Antimicrobial Effect of *Mentha piperita* (Peppermint) Oil against *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*, and *Salmonella* Enteritidis in Various Dairy Foods: Preliminary Study

Hyun-Woo Lim1†, Dong-Hyeon Kim1 †, Se-Hyung Kim1, Jun-Man Lee1, Jung-Whan Chon1,2†, Kwang-Young Song1,3†, Dongryeoul Bae1, Jinhyun Kim1, Hyunsook Kim4, and Kun-Ho Seo1†

1Center for One Health, College of Veterinary Medicine, Konkuk University, Seoul, Korea
2College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
3Dept. of Biological Engineering, Yanbian University of Science & Technology, Yanji, China
4Dept. of Food & Nutrition, College of Human Ecology, Hanyang University, Seoul, Korea

Abstract

There are more than 25 species of *Mentha* plants, which are aromatic perennial herbs. Currently, these species are being widely used with great interest because of various clinical findings regarding their health benefits. This is due to the abundance of volatile compounds that could expedite environmental interactions such as protection against herbivores, parasites, pathogens, and so on. Therefore, in this study, the antimicrobial effect of *Mentha piperita* (peppermint) oil on *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*, and *Salmonella* Enteritidis were investigated using the spot-on-lawn method. The results show that *Mentha piperita* (peppermint) oil exhibited antimicrobial activities against *Bacillus cereus*, *Staphylococcus aureus*, and *Cronobacter sakazakii*; however, it did not inhibit the growth of *Salmonella* Enteritidis. This shows that the antimicrobial effect of *Mentha piperita* (peppermint) oil is effective against both Gram-positive and Gram-negative bacteria. Hence, in the present study, *Mentha piperita* (peppermint) oil was shown to have strong antimicrobial activities; it could be used as a potential food additive for improving the quality of various milk-based products due to its various bioactive properties. Future studies should be conducted for manufacturing functional dairy products with the addition of peppermint oil to prevent and/or alleviate specific diseases.

Keywords

*Mentha piperita*’s (peppermint) oil, antimicrobial effect, *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*

Introduction

Currently, more than 80% of the world population use the traditional medicine and medicinal plants (especially plant extracts and essential oil) for their primary health needs (Loolai et al., 2017). Among them, the plant *Mentha piperita* L. and their extracts is being used in the treatment of several disease from thousands of years (Alankar, 2009; Neeraj et al., 2013; Abdellatief et al., 2017). In general, peppermint or mint (*Mentha piperita* L.), a perennial aromatic herb belonging to the Lamiaceae (Labiatae) family, is a natural hybrid between spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatica* L.) (Fig. 1) (Park et al., 2016; Loolai et al., 2017). The rhizomes are wide-spreading, fleshy, and bare fibrous roots. The leaves are mint green and stand crosswise opposite each other on the stem, and also are elongated, oval with an acute apex (Neeraj et al.,...
Antimicrobial effect of *Mentha piperita*’s oil on various foodborne bacteria

Fig. 1. *Mentha piperita* (peppermint) leaf (Loolaie et al., 2017).

In general, the oil is obtained by water vapors distillation of the leaves and bloom top (Neeraj et al., 2013). Recently, biologically active compounds from peppermint sources have always been a great interest for scientists working on infectious diseases (Mucciarelli et al., 2007). Because *Mentha piperita* have the medicinal values in the treatment of various diseases such as cancer, diabetes, asthma, heart problems, and so on, and also the mint main chemical compounds consist of limonene (1 to 5%), cineole (3.5 to 14%), menthone (14 to 32%), menthofuran (1 to 9%), isomenthone (1.5 to 10%), methyl acetate (2.8 to 10%), isopulegol (0.2%), menthol (30 to 55%), pulegone (4%) and carvone (1%) (Liang et al., 2012; Dagli et al., 2015; Shams et al., 2015; Cash et al., 2016) (Table 1). Furthermore, the plant is rich in a wide variety of secondary metabolites such as tannins, phenols, steroids, flavonoids and volatile oils, which were found in vitro to have antimicrobial properties (Iscan et al., 2002).

