boiss. (*Chenopodiaceae*); *Crataegus aronia* L. (*Rosaceae*); *Mercurialis annua* L. (*Euphorbiaceae*); *Matthiola crassifolia Boiss.* (*Brassicaceae*); *Myristica fragrans* Houtt. (*Myristicaceae*); *Brassica nigra* Koch. (*Cruciferae*); *Coriandrum sativum* L. (*Apiaceae*); *Zingiber officinale* Rosc. (*Zingiberaceae*); and *Achillea fragrantissima* Forssk. (*Asteraceae*) samples were collected during the flowering season from different regions in Syria between March and July 2010, or purchased from local markets (table 1). The samples were cleaned from any strange plants, dust, or any other contaminants.

Essential Oil Extraction

Essential oils from fresh, clean, weighed aerial parts, flowers, leaf fruits, barks, seeds, rhizomes, and bulbs (table 1) extracted by hydro-steam distillation using the Clevenger apparatus were collected and stored in sterile vials.[22] Briefly, 100 to 150 g of each plant was introduced in the distillation flask (1 L), which was connected to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oils were released from the plant material and evaporated into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to condense the steam. The steam was applied for 3 h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was filtered through anhydrous Na_2SO_4 to dry the yielded essential oil. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the oils were done using dimethyl sulfoxide (DMSO).

Preparation of Ethanolic Extracts

Successive solvent extraction was performed for some plants (table 1). Leaves and bulbs were washed, air dried for 7-8 days, and ground into powder before they were placed into the flask of

Table 1: Plants and their families, collection sites, and parts used

Scientific name	Plant family	Collection site	Altitude (m)	Collection time	Extracted part	Extract or oil
Rosmarinus officinalis L.	*Lamiaceae*	Latakia	300	June	Aerial parts	Oil
Origanum syriacum L.	*Lamiaceae*	Kafr Nobol-Idlib	446	July	Aerial parts	Oil
Thymus syriacus Boiss.	*Lamiaceae*	Alsoja Mountain-Damascus	840	July	Aerial parts	Oil
Salvia palaestina Benth.	*Lamiaceae*	Alyarmouk Valley-Konaitera	800	June	Aerial parts	Oil
Mentha piperita,. L.	*Lamiaceae*	Latakia	300	June	Aerial parts	Oil
Lavandula stoechas L.	*Lamiaceae*	Tartous	300	June	Aerial parts	Oil
Citrus aurantium L.	*Rutaceae*	Latakia	300	April	Flowers	Oil
Citrus medica L.	*Rutaceae*	Latakia	300	April	Flowers	Oil
Syzygium aromaticum L.	*Myrtaceae*	Market			Flowers	Oil
Myrtus communis L.	*Myrtaceae*	Latakia	300	June	Leaves	Extract
Eucalyptus camaldulensis Dehnh.	*Myrtaceae*	Tartous	300	June	Flowering branches	Oil
Cinnamomum zeylanicum L.	*Lauraceae*	Market			Barks	Oil
Laurus nobilis L.	*Lauraceae*	Latakia	300	July	Leaves	Extract
Juniperus foetidissima Wild	*Cupressaceae*	Dobaya-Damascus	800	June	Leaves	Oil
Pelargonium roseum L.	*Geraniaceae*	Kodsaya-Damascus	916	May	Aerial parts	Extract
Scilla maritime Squill.	*Liliaceae*	Tartous	300	March	Bulbs	Extract
Allium sativum L.	*Liliaceae*	Market			Bulbs	Oil
Pinus halepensis Miller.	*Pinaceae*	Dobaya-Damascus	900	May	Leaves	Extract
Artemisia herba-alba Asso.	*Compositae*	Alsoja Mountain-Damascus	840	March	Aerial parts	Extract
Anabasis haussknechtii Boiss.	*Chenopodia-ceae*	Alkariatain-Homs	500	March	Aerial parts	Extract
Crataegus aronia L.	*Rosaceae*	Alkonaitera	1100	April	Flowering branches	Extract
Mercurialis annua L.	*Euphorbiaceae*	Kasab-Latakia	800	March	Aerial parts	Extract
Matthiola crassifolia Boiss.	*Brassicaceae*	Latakia	10	March	Aerial parts	Extract
Myristica fragrans Houtt.	*Myristicaceae*	Market			Fruit	Oil
Brassica nigra Koch.	*Cruciferae*	Market			Seeds	Oil
Coriandrum sativum L.	*Apiaceae*	Market			Seeds	Oil
Zingiber officinale Rosc.	*Zingiberaceae*	Market			Rhizome	Oil
Achillea fragrantissima Forssk.	*Asteraceae*	Palmyra	405	July	Aerial parts	Oil

