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Evaluation of the Antibacterial Activity of *Micromeria barbata* in Lebanon

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Abstract: This study aims to evaluate the antimicrobial activity of the essential oil of the plant *Micromeria barbata* (*M. barbata*) against the Gram negative and Gram positive bacterial strains (from wild type strains and strains that have multiple mechanisms of resistance to antibiotics), as well as against a clinical strain of *Candida albicans* (*C. albicans*). The essential oil was obtained by Clevenger steam distillation. The composition of the essential oil in question as well as the concentrations of each component was determined by GC-MS. Clinically isolated strains were used, and their sensitivity to antibiotics was determined using the well diffusion method, while the resistance mechanism was determined through molecular methods. The activity of the essential oil against all tested strains was carried out using the diffusion method, and the Minimum Inhibitory Concentration (MIC) was determined using the dilution method on agar. The results of the antimicrobial activity showed that the oil had a good activity on wild strains and on strains having different mechanisms of resistance. The oil in question has a significant activity on all tested microorganisms. The broad spectrum of the antimicrobial activity of the oil, especially that directed against multi-resistant pathogens involved in human infections, make it useful to use this oil for the formulation of new drugs used to treat microbial infections, especially infections with antibiotic-resistant strains.

Key words: *Micromeriabarbata*, Essential oil, Antimicrobial activity.

Introduction

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans; the emergence of resistant microorganisms have paved the way for the emergence of infections that are treated by a limited number of antimicrobial agents¹⁴. The emergence of resistant Gram positive and Gram negative bacteria

is a major challenge for the antimicrobial treatment of infectious diseases ¹.

Here lies the need for researching new, efficient and cost-effective molecules for the control of infectious diseases. The results of various studies showed that some medicinal plants may indeed be a potential source of new antibacterial agents ²³.

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Essential oils are chemical compounds consisting mainly of hydrogen, carbon and oxygen molecules. They fall into the following two groups: hydrocarbons, which are mostly terpenes, and oxygenated compounds, such as esters, aldehydes, ketones, alcohols, phenols and oxides. For centuries, people have been using essential oils, or fluids extracted from plants, in aromatherapy as well as other topical uses, such as soaps for routine washing and antiseptics for healing wounds¹¹.

Essential oils are strong antimicrobial agents with a broad-spectrum activity and a possible potential for the control of pathogens in plants as of post-harvest spoils of many crops as well as human pathogenic diseases²¹.

M. barbata is a plant that belongs to the genus *Micromeria*, which is a member of the lipid plants family. *Micromeria* species are well known as aromatic species containing considerable amounts of essential oils². The chemical composition of the different species of *Micromeria* was detailed in various articles^{3,2,8,19}. However, the therapeutic effects and especially the antimicrobial effects of this plant has been the subject of a very limited number of studies^{19,15}.

The purpose of this study is to evaluate the antimicrobial activity of the essential oil of the plant *M. barbata* against resistant and sensitive clinical Gram negative and Gram positive bacterial strains as well as against a clinical strain of *Candida albicans* (*C. albicans*).

Materials and methods

Sampling and production of the essential oil

This plant was harvested in the region of North Lebanon, Dennyeh, at an altitude of approximately 1300 m, during the month of July 2012. The essential oil was obtained by Clevenger steam distillation (LMS - Germany, 10-653) with a yield of 2 %, and it was stored in an opaque glass bottle at a temperature of 4°C.

Chemical analysis

The composition of the essential oil in question as well as the concentrations of each component were determined by GC-MS (Shimadzu QP 2010)³. The pH of this oil was measured using a pH meter named Eutech-Ecosan pH 510 (Singapore).

Study of the antimicrobial effect

This method was carried out at the Health and Environment Microbiology Laboratory, (LMSD)-EDST, Lebaenes University. The strains used were all isolated in pathological specimens in the sector of microbiology at Nini hospital in Tripoli, northern Lebanon; after which, they were preserved in the Collection Microbiologique de l'Université Libanaise (CMUL). These strains were preserved and previously identified by various techniques such as the API system (Biomérieux, France) and the pyrosequencing method (Qiagen, Germany) for the variable regions of the 16S ribosomal gene (V1 and V3)⁹.

The resistance profile of the indicator strains used were determined using two methods, the first was the standard method in an agar medium according to the recommendations of comité del'antibiogramme-Société Française de Microbiologie (CA-FSM, 2013), while the other method was the detection of resistance genes by molecular methods (PCR).

The strains used were *Enterobacteria* that are susceptible and resistant to β -lactamines and *Staphylococcus aureus* (*S. aureus*) Meticilin resistant (MRSA) and *S. aureus* Methicilin Sensitive (MSSA). Furthermore, the antimicrobial activity was evaluated against a clinical strain of *C. albicans* (Table 2).