Table 1. Various functions by *Mentha piperita*’s (peppermint) oil

| Type of essential oil | Specific functions | References                                      |
|----------------------|--------------------|-------------------------------------------------|
| ≫ Antimicrobial       |                   |                                                 |
| ≫ Antibacterial       | → Antifungal       | Barrera-Necha et al., 2008                      |
| ≫ Antifungal          | → Antiviral        | Mohsenzadeh, 2007                              |
| ≫ Anticancer          |                   | Baliga and Rao, 2010                           |
| ≫ Antidiabetic        |                   | Neeraj et al., 2013                            |
| ≫ In digestive system |                   | Dalvi et al., 1991; Neeraj et al., 2013         |
| ≫ Cold and fever      |                   | Neeraj et al., 2013                            |
| ≫ Mental capabilities |                   | Neeraj et al., 2013                            |
| ≫ Asthma              |                   | Wilkinson and Beck, 1994; Neeraj et al., 2013   |
| ≫ Heart problems      |                   | Paula, 2000                                    |
| ≫ Cold tea            |                   | Neeraj et al., 2013                            |
| ≫ Reducing irritable bowel syndrome (IBS) | → Application with ethereal oil | Shyu et al., 2007; Gardiner, 2000 |
| ≫ In agriculture as a biopesticide | → Ornamental use | Gupta and Dikshit, 2010 |
| ≫ Cosmetics           | → Facewash         | Mohsenzadeh, 2007                              |
| ≫ Hair and skin care  |                   | Neeraj et al., 2013                            |

*Mentha piperita*’s (peppermint) oil
Among several essential oils, peppermint oil is one of the most widely produced and consumed essential oils (Park et al., 2016; Loolaei et al., 2017), and also peppermint oil and extracts showed a good antimicrobial effect against various foodborne pathogenic bacteria (Singh et al., 2011; Neeraj et al., 2013; Schmitz et al., 2015). According to previous study, the antimicrobial effect of peppermint leaves extract against Gram negative bacilli was higher than of its stem extract (Shaikh et al., 2014; Loolaei et al., 2017).

There are differences in antimicrobial effect against foodborne pathogens in previous studies, which is due to the differences in the chemical composition of peppermint essential oil from different parts of its structure (Shaikh et al., 2014).

Until now, there was no report about the inhibition against Cronobacter sakazakii and Salmonella Enteritidis except for Bacillus cereus and Staphylococcus aureus using Mentha piperita’s (peppermint) oil. Therefore, this objective of this study was investigated the antimicrobial effect of Mentha piperita’s (peppermint) oil against 4 different foodborne pathogenic bacteria for exploring the possibility of food additive and natural antibiotics and for improving the quality of dairy products.

**Materials and Methods**

1. **Mentha piperita’s (peppermint) oil**

   Mentha piperita’s (peppermint) oil was manufactured in Borak Company (Korea) and was purchased from Serim Food Company (Korea). Mentha piperita’s (peppermint) oil was composed of 100% peppermint oil and also was the grade of food additive as natural fragrance. The various concentration of Mentha piperita’s (peppermint) oil was prepared using a concentrator.

2. **Foodborne pathogenic bacteria**

   Four different foodborne bacteria used in the study were prepared with the donation of Center for One Health, College of Veterinary, Konkuk University in Seoul, Korea. Cronobacter sakazakii KCTC2949, Salmonella Enteritidis 110, Bacillus cereus ATCC10876, and Staphylococcus aureus ATCC6538 were grown on nutrient agar (NA) (Oxoid, UK) for over 18 hours and then colonies were transferred into tubes containing cryopreservation fluid according to the instruction of the manufacturer (Original Microbiology Bead Storage System, STS, Technical Service Consultants Limited, UK). Until use, the beads were stored at −70°C.