the Soxhlet apparatus for extraction using ethanol with increasing order of polarity to extract the phytoconstituents separately at 20ºC for 3-4 h. (The ethanol used was HPLC grade obtained from Sigma-Aldrich, Germany.) Whatman No.1 filter papers were then applied to filter the extracts. After that, reduced pressure was applied to evaporate and dry the filtrates, which were stored at -20ºC in labeled, sterile, screw-capped bottles.

Antibacterial Susceptibility Assay

Muller-Hinton Broth (MHB, Merck) medium was used to grow the test isolates for 22 h at 37°C. Final bacterial numbers were standardized to 1×10^6 CFU/ml. A total of 0.1 ml of bacterial suspension was poured on each plate, containing Muller-Hinton Agar (MHA, Merck). The lawn culture was prepared by sterile cotton swab and allowed to remain in contact for 1 min. Thereafter, a 5% concentration of each plant extracts was prepared. The sterile filter paper discs (6-mm diameter) were placed on the lawn cultures, and 24 h after incubation at 37˚C, the inhibition zone was measured in mm.

Antibiotics Minimum Inhibitory Concentration Determination

In order to estimate the antibiotics susceptibility, the well broth microdilution method was used with 96-well plates (TPP, Switzerland). The antibiotics were diluted twofold in LB broth® (Acumedia, Michigan, USA), and the wells were inoculated with 1×10^6 CFU of bacteria (in a 0.2 ml final volume). The incubation period was 24 h at 37°C. The lowest concentration that inhibited 50% of visual growth was recorded and interpreted as the MIC_{50}. The MIC testing was performed according to the recommendations of

the Clinical and Laboratory Standards Institute (CLSI).[23] The range of the concentrations assayed for each antibiotic was 0.064 to 128 µg/ml. The absorbance was determined at 590 nm (Thermo-Lab Systems Reader, Finland). All the tests were performed in triplicate and then averaged. The investigated antibiotics were Ciprofloxacin, Levofloxacin, Ofloxacin, Sparfloxacin, Ceftazidime, Ceftriaxone, and Cefotaxime. Positive control was done without adding any antibiotics.

Plants Extracts and Oils Minimum Inhibitory Concentration Determination

The microdilution broth susceptibility assay was used.[24] Three replicates of the serial dilutions of each essential oil were prepared in LB broth medium in 96-well microtiter plates, using a range of concentrations for each essential oil from 0.75 to 50 µl/ml. Next, 100 µl of freshly grown bacteria, standardized until a bacterial number of 1×10^6 CFU/ml in LB broth was achieved, was added to each well. Positive and negative controls were also done. The plate was incubated with shaking for 24 h at 37˚C. The lowest concentration that inhibited 50% of visual growth was recorded and interpreted as the MIC_{50}.

Statistical Analysis

Optimal concentrations for the most effective essential oils and plant extracts were estimated by Probit Analysis (SPSS Inc. 2010; Finney, 1971). Minimum concentrations to achieve 50% inhibition of the various bacteria (MIC_{50}) were considered significantly different if their 95% confidential limits did not overlap.

