



## ARTICLE



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

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## Abstract

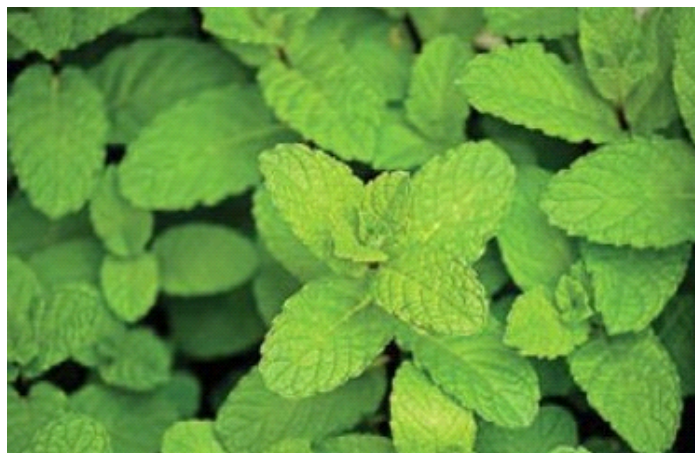
There are more than 25 species of *Mentha* plants, which are aromatic perennial herbs. Currently, these species are being widely used with great interest because of various clinical findings regarding their health benefits. This is due to the abundance of volatile compounds that could expedite environmental 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 (Loolaie *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 aquatic* L.) (Fig. 1) (Park *et al.*, 2016; Loolaie *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.*,



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

2013). 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%), menthyl 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 (Iskan *et al.*, 2002).

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

Type of essential oil	Specific functions	References
<i>Mentha piperita</i> 's (peppermint) oil	⇒ Antimicrobial properties	→ Antibacterial Burt, 2004; Sartoratto <i>et al.</i> , 2004 → Antifungal Barrera-Necha <i>et al.</i> , 2008 → Antiviral Mohsenzadeh, 2007
	⇒ Anticancer	Baliga and Rao, 2010
	⇒ Antidiabetic	Neeraj <i>et al.</i> , 2013
	⇒ In digestive system	Dalvi <i>et al.</i> , 1991; Neeraj <i>et al.</i> , 2013
	⇒ Cold and fever	Neeraj <i>et al.</i> , 2013
	⇒ Mental capabilities	Neeraj <i>et al.</i> , 2013
	⇒ Asthma	Wilkinson and Beck, 1994; Neeraj <i>et al.</i> , 2013
	⇒ Heart problems	Paula, 2000
	⇒ Cold tea	Neeraj <i>et al.</i> , 2013
	⇒ Reducing irritable bowel syndrome (IBS)	Shyu <i>et al.</i> , 2007
	⇒ Application with ethereal oil	Gardiner, 2000
	⇒ In agriculture as a biopesticide	Gupta and Dikshit, 2010
	⇒ Ornamental use	Ansari <i>et al.</i> , 2010
	⇒ Cosmetics	→ Facewash Mohsenzadeh, 2007 → Hair and skin care Neeraj <i>et al.</i> , 2013

Among several essential oils, peppermint oil is one of the most widely produced and consumed essential oils (Park *et al.*, 2016; Loolaie *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; Loolaie *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  $-70^{\circ}\text{C}$ .

### 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  $37^{\circ}\text{C} \pm 0.5$  for 24 hours. The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller-Hinton agar (MHA; Difco)