3. **Antimicrobial susceptibility testing**

   The antimicrobial effect of Mentha piperita’s (peppermint) oil were tested on 4 different foodborne bacteria using by the spot-on-lawn method with some modifications (Cadirci and Citak, 2005). Cronobacter sakazakii KCTC2949, Salmonella Enteritidis 110, Bacillus cereus ATCC10876, and Staphylococcus aureus ATCC6538 were cultured on Mueller–Hinton broth (MHB; Difco) and incubated at 37°C ± 0.5 for 24 hours. The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller–Hinton agar (MHA; Difco)
using sterilized cotton swabs. A total of 0X as negative control, 1X, 2X, and 3X of Mentha piperita’s (peppermint) oil was directly dropped onto the surface of the MHA, respectively. The plates were incubated for one day at 37±0.5°C, and the inhibition zone was observed.

4. Statistical analysis
All experiments were carried out independently in triplicate experiments. The inhibition of various concentration of Mentha piperita’s (peppermint) oil against Cronobacter sakazakii KCTC2949, Salmonella Enteritidis 110, Bacillus cereus ATCC10876, and Staphylococcus aureus ATCC6538 were evaluated by one-way analysis of variance (ANOVA). Statistical significance was accepted at the $p=0.05$ level.

Results and Discussion
The Mentha piperita’s (peppermint) oil showed various levels of antimicrobial effect when tested by the spot-on-lawn method (Fig. 2). In general, the spot-on-lawn method
is known to be practical and suitable technique in antimicrobial effect research (Soomro et al., 2007). These results obtained showed that Mentha piperita’s (peppermint) oil exhibited antimicrobial activities against Cronobacter sakazakii KCTC2949, Bacillus cereus ATCC10876, and Staphylococcus aureus ATCC6538 Bacillus cereus and the inhibitory effect of Mentha piperita’s (peppermint) oil was shown as a whole regardless of the increase in the concentration (Fig. 2). Whereas Salmonella Enteritidis 110 did not show any inhibition by the Mentha piperita’s (peppermint) oil (Fig. 2).

As is generally known, the characteristics of peppermint oil are (1) to increase the stickiness and viscosity of food, (2) to enhance the emulsion stability of food, and (3) to improve the physical properties and texture of food (Alankar, 2009; Cash, 2015; Shams et al., 2015). Hence, it is mainly used as a thickener in foods (Baliga and Rao, 2010). According to Singh et al. (2011), Mentha piperita’s (peppermint) oil showed a wider spectrum of activity but less strong inhibition as compared to the investigated commercial antibiotic. Hence, Mentha piperita’s (peppermint) oil could be used as a good conservation agent by inhibiting some food borne pathogens. Also, Park et al. (2016) screened the different crude organic extracts of nine Mentha species showed that the ethanol extract having more activity against six pathogenic bacteria using disc diffusion method. The ethanol extract would produce more activity than other organic solvents compared to other extracts, and 9 mint species of ethanol extracts having significant activity against S. haemolyticus, A. salmonicida, E. coli, A. hydrophila, and so on (Park et al., 2016). The results of this study were similar to those of previous studies.

The antimicrobial effect of the peppermint oils against various foodborne pathogenic bacteria is thought to be due to two large amount of menthol and menthone (Fig. 3) (Iscan et al., 2002). Namely, the efficiency of antimicrobial effect is correlated with the menthol and menthone percentages (Knowlton et al., 2013; Schmitz et al., 2015). The role of menthol was responsible for mint’s cooling sensation and that of menthone was responsible for mint’s characteristic icy scent (Fig. 3) (Knowlton et al., 2013; Schmitz et al., 2015).

However, antimicrobial evaluations of essential oils are difficult because of their volatility, insolubility in water, and complex chemistry. Some factors are important

![Fig. 3. Chemical structure of menthol (left) and menthone (right) (Knowlton et al., 2013; Schmitz et al., 2015).](image-url)
when testing oils such as the assay technique, growth medium, the microorganism, and the oil itself (Iscan et al., 2002).