Preparation of the inoculum

For the bacterial strains, the inoculum was prepared from a pure culture incubated for 18 hours on an ordinary agar (Biorad - France). The bacterial and the *C. albicans* suspensions were prepared in Muller-Hinton II and Sabouraud dextrose broths respectively (Biorad, France), with a 0.5 McFarland turbidity ($\sim 1 \times 10^8$ UFC/ml).

Muller-Hinton II agar medium provided the necessary nutrients to support the growth of the microorganisms tested and a suitable medium to perform susceptibility testing. It was prepared from a commercially available dehydrated powder as per the manufacturer's instructions.

Evaluation of the antimicrobial activity

The suspension of the indicator strains was diluted by 1/100 and flooded at the surface of Muller-

Hinton agar (bacterial strains) and Sabouraud dextrose agar (*C.albicans*).

Method of diffusion in wells

The wells were produced on the surface of the agar previously seeded with the indicator strain, using a sterile pasteur pipet. 20 μ l of the essential oil was placed in each well. Diameters of zones

of inhibition were measured after 24 hours of incubation at 37°C using a caliper. Each test was performed in duplicates (fig. 1 and 2).

Determination of MIC

To ensure good dissolution of the essential oil in the medium used, the method of dilution in an agar medium was adopted through the use of a Muller-

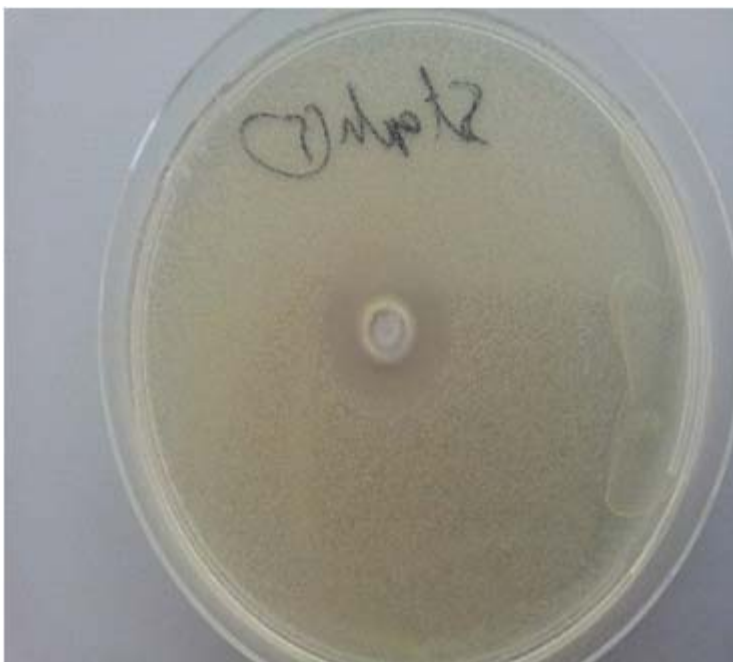


Figure 1. Inhibition zone obtained by 20 μ l of the essential oil in the presence of wild type MSSA



Figure 2. Inhibition zone obtained by 20 μ l of oil deposited in the presence of MRSA

Hinton II agar supplemented with 0.05 % Tween 80 (Fluka - Germany). A range of dilutions of the essential oil with the agar was carried out in order to obtain the final concentrations: 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 and 1/1280.

The inoculum of each indicator strain was prepared as described above and 10 μ l of each inoculum were deposited in spots on the surface of the Muller-Hinton agar containing different concentrations of the essential oil. The plates were incubated at 37°C for 18 hours.

Results

The Clevenger hydrodistillation of *M. barbata* plant gave an essential oil that was stored immediately at 4°C. To determine the organic and chemical composition of the essential oil, chromatographic analysis was performed on the gas chromatography (GC Shimadzu QP - 2010). Table 1 shows the composition of the essential oil in question as well as the concentration of each component in terms of percentage.

Antimicrobial activity

The antimicrobial activity was evaluated using

the two methods specified previously. Table 2 shows the results of the antimicrobial effect against the different bacterial strains as well as the *Candida albicans* strain obtained by the two methods used.

Discussion

The aerial part of the plant was harvested in July 2012, in Dennyeh region in North Lebanon and it was identified by Professor Georges Tohme, a botanist at the National Council for Scientific Research (CNRS) in Lebanon.

The oil was extracted through steam distillation, and the resulting oil gave off a pleasant odor similar to that of the essential oil of peppermint.

The essential oil of *M. barbata* was characterized by the presence of a large number of components which may include: Pulegone - Limonene - Menthol - Neomenthol - β -pinene - Piperitone and other ketone components that are the source of the characteristic odor of the oil (Table 1).

