4-2016

Antibacterial effects of peppermint, *Mentha piperita* essential oil in free-form and in nanoparticles on *Pseudomonas fluorescens*

Car Reen Kok
University of Nebraska-Lincoln, carreen.k@huskers.unl.edu

Junsi Yang
University of Nebraska-Lincoln, jyang12@unl.edu

Ozan Ciftci
University of Nebraska-Lincoln, ciftci@unl.edu

Robert W. Hutkins
University of Nebraska-Lincoln, rhutkins1@unl.edu

Antibacterial effects of peppermint, *Mentha piperita essential oil in free-form and in nanoparticles on Pseudomonas fluorescens*

Kok, C. [1], Yang, J. [2], Ciftci, O.N. [2], Hutkins, R. [2]

[1.] Department of Biochemistry, University of Nebraska-Lincoln [2.] Department of Food Science & Technology, University of Nebraska-Lincoln

Introduction

Mentha piperita , commonly known as peppermint originally came from Europe and the Middle East and its oil is commonly used in traditional medicine and aromatherapy. Like many other free essential oils (EO), peppermint oil is believed to possess antimicrobial properties.

However, the application of EO in food as an antimicrobial agent has yet to be established. This would require proper formulation of the EO for it to be viable in foods. **This study aims to test the effects of both free *Mentha piperita* EO and *Mentha piperita* EO nanoparticles against *Pseudomonas fluorescens* and compare their inhibitory effects.** We used nanoparticles of encapsulated *Mentha piperita* EO in a shell of fully hydrogenated soybean oil (FHSO) and expect that the inhibitory effects of these nanoparticles would be greater than that of the free EO due to the ability of the shell to protect the EO and control its dispersion. We observed for the presence of antibacterial properties using various methods such as well diffusion, disc diffusion, optical density measurements and plate counting of serial dilutions. We found that the best method that produced quantifiable results was to perform serial dilutions and plate count them after incubation.

In these series of experiments, we demonstrated that free *Mentha piperita* EO inhibits *P. fluorescens* growth but have yet to demonstrate a similar effect with the EO nanoparticles.

Results

Free *Mentha Piperita* EO inhibits *P. fluorescens* growth

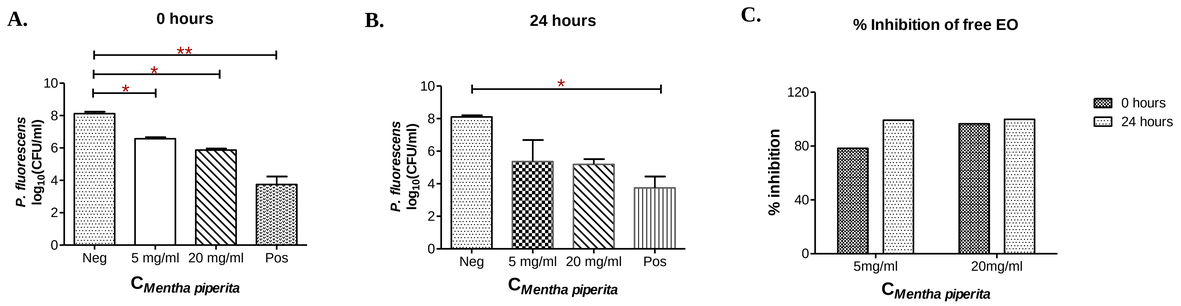


Figure 1. Aerobic plate count for free EO at 0 and 24 hours and its calculated % inhibition. Positive control plates were spread with inoculum containing Kanamycin (50mg/ml). Aerobic plate count of *P. fluorescens* at (A) 0 hours and (B) 24 hours incubation with different concentrations of essential oil . (C) % Inhibition essential oil against *P. fluorescens*. (One-way ANOVA analysis: * P≤0.05, ** P ≤ 0.01)

At 0 hour, the plate counts showed that the number of colonies were significantly different between the negative control and all other treatments **(Fig 1A)**. Both Kanamycin and free EO showed inhibitory effects even at 0 hour. At 24 hours, the negative controls were significantly different when compared to the positive controls **(Fig 1B)**. There is a slight increase in the % of inhibition in 20mg/ml EO compared to 5mg/ml at both 0 and 24 hours but this was not significant. **(Fig 1C)**.

Mentha Piperita EO nanoparticles loses inhibitory effect after 24 hours

Treatment	Aerobic plate count (CFU/mL)	
	0 hour	24hours
Negative Control	2.98×10^5	$> 10^8$
Positive Control	3.9×10^5	$< 10^3$
Carrier	1.09×10^6	$> 10^8$
EO-loaded particles 10mg/ml	$< 10^3$	$> 10^8$
EO-loaded particles 20mg/ml	$< 10^3$	$> 10^8$

Figure 2. Aerobic plate count for experimental set up using EO nanoparticles containing 10mg/ml and 20mg/ml of EO to observe the presence of antibacterial effects.

