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Research Article

Development of a hand sanitizing spray using peppermint oil

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Abstract

Antimicrobial activities of peppermint oil against major bacteria commonly found on hands (*Escherichia coli*, *Staphylococcus aureus* and coliform) were investigated. The disc diffusion method was employed to determine the minimal inhibitory concentration (MIC) and the broth dilution method was used for minimal bactericidal concentration (MBC) using the concentration of essential oil between 50-800 µL/mL. It was found that peppermint oil had potential in inhibiting all tested bacteria with the MIC of 450 µL/mL and the MBC of 460 µL/mL. Inhibitory effect of the essential oils against these bacteria on hands was developed in the form of a peppermint oil spray. The formula of peppermint oil spray was 51% water, 46% essential oil, 3% sorbitol and 0.02% sodium laureth sulphate. In addition, results from the sensory test showed that 95% of the consumers accepted this product as 'moderately liked' (hedonic score 7).

Keywords: food contamination, sanitation, peppermint oil, *Escherichia coli*, *Staphylococcus aureus*, coliform, antimicrobial

Introduction

Food safety constitutes a growing concern for regulatory agencies, producers and the public due to the incidence of foodborne illness caused by enteric human pathogens in various food products at retail and commercial food service facilities**.** In recent years,

outbreaks of illness linked to the consumption of food have been increasing [1]. A number of these outbreaks have also been linked to packaging, pre-washing and cleanliness of workers. Employees who are in direct contact with food is a major factor in foodborne illness. Because there is a constant risk of spreading pathogens via hand contact and food surfaces, hygiene measures are important for these surfaces. Washing hands with sanitizing solutions is the only step where a reduction in spoilage microorganisms and potential pathogens can be achieved. However, limited scientific information is available on the efficacy of many disinfection methods for reducing the populations of pathogenic bacteria on hand.

Escherichia coli, coliform and *Staphylococcus aureus* are living on human skin and in food. *E. coli* especially is used as reliable indicator of fecal contamination and as a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds [2]. Moreover, the presence of fecal coliforms in food indicates post-sanitization or post-process contamination, often caused by a lack of hand hygiene on the part of food handlers [3]. S. *aureus* is also among the most common causes of foodborne illnesses. Foodborne disease caused by *S. aureus* is typically due to enterotoxin ingestion preformed in food by the enterotoxigenic strain [4]. A conventional method of hand sanitizing procedures is washed with plain water or water containing a sanitizer, such as chlorine. Chlorine has however, a minimal effect in killing bacteria on these surfaces. In recent years, essential oils as a natural compound have been studied for potential uses in hand protection. Over the past few years, increasing consumer demand for more natural, "synthetic preservative-free" products, has led the food industry to consider the incorporation of natural preservatives in a range of products. The use of natural antimicrobial compounds has the advantage of being more acceptable to the consumers as these are considered as "non chemical" [5]. This study was designed to evaluate the effectiveness of peppermint oil for reducing *E.coli*, *S. aureus* and coliform on hands during washing.

Materials and Methods

Bacterial culture

Strains of *Escherichia coli*, *Staphylococcus aureus* and coliform obtained from the Microbiology Laboratory, Walailak University, Nakhon Si Thammarat province, Thailand was used as test microorganisms. Inocula used in antimicrobial assays was obtained from overnight cultures grown on nutrient agar (NA) slants at 37°C. A loopfull of the culture was diluted in sterile saline solution (0.85 g 100 mL^{-1}) to have a final concentration of approximately 10^8 cfu mL^{-1}.

Antibacterial assay

Antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and coliform were investigated by the agar disc diffusion method. Each strain of *Escherichia coli*, *Staphylococcus aureus* and coliform (0.1 mL) was separately cultured in NA used for inoculation. Sterile paper discs (7 mm diameter) containing peppermint oil at concentration of 50-800 μL/mL were placed on the agar. Plates were at room temperature for 30 min to allow liquid absorption into the disc. Vegetable oil was

used as a control. Different dilutions of the oils (including controls) were made with methanol. Then, plates were incubated at 35°C for 24 h. Following incubation, the diameters of the inhibition zones were measured.

A minimal inhibitory concentration (MIC) and a minimal bactericidal concentration (MBC) of peppermint oil were performed by the broth dilution method in test tubes. Fifty microlitres of each substance at a concentration of 50-800 μL/mL was added to 5 ml of nutrient broth (NB) tubes containing inoculums of 10^8 cfu mL^{-1}. Different dilutions of the oils (including controls) were made with methanol. The preliminary work revealed that methanol had no effect on bacterial growth. The tubes were then incubated at 35°C for 24 h. on an incubator shaker (Gallenkamp, Loughborough, England) to evenly disperse the oil throughout the broth. The lowest concentration that showed no visible growth was regarded as the MIC. Cells from the tubes showing no growth were subcultured on NA agar plates to determine if the inhibition was reversible or permanent. The MBC was determined as the lowest concentration at which no growth occurred on the plates.

Peppermint oil spray for hand protection

Fifty participants from local households were requested to decontaminate their hands by washing thoroughly for 2 min using a peppermint oil spray (containing 460 μL/mL of peppermint oil, 510 μL/mL of water, plus with sorbitol and sodium laureth sulphate as a surfactant), followed by rinsing for 20 s with de-ionized water. Sampling of contaminated hands was done before and after hand washing. The inside surface of parts of the palms and fingers (5 × 5 cm area) of one hand was swabbed by cotton swabs pre-moistened in peptone water. The cotton swabs and the 10 ml peptone water rinse were transferred into 100 ml sterile bottles.

