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Antimicrobial Activity of a Blend of Essential Oils Extracted from Oregano and Cinnamon

Chaiting See and Ekachai Jenwitheesuk*

Department of Microbiology, Pacific Lab Services, 5 Woodlands Terrace, Singapore, 738430

***Corresponding author:** Ekachai Jenwitheesuk, Department of Microbiology, Pacific Lab Services, 5 Woodlands Terrace, Singapore, 738430; **E-mail:** ekachai.jenwitheesuk@pacificlab.com.sg

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Abstract

The antimicrobial property of essential oils extracted from oregano and cinnamon have been documented and widely reviewed. However, most of the *in vitro* studies determined the inhibitory activity based on a single essential oil and/or purified active compound. The aim of this study was to determine the overall inhibitory activity in terms of minimum inhibitory concentration (MIC) of a blend of oregano and cinnamon essential oils in a commercial product against pathogenic bacteria. The result showed that the blend of the essential oils inhibited *Escherichia coli, Escherichia coli O157:H7, Salmonella enteritidis, Salmonella typhimurium, Vibrio cholerae,* and *Vibrio parahaemolyticus* at MICs ranging from 31.25 to 250 mg/L. The MICs of the examined blend against *Bacillus cereus, Campylobacter jejuni, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were greater than 1,000 mg/L. This finding suggests that the examined blend of essential oils could, to some extent, inhibit *Escherichia coli*, *Salmonella*, and *Vibrio*, and could be therefore applicable for use as a bacteriostatic agent.

Keywords: Antimicrobial; Essential oils; Oregano; Cinnamon; Carvacrol; Thymol; Cinnamaldehyde, Livestock; Feed additives

Abbreviations: MIC-Minimum inhibitory concentration; CLSI-Clinical and Laboratory Standard Institute; ATCC-American Type Culture Collection; ppm-Parts Per Million; CFU: Colony forming unit

Background

Medicinal plants have been widely used as growth promoting agents in livestock production industry [1] as they possess a wide range of biological activities such as antioxidative, anti-inflammatory, and antimicrobial that positively affects livestock growth [2]. Essential oil of medicinal plants exerts intense antimicrobial activity against pathogenic bacteria [2]. *In vitro* inhibition of bacteria development by the essential oils has been extensively studied, and their antimicrobial activities have been confirmed [2-5].

Oregano and cinnamon have been used as growth promoting agents in livestock industry as they are among the medicinal plants well-known for their antimicrobial properties against animal pathogenic bacteria [1]. Essential oils of these plants are mainly responsible for this activity. Carvacrol and thymol in oregano, and cinnamaldehyde in cinnamon are the major active compounds in the essential oil of these plants [6]. Inhibitory activity in terms of minimum inhibitory concentration (MIC) of carvacrol, thymol and cinnamaldehyde against pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Vibrio*, etc., have been extensively reported [3-6]. Other compounds in the extracted essential oils of oregano and cinnamon have also been found to possess antimicrobial activity though these compounds are not the major components of the extract [7-12]. However, these studies determined the inhibitory activity based on a single essential oil and/or purified active compound, which may not represent the actual inhibitory activity of the essential oils in the commercial products. This study aimed to determine the inhibitory activity of a commercial product containing a blend of essential oils extracted from oregano and cinnamon to examine if the inhibitory profile against pathogenic bacteria is similar to that of the previous literature.

Methods

Essential oils blend

The blend of essential oils used in this study was a commercial feed additive product for livestock (Orecim; Zagro Singapore Pte Ltd., Singapore). The product contained 3.75% of oregano and 2.5% of cinnamon essential oils. These two essential oils provided three active compounds; carvacrol 3%, cinnamaldehyde 2% and thymol 0.06%. The working essential

oil solution was prepared by diluting the product 1:1000 (according to manufacturer's recommendation) with sterile deionized water. The working essential oil solution was then used for MIC determination against pathogenic bacteria.

Tested microbes

We determined the inhibitory activity of the examined blend of essential oils against nine strains of Gram negative and two strains of Gram positive bacteria. The bacterial names and their American Type Culture Collection (ATCC) reference numbers are listed in table 1.

MIC determination

The MIC was determined according to the broth micro dilution method described in the Fourth Edition of Clinical and Laboratory Standard Institute (CLSI) VET01-A4 [13]. In brief, the working essential oil solution was added to the 96-well plate, followed by two-fold dilution using sterile water. Bacterial suspension containing approximately 5×10^5 CFU/mL was prepared and added to the plates. The plates were sealed with plastic tape to prevent drying and then incubated at 37 °C for 24 hours. After the incubation was over, the plates were observed for bacterial growth inhibition. The MIC value was the concentration value of the examined blend of essential oils in the well that completely inhibited bacterial growth.