Results

Table 2 demonstrates that *O. syriacum.* L., *T. syriacus*, *S. aromaticum*, *C. zeylanicum*, *L. nobilis* L., *J. foetidissima*, *A. sativum* L., and *M. fragrans* Houtt. had good antibacterial activities against the Gram-negative bacteria, whereas the rest of the studied extracts were ineffective.

The MIC_{50} values for these plant extracts and oils were 12.5, 12.5, 25, 12.5, 12.5, 25, 12.5, and 6.25 µl/ml, respectively, against *E. coli* O157:H7; and 1.5, 6.25, 6.25, 6.25, 6.25, 25, 6.25, and 12.5 µl/ml, respectively, against *Y. enterocolitica* O9; and 1.5, 3.125, 1.5, 1.5, 3.125, 12.5, 3.125, and 12.5 µl/ml, respectively, against *Proteus* spp.; and 6.25,

Table 2: Number of Gram-negative isolates susceptible to each plant extract

	Number of isolates susceptible to plant extracts			
	***E. coli* O157:H7**	***Y. enterocolitica* O9**	***Proteus* spp**	***K. pneumoniae***
Rosmarinus officinalis L.	1	2	2	2
Origanum syriacum L.	12	12	13	12
Thymus syriacus Boiss.	12	15	15	11
Salvia palaestina Benth.	0	0	0	0
Mentha piperita. L.	1	0	2	1
Lavandula stoechas L.	3	3	5	6
Citrus aurantium L.	1	0	0	0
Citrus medica L.	1	1	0	0
Syzygium aromaticum L.	9	14	13	14
Myrtus communis L.	0	3	2	3
Eucalyptus camaldulensis Dehnh.	1	2	2	2
Cinnamomum zeylanicum L.	14	15	15	13
Laurus nobilis L.	14	13	13	15
Juniperus foetidissima Wild	11	11	12	13
Pelargonium roseum L.	2	2	3	5
Scilla maritime Squill.	2	1	1	2
Allium sativum L.	14	15	15	15
Pinus halepensis Miller.	0	0	0	0
Artemisia herba-alba Asso.	0	0	0	0
Anabasis haussknechtii Boiss.	0	0	0	0
Crataegus aronia L.	1	0	0	0
Mercurialis annua L.	0	0	1	0
Matthiola crassifolia Boiss.	3	4	2	3
Myristica fragrans Houtt.	13	13	13	12
Brassica nigra Koch.	0	0	0	0
Coriandrum sativum L.	3	3	2	0
Zingiber officinale Rosc.	3	3	4	5
Achillea fragrantissima Forssk.	0	0	0	0

3.125, 1.5, 3.125, 6.25, 12.5, 6.25, and 6.25 µl/ml, respectively, against *K. pneumoniae* (table 3).

In contrast, when studying the optimal concentrations that could inhibit 50% of the bacterial isolates, the X^2 values were not significant ($P>0.05$) for all the studied concentrations, indicating adequate fit of the Probit regression models (table 4).

Table 5 also shows that Ceftazidime, Cefotaxime, and Ciprofloxacin were the most effective antibiotics against *E. coli* O157:H7 (MIC_{50}= 0.25, 0.5, and 2 µg/ml, respectively). Moreover, Ceftazidime and Ciprofloxacin were the most effective antibiotics against *Y. enterocolitica* O9 (MIC_{50}= 0.25 and 0.5 µg/ml, respectively) and against *Proteus* spp. (MIC_{50}= 4 and 2 µg/ml, respectively) and Ceftriaxone, Cefotaxime, and Ciprofloxacin were the most effective antibiotics against *K. pneumoniae* (MIC_{50}= 0.25, 0.25, and 0.5 µg/ml, respectively).