E. coli and coliform were cultured on the 3M™ Petrifilm™ E.coli/Coliform Count Plate (3M Company, Thailand) and incubated at 35°C for 24 hours. Coliform was revealed as red and blue colonies with associated gas bubbles. Confirmed *E.coli* was blue colonies with associated gas bubbles.

Sensory Evaluation

Sensory-derived effects of peppermint spray to the participants from households (50 panelists) were evaluated by an acceptance test using a nine-point hedonic scale where 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely. The scale was used to determine their degrees of acceptance of the hand spray in terms of flavour, appearance, after use and overall liking.

Results and Discussion

Antimicrobial activity

The diameter of the zones of inhibition and the MICs and MBCs of the peppermint oil are shown in Table 1. These results indicate that the oil has strong antibacterial activity against all bacteria. Coliform was inhibited by peppermint oil with MIC of 450 μL/mL, which was significantly active against *Escherichia coli* and

Staphylococcus aureus (MIC 300 µL/mL). The MIC values ranged from 300 to 450 µL/mL. In most than cases, the MBC values of peppermint oil were about 10µL/mL higher than the MIC values. In this study, the MBC for *Escherichia coli* and *Staphylococcus aureus* were 310 µL/mL and 460 µL/mL for coliform.

The antimicrobial activities of the peppermint oil can be attributed to the presence of high concentrations of menthol, which is known to occur at very high concentrations in many plant oils, such as min oil [6]. The pharmacological actions of the peppermint oils are suggested to parallel their menthol contents [7]. It has also been suggested that minor components interact with the other components, not affecting the antimicrobial activities of the oils. It is possible that the activity of the main components is regulated alone. [8].

Peppermint oil spray for hand protection

Antibacterial activity of the peppermint oil spray (460 µL/mL) on hands has been summarised in Table 2. Data show differences between number of *E.coli* and coliform of the control and the treated hands, indicating that peppermint oil did completely remove all of the bacteria on hands.

Table 1. Antimicrobial activity of the peppermint oil.

Microorganism	Clear zone (mm)[a]	MIC[b]	MBC[c]
E. coli	10.03± 0.19	300	310
S. aureus	16.42±1.42	300	310
Coliform	10.87±0.49	450	460

[a] Diameter of inhibition zone including disc diameter of 6 (mm).
[b] MIC, minimum inhibitory concentration (as µL/mL).
[c] MBC, minimal bactericidal concentration (as µL/mL).

Table 2. Effectiveness of peppermint oil spray hand sanitizer.

Microorganism	No. of Participants	Before washing (+) results	Peppermint oil spray	Control (water)
			After washing (+) results	After washing (+) results
E. coli	50	21 (42%)	0 (0%)	18 (36%)
Coliform	50	17 (34%)	0 (0%)	15 (30%)

In the present study, peppermint oil sanitizer agents were tested to determine their efficiency in reducing microbial load on hands. As expected, the tested hand washing solutions showed higher antimicrobial activity compared to water washes. Significant differences in microbial reductions were observed after washing hands with peppermint oil

Sensory Evaluation

The average scores for all attributes of peppermint oil spray hand sanitizer are presented in Figure 1. It was observed that hand washing with peppermint oil at a concentration of 460 μL/mL produced an average score of 7.54 ± 0.95 on hedonic scale. Flavour, appearance and after use were scored as like very moderately or like very much (scores ≥ 7) for overall attributes.

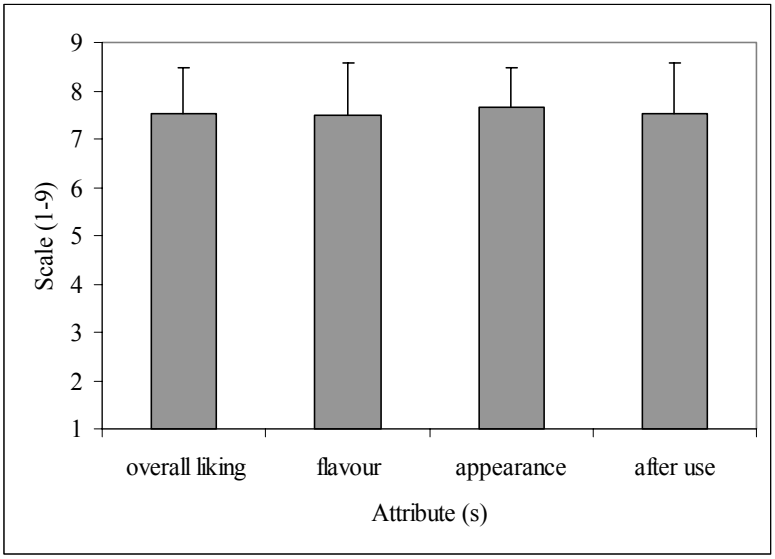

Figure 1. Sensory evaluation test of peppermint oil spray hand sanitizer.

Conclusions

Our study reports the effect of peppermint oil on *Escherichia coli*, *Staphylococcus aureus* and coliform species on agar. Both *Escherichia coli* and coliform were inhibited on hands. The spray hand sanitizer of peppermint oil appears to have good preventive treatment with regard to pathogens on hands and would have wider application in food safety.

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