At 0 hour, *P. fluorescens* growth in the plates containing carrier particles were significantly different compared to all other treatments and plates containing EO nanoparticles have no visible colonies at the minimum dilution of 10^{-3} **(Fig 2)**. However, at 24 hours, the growth on all plates were more than 10^8 CFU/mL. **(Fig 2)**. Only the positive control plates with Kanamycin showed counts of less than 10^3 CFU/mL.

Discussion & Conclusion

Free *Mentha Piperita* EO inhibits *P. fluorescens* growth

- Free EO demonstrated inhibitory effect against *P. fluorescens* and was consistent with antibacterial effects of Kanamycin.
- The inhibitory effect of the EO showed effectivity even at 0 hour but plateaued with time.
- At 24 hours, inhibitory effect of 5mg/ml EO is similar to that of 20mg/ml.

Mentha Piperita EO nanoparticles loses inhibitory effect after 24 hours

- At 0 hour, plates containing both 10 and 20mg/ml of EO nanoparticles had almost close to no growth at the minimal dilution of 10^{-3}.
- This was unexpected as 20mg/ml of free EO was unable to completely inhibit growth.
- At 24 hours, bacteria growth in all plates were more than 10^8 CFU/mL.
- The particles have lost its effectiveness after a certain period of time, allowing bacteria to grow freely.

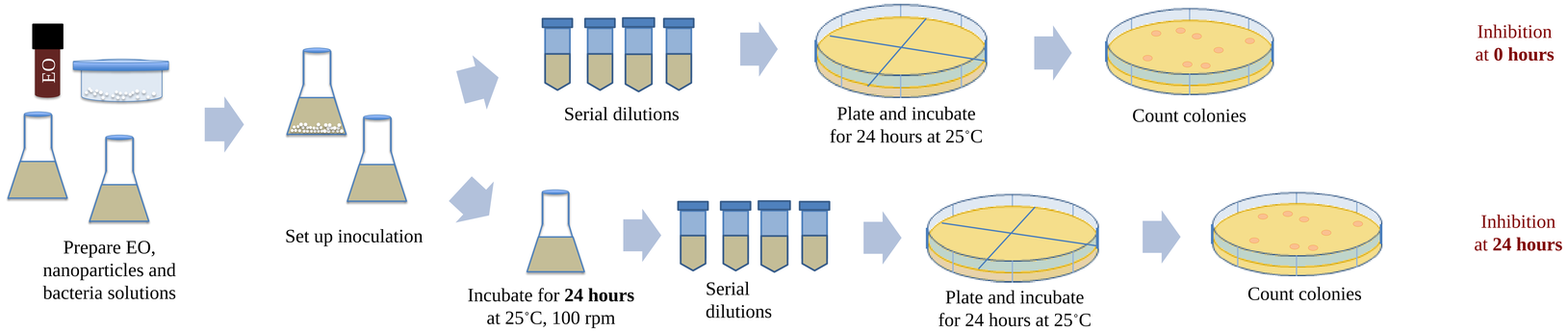
Preliminary Conclusions

- ***Mentha Piperita*** EO demonstrates potential as antibacterial agent.
- The control and release of EO is dependent on the encapsulation of the nanoparticles.
- Study can be extended by testing for antimicrobial activities of EO against different bacteria, fungi and yeast.
- Studies can be conducted to select for more suitable oils to be used in food and to investigate the possibility of enhanced antimicrobial activity when combined with other known antimicrobial agents.

Acknowledgements

I would like to thank the UNL UCARE program for funding this project; Maria X. Maldonado-Gomez for her guidance throughout the project; the Hutkins Lab for their support as well as Dr. Ciftci and Junsi Yang for providing the essential oil and nanoparticles for the study.

Method



References

1. Ghabraie M., Vu K.D., Tata L., Salmieri S., Lacroix M. Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. LWT-Food Science and Technology, 66. 332-339 (2016).
2. Varona S., Rojo S.R., Martin A., Cocero M.J., Serra A.T., Crespo T., Duarte C.M.M. Antimicrobial activity of lavandin essential oil formulations against three pathogenic food-borne bacteria. Industrial Crops and Products, 42. 243-250 (2013).
3. Tyagi A.K., Malik A. Antimicrobial potential and chemical composition of Mentha piperita oil in liquid and vapour phase against food spoiling microorganisms. Food Control, 22. 1707-1714 (2011).
4. Moghimi R., Ghaderi L., Rafati H., Aliahmadi A., McClements D.J. Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. Food Chemistry, 194. 410-415 (2016).