Generally, mint oil and menthol have moderate antimicrobial effect against both Gram-positive/negative bacteria (Loolair et al., 2017). Hence, it seems peppermint can become a novel target for synthesis of plant-derived drugs against a large spectrum of multidrug resistance bacteria (Cash et al., 2016).

Until now, peppermint plant had great beneficial and economical role in human society, and the peppermint industry was the largest commercial herb industry in the United States and the annual output of peppermint was over 4000 tons (Esetli et al., 2015; Loolair et al., 2017). In the world, the annual average production of peppermint essential oil was about 7000 tons, and this meant that production of peppermint oil was ranked second after the citrus oil (Esetli et al., 2015). The largest producer of peppermint oil was United States, followed by France, Brazil, Argentina, Western European countries, China, Peru, Thailand and South Korea (Esetli et al., 2015).

Hence, to maintain and develop various benefits points of peppermint, two things must be considered: (1) researches must be considered its minor side effects and toxicity, and (2) various dosage forms are available in market for treatment of various human lifestyle diseases (Alankar et al., 2009; Abdellatief et al., 2017; Loolair et al., 2017).

Next, recently, it has been reported that using peppermint oil has a great effect on irritable bowel syndrome (IBS) (Neeraj et al., 2013; Cash, 2015; Loolae et al., 2017).

Generally, IBS is defined as a chronic disorder of altered bowel function characterized by symptoms of diarrhea, constipation, or alternating bowel habits accompanied by pain or discomfort and may include a constellation of other symptoms, e.g., bloating, urgency, and incomplete evacuation (Cash, 2015; Cash et al., 2016; Loolae et al., 2017). This syndrome affects 9 to 23% of the population across the world (Shams et al., 2015; Loolae et al., 2017). It was reported that Mentha piperita’s (peppermint) oil is a safe and effective short-term treatment for IBS (Khanna et al., 2014). Also, Mentha piperita’s (peppermint) oil acts as inhibitor for calcium channel effect in the intestine and therefore it can able to reduce symptoms of IBS (Harris, 2016). Other postulated mechanisms for Mentha piperita’s (peppermint) oil in treatment of IBS include inhibition of potassium depolarization-induced and electrically stimulated responses in the ileum (Kline et al., 2001). Also, it was reported that Mentha piperita’s (peppermint) oil has crucial effects on histamine, serotonin, and cholinergic receptors in the gastrointestinal tract may also mediate some of its antiemetic effects (Egan et al., 2015). Cappello et al. (2007) showed that a four weeks treatment with Mentha piperita’s (peppermint) oil improved abdominal symptoms in patents with IBS. The similar results also were reported in other studies (Tate, 1997; Sagduyu, 2002; Madisch et al., 2004).

Taken together, peppermint is the most encouraged plant for treatment of gastro-intestinal disorders.

In conclusion, this study demonstrated the potential of Mentha piperita’s (peppermint) oil to inhibit the growth of Bacillus cereus, Staphylococcus aureus, Cronobacter sakazakii as antimicrobial effect, except for only Salmonella Enteritidis. Hence, this study indicated that Mentha piperita’s (peppermint) oil could be applicable in various
dairy foods with improved bioactive additives for alleviating various lifestyle disease. Additionally, *Mentha piperita*’s (peppermint) oil is most frequently traded essential oil in the entire world and in many developed and developing countries, because it considered as a valuable target for both food and pharmaceutical studies. And then further studies are need to exploration of cellular and molecular mechanisms of peppermint and its compounds on human body

**Acknowledgement**

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03035427).

**References**

Abdellatif, S. A., Beheiry, R. R. and El-Mandrawy, A. M. 2017. Peppermint essential oil alleviates hyperglycemia caused by streptozotocin-nicotinamide-induced type 2 diabetes in rats. Biomedicine & Pharmacotherapy 95:990-999.

