MICROMERIA BARBATA : ANTIMICROBIAL AND SPORICIDAL ACTIVITY AGAINST BACILLUS CEREUS.

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ABSTRACT

The present investigation seeks to evaluate the antimicrobial and sporicidal activity of Micromeria barbata essential oil against spore-forming bacteria (Bacillus cereus) . It tries to respond to the increasing needs of alternative methods to control bacterial endospore contamination in a range of industries and applications .

The extraction of essential oil was performed by Hydrodistillation from the leaves of Micromeria barbata . This oil was found to be highly antimicrobial since results have shown low Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values as well as a high growth inhibition diameter . In addition , the oil was found to show a sporicidal activity since bacterial spore reduction was observed with different concentrations of the oil .

Keywords : Micromeria barbata , Bacillus cereus , Endospore , Essential oil , Antiomicrobial , Sporicidal .

1-INTRODUCTION

Many naturally occurring compounds have been shown to possess antimicrobial and sporicidal effect against food borne pathogens. Food industries require the design of food preservation treatments capable of ensuring microbial inactivation. Heat is still the most powerful food processing technique , but at the intensity needed to assure food safety , undesirable changes in properties of food could occur . Therefore , researchers are proposing the use of milder heat treatments in combination with other barriers such as natural antimicrobial substances , preferably of natural origin ( Espina et al , 2011 ) .

Spore-forming bacteria are gram-positive , usually , rod -shaped bacteria capable of producing spores –also called endospores – within the cell . There are two important genera of spore-forming bacteria : Bacillus and Clostridium . The production of spores allow the bacterium to survive under severe conditions until it becomes able to regenerate its vegetative form whenever possible (Turnbull P. , 1996) .

Bacillus cereus is well-recognized as a significant source of food spoilage ( EFSA journal , 2005; Ramanathan , 2010 ; Granum et al . , 1997) . Its wide distribution in the environment contributes to its transmission to wide variety of foods ( Hendrickx M. , 2011) . It has actually become one of the major pathogens of food-borne illness since endospores are extremely resistant to most food preservation techniques ( FSM , 2007) .

In the recent years , the essential oils deriving from plants are being thoroughly investigated worldwide to seek their ability to replace the synthetic antimicrobial agents that are facing increasing resistance . Big number of studies published in the last few years have had the aim of finding reasonable answers ( Jeyakumar et al . , 2011 ; Khatib et al . , 2013 ; Milosevic et al . , 2007; Upadhyay et al , 2010 ) . Spore-forming bacteria and particularly their endospores are among the main concerns of these investigations (Lawrence et al , 2009) .

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In this context, since the powerful antimicrobial activity of the *Micromeria barbata* essential oil is being elucidated through previous studies that have proven its efficacy against wild types as well as highly resistant pathogens (Bakkour et al., 2012), it becomes interesting to further investigate the activity of this oil against bacterial endospores.

This study aims to evaluate the activity of the *Micromeria barbata* essential oil against *Bacillus cereus* bacteria encountered in a routine culture of a previously filtered water. It tries to respond to the increasing need of alternative methods to control bacterial endospore contamination in a range of industries and applications.

2-MATERIALS AND METHODS

2.1 Collection and Extraction

The aerial parts of *Micromeria barbata* were collected from North-Lebanon, Denniyeh zone at 1300m altitude above sea level. The extraction of essential oil was performed by Clevenger-type (LMS –Germany, 10-563) hydrodistillation with 2% yield. The extract was preserved in a bottle of opaque glass at a temperature of 4°C.

2.2 Microbial Strains

The essential oil was tested against *Bacillus cereus*, spore-forming bacteria found while testing water intended to be used in cosmetic industry. The bacteria was identified using Api ID 32 (Biomerieux, France).

2.3 Antimicrobial activity

To determine MBC, inoculum from each non turbid tube was cultivated on blood agar at a temperature of 37 degrees for 24 hours. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganism was completely killed. The number of colonies from each non turbid on each agar were calculated and compared to the control positive plate. Each test was performed in three replicates and repeated twice.