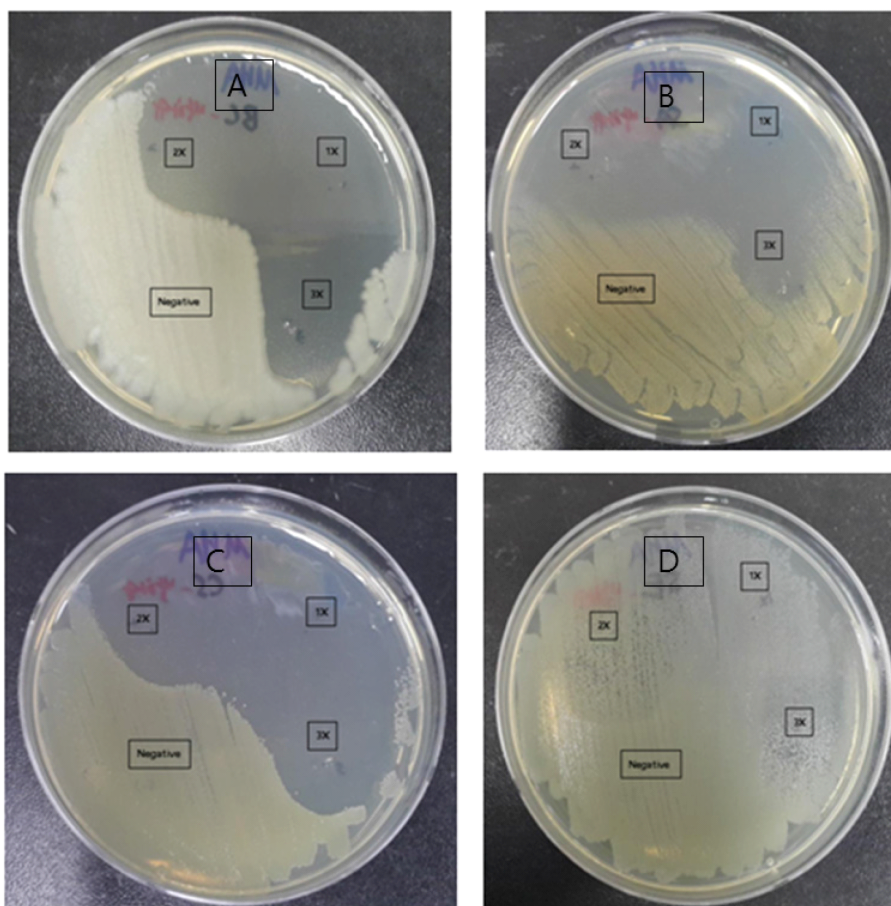
using sterilized cotton swabs. A total of 0X as negative control, 1X, 2X, and 3X of *Mentha piperita*'s (peppermint) oil was directly dropped onto the surface of the MHA, respectively. The plates were incubated for one day at  $37\pm 0.5^\circ\text{C}$ , and the inhibition zone was observed.

#### 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



**Fig. 2.** The antimicrobial effect on various concentration of *Mentha piperita*'s (peppermint) oil against *Bacillus cereus* ATCC10876 (A), *Staphylococcus aureus* ATCC6538 (B), *Cronobacter sakazakii* KCTC2949 (C), and *Salmonella* Enteritidis 110 (D) tested by the spot-on-lawn methods.

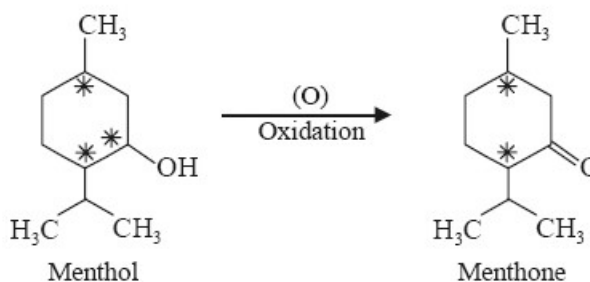
is known to be practical and suitable technique in antimicrobial effect research (Soomro *et al.*, 2007).

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).



when testing oils such as the assay technique, growth medium, the microorganism, and the oil itself (Iskan *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 (Esetlilil *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 (Esetlilil *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 (Esetlilil *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; Loolaie *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; Loolaie *et al.*, 2017). This syndrome affects 9 to 23% of the population across the world (Shams *et al.*, 2015; Loolaie *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 patients 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 gastrointestinal 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

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