Table 3: Minimum inhibitory concentrations (MICs) for the selected essential oils and extracts against some Gram-negative bacteria

	MIC_{50} and MIC_{90} of plant extracts (µl/ml)							
	***E. coli* O157:H7**		***Y. enterocolitica* O9**		***Proteus* spp**		***K. pneumoniae***	
	MIC_{50}	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
Origanum syriacum.	12.5	NA	1.5	12.5	1.5	12.5	6.25	NA
Thymus syriacus. Boiss.	12.5	NA	6.25	25	3.125	25	3.125	NA
Syzygium aromaticum	25	50	6.25	25	1.5	50	1.5	25
Cinnamomum zeylanicum	12.5	25	6.25	25	1.5	25	3.125	NA
Laurus nobilis L.	12.5	NA	6.25	50	3.125	50	6.25	12.5
Juniperus foetidissima Wild	25	50	25	50	12.5	50	12.5	25
Allium sativum L.	12.5	25	6.25	50	3.125	50	6.25	50
Myristica fragrans Houtt	6.25	50	12.5	50	12.5	NA	6.25	NA

Table 4: Optimal inhibitory concentrations of the selected essential oils and extracts against some Gram-negative bacteria

Bacteria	**Plant**	**MIC_{50} (µl/ml)**	**X^2**	***Significance***
E. coli	*O. syriacum.* L.	9.48	1.33	0.932
	T. syriacus. Boiss.	7.85	2.42	0.788
	S. aromaticum	16.11	2.8	0.732
	C. zeylanicum	9.48	1.33	0.932
	L .nobilis L.	20.43	6.32	0.276
	J. foetidissima Wild	21.82	2.98	0.703
	A. sativum L	8.41	1.71	0.888
	M. fragrans Houtt.	7.91	3.01	0.699
Y. enterocolitica	*O. syriacum.* L.	1.59	1.36	0.929
	T. syriacus. Boiss.	5.76	0.69	0.983
	S. aromaticum	5.22	1.28	0.937
	C. zeylanicum	5.76	0.69	0.983
	L .nobilis L.	4.30	2.99	0.702
	J. foetidissima Wild	12.57	1.87	0.867
	A. sativum L	5.52	2.05	0.842
	M. fragrans Houtt.	7.00	0.63	0.986
Proteus spp.	*O. syriacum.* L.	1.12	1.93	0.859
	T. syriacus. Boiss.	4.68	3.72	0.591
	S. aromaticum	2.21	4.92	0.426
	C. zeylanicum	1.35	1.73	0.885
	L .nobilis L.	4.68	3.72	0.591
	J. foetidissima Wild	6.98	0.78	0.978
	A. sativum L.	4.68	3.72	0.591
	M. fragrans Houtt.	6.03	0.63	0.986
K. pneumoniae	*O. syriacum.* L.	5.20	1.38)	0.927
	T. syriacus. Boiss.	3.03	3.58	0.612
	S. aromaticum	1.33	1.79	0.877
	C. zeylanicum	2.97	4.91	0.427
	L .nobilis L.	3.51	1.20	0.954
	J. foetidissima Wild	9.81	5.22	0.390
	A. sativum L.	8.75	3.86	0.570
	M. fragrans Houtt.	12.4	6.53	0.258

Table 5: Minimum inhibitory concentrations (MICs) of some antibiotics against Gram-negative bacteria

	MIC_{50} and MIC_{90} of some antibiotics (µg/ml)							
	E. coli O157:H7		*Y. enterocolitica* O9		*Proteus* spp		*K. pneumoniae*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Ciprofloxacin	2	64	0.5	NA	2	NA	0.5	NA
Levofloxacin	4	NA	4	NA	4	NA	4	NA
Ofloxacin	4	NA	2	64	2	NA	4	NA
Sparfloxacin	NA	NA	32	NA	32	NA	32	NA
Ceftazidime	0.25	NA	0.25	64	4	NA	4	NA
Ceftriaxone	32	NA	32	NA	32	NA	0.25	NA
Cefotaxime	0.5	NA	8	NA	64	NA	0.25	NA