Alankar, S. 2009. A review on peppermint oil. Asian J. Pharmaceutical and Clinical Research 2:27-33.

Ansari, M. A., Vasudevan, P., Tandon, M. and Razdan, R. K. 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Bioresource Technology 71:267-271.

Baliga, M. S. and Rao, S. 2010. Radio protective potential of mint: A brief review. J. Cancer Res. Ther. 6:255-262.

Barrera-Necha, L. L., Garduno-Pizana, C. and Garcia-Barrera, L. Z. 2008. *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporium* f.sp. Gladioli (Massey) Synder and Hansen. J. Plt. Pathol. 7:1-5.

Burt, S. A. 2004. Essential oils: Their anti-bacterial properties and potential applications in foods: A review. International Journal of Food Microbiology 94:223-253.

Cadirci, B. H. and Citak, S. 2005. A comparison of two methods used for measuring antagonistic activity of lactic acid bacteria. Pakistan Journal of Nutrition 4:237-241.

Cappello, G., Spezzaferro, M., Grossi, L., Manzoli, L. and Marzio, L. 2007. Peppermint oil (mintoil®) in the treatment of irritable bowel syndrome: A prospective double blind placebo-controlled randomized trial. Dig. Liver Dis. 39:530-536.

Cash, B. 2015. Novel peppermint oil formulation for dietary management of irritable bowel syndrome. Gastroenterology & Hepatology 11:631-633.

Cash, B. D., Epstein, M. S. and Shah, S. M. 2016. A novel delivery system of peppermint oil is an effective therapy for irritable bowel syndrome symptoms. Dig. Dis. Sci. 61:560-571.