The results of the antimicrobial activity showed that the oil had a good activity (Table 2) on wild strains and on strains having different mechanisms of resistance. The essential oil of *M. barbata*

Table 1. Various chemical compounds found in the essential oil obtained after a GC analysis

Compounds	RT	Ret Index	% Area
α -Pinene	7.967	933	1.76
Camphene	8.183	953	0.10
β -Pinene	8.617	978	3.29
β -Myrcene	8.808	991	1.56
3-octanol	8.883	999	1.16
Limonene	9.525	1030	16.59
Ocimene	9.792	1046	0.17
Terpinolene	10.558	1052	0.10
Menthone	11.467	1158	0.16
Neomenthol	11.642	1170	12.37
Menthol	11.833	1184	6.19
Pulegone	12.442	1241	20.19
Piperitone	12.542	1267	4.22
Menthyl acetate	12.808	1290	0.35
Isomenthol acetate	12.933	1305	0.89
Cadinene	14.150	1512	1.06
Cedrol	14.858	1610	0.49
Total			70.65

Table 2. Results of the antibacterial effect of the essential oil obtained by the two methods

Name	Sensibility profile	Diameters measured	MIC
<i>Esherichia coli (E. coli)</i>	wild	11 mm	1/40
<i>E. coli</i>	β -lactamases with an extended spectrum (ESBL)	11 mm	1/40
<i>E. coli</i>	producer of carbapenemases	10 mm	1/40
<i>S. aureus</i>	wild	18 mm (figure 1)	1/40
<i>S. aureus</i>	MRSA	18 mm (figure 2)	1/40
<i>K. pneumonia</i>	Producer of hyper produced cephalosporinase	10 mm	1/40
<i>Salmonella spp.</i>	wild	13mm	1/40
<i>Listeria innocua</i>	wild	18mm	1/40
<i>Enterococcus faecalis</i>	wild	12mm	1/20
<i>C. albicans (clinical strain)</i>	wild	30 mm	1/1280

showed an MIC of 1/40 for all tested bacteria; however, the *E. faecalis* strain gave an MIC of 1/20 while the MIC of *C. albicans* was very low (1/1280).

The inhibition diameters varied between 10-18 mm for all the bacteria tested, with the exception for *C. albicans*, where the activity of the essential oil showed a very important anti-*Candida* effect (30 mm diameter).

The results of the antibacterial effect of this essential oil showed that this activity was not specific, since the essential oil gave almost the same diameters of inhibition zones and values of MIC on all Gram negative and Gram positive bacteria. For example, the MIC and inhibition diameters were similar to wild *Esherichia coli*, (*E. coli*), *E. coli* with extended spectrum β -lactamases (ESBL) and *E coli* carbapenemases positive; this was similar to wild *S. aureus* and MRSA.

These results are very interesting when it comes to the efficiency on the bacteria that are resistant to β -lactamines; in fact, infections by ESBL-producing strains, hyperproduced cephalosporinase and carbapenemase constitute a real challenge to public health¹⁸.

Currently, we are observing an outburst in the isolation of ESBL-producing Enterobacteria and especially the emergence of carbapenemase-producing strains in our region, in particular those of the OXA-48 type¹⁸. Since carbapenems exist

among the latest therapeutic reserves against severe infections, resistance to carbapenems reduces the possibility of treating such infections caused by multi-resistant strains. Carbapenemase Enterobacteria and MRSA are increasingly being revealed around the world and are becoming one of the major concerns of public health; they constitute the last step towards a therapeutic impasse^{18,6,4}.

Plant essential oils and extracts have been used for thousands of years¹⁰, in food preservation, pharmaceuticals, alternative medicine and natural therapies^{20,12}. It is necessary to investigate those plants that have been used in traditional medicine to improve the quality of healthcare scientifically. Essential oils are potential sources of novel antimicrobial compounds¹⁶ especially against bacterial pathogens. Many studies report the importance of the activity of essential oils against the wide range of resistance Gram positive and Gram negative bacteria^{7,13}, particularly against Multi Drug resistant bacteria^{17,22}.

In addition, the antimicrobial effect of the oil was remarkable on *C. albicans*, where the inhibition diameter measured was 30 mm and the MIC was 1/1280. This indicates that the activity of the essential oil of *M. barbata* may be specific against yeasts and especially against *C. albicans*.

This work may be continued further by seeking the active molecule, or molecules, responsible for

the antimicrobial activity. Moreover, the search for the cytotoxicity of these molecules may give us the opportunity of reproducing these molecules in any eventual human medical application.

Conclusion

The oil in question has a significant activity on all tested microorganisms. The broad spectrum of antimicrobial activity of the oil, especially that directed against multi-resistant pathogens involved in human infections, make it useful to use this oil for the formulation of new drugs used to treat microbial infections and especially by antibiotic-resistant strains. Furthermore, it is very important

to note that the pH of this oil, which is similar to the oil produced by the human skin, paves the way for new studies that make it possible to develop a topical form of this oil. This study showed a significant effect on *C. albicans*, and this subsequently leads us to study the effect of this oil on fungi and dermatophytes, which are involved in the infections of the appendages of both humans and animals.

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