NA: No effect

Discussion

Because of their safety and low cost as well as their impact on a large number of microbes,[25] medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics. The antimicrobial effects of aromatic oils extracted from a large number of plants have been evaluated and reviewed,[26,27] and the mechanisms that enable the natural ingredients of herbs and spices to resist microbes have been discussed.[28] The results show that these mechanisms vary greatly depending on the components of the essential oil.[29,30]

In the present study, the efficacy of some plant extracts and oils was determined, quantitatively, by measuring the diameter of the inhibition zones around the discs (table 2). Only *O. syriacum.* L., *T. syriacus* Boiss., *S. aromaticum* L., *C. zeylanicum* L., *L. nobilis* L., *J. foetidissima Wild*, *A. sativum* L., and *M. fragrans* Houtt. extracts inhibited the growth of the tested bacteria. In addition, *O. syriacum.* L., *T. syriacus* Boiss., *S. aromaticum* L., and *C. zeylanicum* L. essential oils were the most effective, and their MIC_{50} values varied from 1.5 µ**l/ml** to 25 µ**l/ml** against various kinds of bacteria. Because the values of minimum bactericidal concentration (MBC) and MIC are usually very similar,[31] it can be logically assumed that the above-mentioned plant extracts and oils have a bactericidal effect on Gram-negative bacteria, especially against *Proteus* spp. and *K. pneumoniae*.

The Probit Analysis (table 4) revealed that the minimum concentrations of the essential oils that could inhibit 50% of the various bacteria were *T. syriacus Boiss*. for *E. coli* O157H7 (7.85 µl/ml), O. syriacum. L. for *Proteus* spp. and *Y. enterocolitica* (1.12 and 1.59 µl/ml, respectively), and *S. aromaticum* for *K. pneumoniae* (1.33 µl/ml).

Ooi et al.[32] reported that *Cinnamomum verum* shows excellent activities against *E. coli* and *Proteus vulgaris*. Preuss et al.[33] found that *origanum* essential oil proves cidal to *E. coli* and *K. pneumoniae*. In addition, Barbosa et al.[34] found that the MIC_{90} of *Origanum vulgare* essential oil is 0.46% (v/v) against *E. coli*. López et al.[35] found that 8-10% (v/v) concentrations of *Origanum vulgare* essential oil can completely inhibit the growth of *E. coli* and other Gram-negative bacteria. Elsewhere, Mkaddem et al.[36] reported that *Mentha* essential oils are very active against *K. pneumoniae* bacteria, whereas they are less effective against *E. coli*. Furthermore, *Mentha longifolia* oil is thought to exhibit an antimicrobial activity against some Gram-positive bacteria such as *Streptococcus mutans* and *Staphylococcus aureus*, but without affecting *Pseudomonas aeruginosa*.[37]

Since the antibacterial effectiveness of medicinal plants varies dramatically depending on the phytochemical characteristics of plant families and subfamilies, it is not surprising to note the difference in this efficacy even when using samples taken from the same plant, but from two different regions.[38] Our results reveal that the cephalosporins were the most effective antibiotics against almost all the studied bacteria, and only Ciprofloxacin, one of the fluoroquinolones group, was effective against these bacteria.

Conclusion

O. syriacum. L., *T. syriacus* Boiss., *S. aromaticum* L., *C. zeylanicum* L., *J. foetidissima Wild*, *A. sativum* L., and *M. fragrans* Houtt. oils and *L. nobilis* L. extract were the most effective plant extracts against the Gram-negative bacteria studied in this work. These plant extracts could be a potential source of new antibacterial agents.

Further and more specific studies, in vivo, are recommended to determine the efficacy of these essential oils in the treatment of gram-negative bacterial infections.

Acknowledgment

The authors would like to thank the Director General of the Atomic Energy Commission of Syria (AECS) and the head of the Department of Molecular Biology and Biotechnology for their support.

Conflicts of Interest: None declared.

References

1 Tepe B, Daferera D, Sökmen M, Polissiou M, Sökmen A. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii M. Zohary et P.H. Davis. J Agric Food Chem. 2004;52:1132-7. PubMed PMID: 14995110.