Dagli, N., Dagli, R., Mahmoud, R. S. and Baroudi, K. 2015. Essential oils, their therapeutic properties, and implication in dentistry: A review. J. Int. Soc. Prevent Com-
munit Dent. 5:335-340.
Dalvi, S. S., Nadkarni, P. M., Paradesi, R. and Gupta, K. C. 1991. Effect of peppermint oil on gastric emptying in man: A preliminary study using a radiolabel led solid test meal. Indian J. Physiol. Pharmacology 35:212-214.
Egan, M., Connors, E. M., Anwar, Z. and Walsh, J. J. 2015. Nature’s treatment for irritable bowel syndrome: Studies on the isolaton of (-)-menthol from peppermint oil and its conversion to (-)-methyl acetate. J. Chem. Educ. 92:1736-1740.
Esetliyi, B. Ç., Çobanoğlu, Ö., Tepeck, M., Öztürk, B. and Anaç, D. 2015. Yield, essential nutrients and essential oils of peppermint (Mentha piperita L.) grown under organic farming conditions. Ziraat Fakültesi Dergisi, Uludağ Üniversitesi (Journal of Agricultural Faculty of Uludağ University) 29:29-36.
Gardiner, P. 2000. Peppermint (Mentha piperita). Longwood Herbal Task Force. pp. 1-22.
Gupta, S. and Dikshit, A. K. 2010. Biopesticides: An eco-friendly approach for pest control. Journal of Biopesticides 3:186-188.
Harris, L. A. 2016. Treating irritable bowel syndrome: A fresh and minty approach to an old therapy. Dig. Dis. Sci. 61:334-336.
Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, H. C. and Demirci, F. 2002. Antimicrobial screening of Mentha piperita essential oils. J. Agric. Food Chem. 50:3943-394.
Khanna, R., MacDonald, J. K. and Levesque, B. G. 2014. Peppermint oil for the treatment of irritable bowel syndrome: A systematic review and meta-analysis. J. Clin. Gastroenterol 48:505-512.
Kline, R. M., Kline, J. J., Di Palma, J. and Barbero, G. J. 2001. Enteric-coated, phosphorous-coated peppermint oil capsules for the treatment of irritable bowel syndrome in children. J. Pediatr 138:125-128.
Knowlton, W. M., Palkar, R., Lippoldt, E. K., McCoy, D. D., Baluch, F., Chen, J. and McKemy, D. D. 2013. A sensory labeled-line for cold: TRPM8-expressing sensory neurons define the cellular basis for cold, cold pain, and cooling-mediated analgesia. J. Neurosci. 33:2837-2848.
Liang, R., Xu, S., Shoemaker, C. F., Li, Y., Zhong, F. and Huang, Q. 2012. Physical and antimicrobial properties of peppermint oil nanoemulsions. J. Agric. Food Chem. 60:7548-7555.
Loolaei, M., Mosef, N., Rasouli, H. and Adibi, H. 2017. Peppermint and its functionality: A review. Arch. Clin. Microbiol. 8:54.
Madisch, A., Holtmann, G., Plein, K. and Hotz, J. 2004. Treatment of irritable bowel syndrome with herbal preparatons: Results of a doubleblind, randomized, placebo-controlled, multicentre trial. Aliment Pharmacol. Ther. 19:271-279.
Mohsenzadeh, M. 2007. Evaluation of antibacterial activity of selected Iranian essential oils against Staphylococcus aureus and Escherichia coli in nutrient broth medium. Pak. J. Biol. Sci. 10:3693-3697.
Muccioni, M., Camusso, W., Maffei, M., Panicco, P. and Bicchi, C. 2007. Volatile terpenoids of endophyte-free and infected peppermint (Mentha piperita L): Chemical
parttoning of a symbiosis. Microb. Ecol. 54:685–696.
Neerai, R., Prakash, A. and Seema, Y. 2013. Antimicrobial activity and medicinal values of essential oil of Mentha piperita L. Int. J. Eng. Innov. Technol. 2:214–218.
Park, Y. J., Baskar, T. B., Yeo, S. K., Arasu, M. V., Al-Dhabi, N. A., Lim, S. S. and Park, S. U. 2016. Composition of volatile compounds and in vitro antimicrobial activity of nine Mentha spp. SpringerPlus 5:1628.
Sagduyu, K. 2002. Peppermint oil for irritable bowel syndrome. Psychosomatcs 43:508–509.
Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T. and Rehder, V. L. G. 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz. J. Microbiol. 34:1517–1520.
Schmitz, D., Alvin Shubert, V., Betz, T. and Schnell, M. 2015. Exploring the conformational landscape of menthol, menthone, and isomenthone: A microwave study. Front Chem. 3:15.
Shaikh, S., Yaacob, H. B. and Rahim, Z. H. A. 2014. Prospective role in treatment of major illnesses and potential benefits as a safe insecticide and natural food preservative of mint (Mentha spp.): A review. Asian J. Biomed. Pharm. 4:1.
Shams, R., Oldfield, E. C., Copare, J. and Johnson, D. A. 2015. Peppermint oil: Clinical uses in the treatment of gastrointestinal diseases. JSM. Gastroenterol Hepatol 3:1035.
Shyu, M. K., Yuan, R. H., Shih, J. C., Wu, M. Z., Chen, H. L., Kuo, Y. C., Chien, C. L., Chow, L. P., Chen, H. L and Hsieh, F. J. 2007. Kinetics and functional assay of liver repopulation after human cord blood transplantation. Digestive and Liver Disease. 39:455–466.
Singh, R., Shushni, M. A. M. and Belkheir, A. 2011. Antibacterial and antioxidant activities of Mentha piperita L. Arabian Journal of Chemistry 8:322–328.
Soomro, A. H., Masud, T., Samimi, S. and Rathore, H. A. 2007. Comparison of different methods for detection of antimicrobial activity of Lactobacillus spp. Pakistan J. Zool. 39:265–268.
Tate, S. 1997. Peppermint oil: A treatment for postoperative nausea. J. Adv. Nurs. 26:543–549.
Wilkinson, S. M. and Beck, M. H. 1994. Allergic contact dermatitis from menthol in peppermint. Contact Dermatitis 30:42–43.