2.5 Sporicial Activity

The suspension of tested microorganisms were cultivated on blood agar. Sporulation process

The screening diffusion method was employed to determine the antimicrobial activity of the essential oil (NCCLS, 1997). From a pure culture on blood agar (Biomerieux) aged 18-24 hours, the bacterial suspension was prepared with 0.5 Mcfarland turbidity (10⁶ CFU/ml). The bacterial suspension was diluted to 1/100 and inoculated on the surface of blood agar. Wells were created with the use of a sterile Pasteur pipet on the top of agar cultivated and dried with sterile tongs. Twenty micra were palced in each well. Dishes were incubated for 24 hours at 37°C. The inhibition zone was measured with the use of caliper. A duplicate test was performed.

2.4 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A broth micro dilution method was used to determine the MIC and MBC (NCCLS, 1997; Yu et al., 2004). All tests were performed in nutrient agar broth supplemented with Tween 20 at final concentration of 0.5% (v/v) to assure good homogenization.

Serial doubling dilutions of the oil were prepared in a series of 11 tubes ranged from 1 to 1/2048. The final concentration of the bacteria was adjusted to 10⁶ CFU/ml. Plates were incubated at 37°C for 24 hours. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity. The microorganism growth was assessed by optical microscopy following the staining of spores. Then the produced spores were exposed to various concentrations of the oil and finally the remaining spores were counted. The number of spores declined each time the oil concentration increased.

3-RESULTS AND DISCUSSION

The disc diameter of zone of inhibition (DDs), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Micromeria barbata* essential oil for the microorganism tested are shown in table 1.
Table 1 shows the disc diameter, the MIC and MBC of Bacillus cereus.

Figure 1 shows the inhibition zone of Bacillus cereus.

Figure 2 shows the MIC of Bacillus cereus.

The essential oil of Micromeria barbata showed inhibition zones against Bacillus cereus bacteria. Generally, large diameters are correlated with lower Minimum Inhibitory Concentration (MIC). The data obtained from the disc diffusion method showed that Bacillus cereus had a zone of inhibition of 20 mm, MIC 1/512 and MBC 1/256. These results indicate a significant activity of the oil against Bacillus cereus. The MBC/MIC ratio of the tested microorganism was 0.5. In addition, there was an inverse relation between the spores count and the oil concentrations. The most effective extract concentration was the highest one (pure oil).

This study allows us to show the antimicrobial activity, growth inhibition and spores inactivation. The disc diffusion method might not be adequate to determine if Micromeria barbata essential oil has an excellent antimicrobial activity or not. However, the determination of the MIC and MBC revealed an excellent antimicrobial activity against sporulated bacteria in addition to the sporicidal activity of the oil that decreased the number of spores with increased concentration of the oil.

Moreover, the antioxidant activity of the Micromeria barbata essential oil was evaluated in a previous study conducted by our research team (Bakkour et al., 2012). The essential oil has shown a significant antioxidant activity.

The combination of all these results are very interesting since the bacterial endospores are normally resistant to antimicrobial agents that do not have the ability to kill the spores. This will encourage for more investigations to seek the effect of this oil against endospores of other spore-forming microbes. Finally, the significant antioxidant activity result of the oil will be very helpful in the search for new food processing techniques that carry the most important characteristics: efficacy, safety and low cost.

4- CONCLUSION

Although this study was conducted on one type of bacteria, it was designed to be the beginning of a new series of investigations concerning the activity of our essential oil against spore-forming microbes including bacteria and fungi.

The significant antimicrobial, sporicidal and antioxidant activities of the oil suggest the possibility of using it in the formulation of new food processing techniques that could be better than available ones and can have the ability to prevent contamination from ingestion of spores.

5- ACKNOWLEDGEMENTS

The authors appreciate the valuable assistance of Mrs Tharwat Melhem Darwich.

6- REFERENCES


6. Granum E., Lund T., "Bacillus cereus and its food poisoning toxins ". FEMS


Table 1:

Table 1 shows the disc diameter, the MIC and MBC of Bacillus cereus.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Diameter</th>
<th>MIC</th>
<th>MBC</th>
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<tr>
<td>Bacillus cereus</td>
<td>20</td>
<td>1/512</td>
<td>1/256</td>
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Table 1: Results of diameter, MIC and MBC

Diameter is expressed in mm.
Figures:

Figure 1 shows the inhibition zone of Bacillus cereus

Figure 2 shows the MIC of Bacillus cereus