2 Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot. 2001;64:1019-24. PubMed PMID: 11456186.

3 Singh D, Gupta R, Saraf SA. Herbs-are they safe enough? an overview. Crit Rev Food Sci Nutr. 2012;52:876-98. doi: 10.1080/10408398.2010.512426. PubMed PMID: 22747079.

4 Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al. Plants and human health in the twenty-first century. Trends Biotechnol. 2002;20:522-31. doi: 10.1016/S0167-7799(02)02080-2. PubMed PMID: 12443874.

5 Bankole MA, Shittu LA, Ahmed TA, Bankole MN, Shittu RK, Kpela T, et al. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. Afr J Tradit Complement Altern Med. 2007;4:427-33. PubMed PMID: 20161911; PubMed Central PMCID: PMC2816499.

6 Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol. 2007;117:112-9. doi: 10.1016/j.ijfoodmicro.2007.03.003. PubMed PMID: 17449125.

7 Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006;6:39. doi: 10.1186/1472-6882-6-39. PubMed PMID: 17134518; PubMed Central PMCID: PMC1693916.

8 Aboaba OO, Smith SI, Olude FO. Antibacterial Effect of Edible Plant Extract on Escherichia coli 0157:H7. Pakistan Journal of Nutrition. 2006;5:325-7. doi: 10.3923/pjn.2006.325.327.

9 Mirjana S, Nada B, Valerija D. Variability of Satureja cuneifolia ten essential oils and their antimicrobial activity depending on the stage of development. Eur Food Res Technol. 2004;218:367-71. doi: 10.1007/s00217-003-0871-4.

10 Hamann MT. Enhancing marine natural product structural diversity and bioactivity through semisynthesis and biocatalysis. Curr Pharm Des. 2003;9:879-89. doi: 10.2174/1381612033455297. PubMed PMID: 12678872.

11 Subash Babu P, Prabuseenivasan S, Ignacimuthu S. Cinnamaldehyde--a potential antidiabetic agent. Phytomedicine. 2007;14:15-22. doi: 10.1016/j.phymed.2006.11.005. PubMed PMID: 17140783.

12 Chang ST, Cheng SS. Antitermitic activity of leaf essential oils and components from Cinnamomum osmophleum. J Agric Food Chem. 2002;50:1389-92. PubMed PMID: 11879008.

13 Nogueira de Melo GA, Grespan R, Fonseca JP, Farinha TO, Silva EL, Romero AL, et al. Rosmarinus officinalis L. essential oil inhibits in vivo and in vitro leukocyte migration. J Med Food. 2011;14:944-6. doi: 10.1089/jmf.2010.0159. PubMed PMID: 21663474.

14 Arias BA, Ramón-Laca L. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. J Ethnopharmacol. 2005;97:89-95. doi: 10.1016/j.jep.2004.10.019. PubMed PMID: 15652281.

15 Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol. 2002;79:213-20. doi: 10.1016/S0378-8741(01)00384-1. PubMed PMID: 11801384.

16 Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol. 2004;19:61-4. doi: 10.1046/j.0902-0055.2003.00111.x. PubMed PMID: 14678476.

17 Maksimović Z, Milenković M, Vučićević D, Ristić M. Chemical composition and antimicrobial activity of Thymus pannonicus All. (Lamiaceae) essential oil. Cent Eur J Biol. 2008;3:149-54. doi: 10.2478/s11535-008-0013-x.

18 Burt S. Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol. 2004;94:223-53. doi: 10.1016/j.ijfoodmicro.2004.03.022. PubMed PMID: 15246235.