Results and Discussion

In this study, we determined the MIC of a blend of oregano and cinnamon essential oils against 11 pathogenic bacteria by taking into account major and minor active compounds as well as other substances such as excipients in the commercial product. The MICs of the examined blend of essential oils are shown in table 1. The MIC range varied; 31.25 mg/L for *Vibrio parahemolyticus*; 125 mg/L for *Vibrio cholerae, Salmonella enteritidis* and *Escherichia coli O157:H7*; 250 mg/L for *Escherichia coli* and *Salmonella typhimurium*; and >1000 mg/L for *Bacillus cereus, Campylobacter jejuni, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The MICs for *Escherichia coli, Salmonella* and *Vibrio* found in this study are consistent with previous reports [14]. However, high MICs of *Bacillus, Campylobacter, Klebsiella, Pseudomonas* and *Staphylococcus* in this study indicated that these bacteria were resistant to the blend of oregano and cinnamon essential oils, finding that is inconsistent with previous reports [15-17]. We speculate that high MICs of these bacteria were mainly due to the high dilution of working essential oils solution used for MIC determination. After 1:1000 dilution according to manufacturer's recommendation, the working essential oil solution contained only 0.00375% or 37.5 ppm oregano oil and 0.0025% or 25 ppm cinnamon oil concentration that are possibly not sufficient to effectively inhibit growth of these bacteria.

Table 1: The MICs of the examined blend of oregano and cinnamon essential oils extracted.

Microbes	ATCC reference number	MIC (mg/L)
Escherichia coli O157:H7	ATCC 43888	125
Escherichia coli	ATCC 25922	250
Salmonella enteritidis	ATCC 13076	125
Salmonella typhimurium	ATCC 14028	250
Vibrio parahaemolyticus	ATCC 17802	31.25
Vibrio cholerae	ATCC 51394	125
Campylobacter jejuni	ATCC 33291	>1,000
Klebsiella pneumoniae	ATCC 27736	>1,000
Pseudomonas aeruginosa	ATCC 15442	>1,000
Bacillus cereus	ATCC 11778	>1,000
Staphylococcus aureus	ATCC 29213	>1,000

However, high dilution of working essential oils solution did not explain the resistance of *Staphylococcus aureus* in this study. *Staphylococcus aureus* is usually used as a representative of Gram positive bacteria, while *Escherichia coli* is used as a representative of Gram negative bacteria in MIC determination of essential oils [5,18]. It is widely accepted that Gram positive are generally more sensitive to essential oils than Gram negative bacteria [19]. Nevertheless, the MICs of these two representatives in this study showed the opposite trend since *Escherichia coli* was inhibited at 125 mg/L while *Staphylococcus aureus* was not inhibited at 1000 mg/L. This is not uncommon since inconsistent results of the *in vitro* MIC of essential oils against pathogenic bacteria have been already reported [20]. The reported MICs vary among experiment because there are differences in species and parts of plant used for essential oils extraction, and other laboratory test conditions that prevent direct comparison of the MICs and inhibition patterns. However, we presume that these Gram positive and Gram negative bacteria would show similar inhibition patterns as previous reports when the working solution of essential oils blend is used at higher concentration. Mild *in vitro* inhibitory activity of the essential oils blend suggests that this type of product might be applicable for use as bacteriostatic agent that could be beneficial to livestock and aquaculture industries, where new sources of feed additives have been sought to replace antibiotic growth promoters. Essential oils might contribute in the control of *Escherichia coli* load in piglet gut during weaning period and alleviate diarrhea severity and mortality rate of the piglet. Essential oils might also help limit colonization of *Salmonella* in poultry gut, which could reduce shedding of *Salmonella* to the environment as poultry are considered one of the most important *Salmonella* reservoirs. Finally, essential oils might help control *Vibrio* load in shrimp's hepatopancreas and gut, where *Vibrio* colonizes, disrupts nutrient absorption, and causes cell death and tissue necrosis. Further efficacy trials in these animals would prove the economic benefits of essential oils blend for livestock and aquaculture industries.

Conclusion

This study determined the MIC values of essential oil blend against pathogenic bacteria. The *in vitro* inhibitory activity observed was the overall activity of carvacrol, thymol,

cinnamaldehyde, and other minor active compounds in the oregano and cinnamon extracts and other excipients in the product. The essential oils blend showed inhibitory activity, to some extent, against *Escherichia coli, Salmonella*, and *Vibrio*, which suggested that it might be applicable for use as bacteriostatic agent, especially as a feed additive to control pathogenic bacteria in gastrointestinal tract of livestock and shrimp.

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of supporting data

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CTS performed laboratory tests. EJ mainly carried out this study and drafted the manuscript. All authors read and approved the final manuscript.

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