19 Tamokou Jde D, Kuiate JR, Tene M, Kenla Nwemeguela TJ, Tane P. The Antimicrobial Activities of Extract and Compounds Isolated from Brillantaisia lamium. Iran J Med Sci. 2011;36:24-31. PubMed PMID: 23365474;

PubMed Central PMCID: PMC3559120.
20 Gatsing D, Tchakoute V, Ngamga D, Kuiate JR, Tamokou JD, Nji-Nkah BF, et al. In vitro antibacterial activity of Crinum Purpurascens Herb. leaf extract against the Salmonella species causing typhoid fever and its toxicological evaluation. Iran J Med Sci. 2009;34:126-36.
21 Al-Mariri A. Ultraviolet C lethal effect on Brucella melitensis. New Microbiol. 2008;31:47-55. PubMed PMID: 18437841.
22 Council of Europe. European Pharmacopoeia. 7th ed. France: Council of Europe; 2010. p. 99.
23 National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. 6th ed. Wayne: National Committee for Clinical Laboratory Standards; 2003.
24 Koneman EW, Allen SD, Janda WM. Color Atlas and Textbook of Diagnostic Microbiology Hiladelphia. Philadelphia: Lippincott-Raven Publ; 1997. p. 785-856.
25 Hassawi D, Kharma A. Antimicrobial activity of some medicinal plants against Candida albicans. J Biol Sci. 2006;6:109-14.
26 Koutsaviti A, Milenković M, Tzakou O. Antimicrobial activity of the essential oil of Greek endemic Stachys spruneri and its main component, isoabienol. Nat Prod Commun. 2011;6:277-80. PubMed PMID: 21425694.
27 Stefanello MÉ, Pascoal AC, Salvador MJ. Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. Chem Biodivers. 2011;8:73-94. doi: 10.1002/cbdv.201000098. PubMed PMID: 21259421.
28 Montanari RM, Barbosa LC, Demuner AJ, Silva CJ, Andrade NJ, Ismail FM, et al. Exposure to Anacardiaceae volatile oils and their constituents induces lipid peroxidation within food-borne bacteria cells. Molecules. 2012;17:9728-40. doi: 10.3390/molecules17089728. PubMed PMID: 22893019.
29 Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol. 2005;22:273-92. doi: 10.1016/j.fm.2004.08.006.
30 Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties--an overview. Forsch Komplementmed. 2009;16:79-90. doi: 10.1159/000207196. PubMed PMID: 19420953.
31 Reuben KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO. Phytochemical screening and in vitro antimicrobial investigation of the methanolic extract of Croton zambesicus Muell ARG, stem bark. Eur J Sci Res. 2008;23:134-40.
32 Ooi LS, Li Y, Kam SL, Wang H, Wong EY, Ooi VE. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb Cinnamomum cassia Blume. Am J Chin Med. 2006;34:511-22. PubMed PMID: 16710900.
33 Preuss HG, Echard B, Enig M, Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Mol Cell Biochem. 2005;272:29-34. doi: 10.1007/s11010-005-6604-1. PubMed PMID: 16010969.
34 Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva Probst I, Fernandes A Jr. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog Dis. 2009;6:725-8. doi: 10.1089/fpd.2009.0282. PubMed PMID: 19580445; PubMed Central PMCID: PMC3145167.
35 López P, Sánchez C, Batlle R, Nerín C. Development of flexible antimicrobial films using essential oils as active agents. J Agric Food Chem. 2007;55:8814-24. doi: 10.1021/jf071737b. PubMed PMID: 17880148.
36 Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F, Romdhane M. Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils. J Food Sci. 2009;74:M358-63. doi: 10.1111/j.1750-3841.2009.01272.x. PubMed PMID: 19895481.
37 Al-Bayati FA. Isolation and identification of antimicrobial compound from Mentha longifolia L. leaves grown wild in Iraq. Ann Clin Microbiol Antimicrob. 2009;8:20. doi: 10.1186/1476-0711-8-20. PubMed PMID: 19523224; PubMed Central PMCID: PMC2707363.
38 Sarac N, Ugur A. The in vitro antimicrobial activities of the essential oils of some Lamiaceae species from Turkey. J Med Food. 2009;12:902-7. doi: 10.1089/jmf.2008.0089. PubMed PMID: 19735193.