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Antimicrobial Effect of Cinnamon Essential Oil Against *Escherichia Coli* and *Staphylococcus aureus*

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Background: Various studies have been conducted to determine the effects of essential oils and other natural antimicrobials on foodborne pathogens in culture media.
Objectives: The present study aimed to determine the antibacterial effects of cinnamon essential oil, monolaurin, nisin, and ethylenediaminetetraacetic acid (EDTA) alone and in combination, in culture media.
Materials and Methods: Cinnamon essential oil was analyzed by gas chromatography/mass spectrometry (GC/MS) and the major component was identified as cinnamaldehyde. Broth microdilution assay and agar disk diffusion method were used to evaluate the antibacterial effect of cinnamon essential oil, monolaurin, nisin, and EDTA alone and in combination against *Staphylococcus aureus* and *Escherichia coli.*
Results: The MIC of cinnamon essential oil, monolaurin, nisin, and EDTA for *S. aureus* was 3125.00, > 500.00, > 125.00, and > 250.00 μg/mL, respectively, while the MIC of the aforementioned materials for *E. coli* was 780.00, 31.25, 15.60, and 250.00 μg/mL. In the present study, *S. aureus* was found to be more sensitive than *E. coli* and monolaurin and nisin showed the lowest MIC for *E. coli.* Increased antimicrobial effect was observed when cinnamon essential oil was used in combination with nisin, monolaurin, and EDTA.
Conclusions: The present study showed that cinnamon essential oil when used in combination with nisin, monolaurin or EDTA demonstrated stronger antimicrobial effect against foodborne pathogens than when used alone.

Keywords: Nisin; Monolaurin; Antibacterial Effect; Cinnamon Essential Oil; EDTA

1. Background

Antimicrobial compounds are chemical or natural components, which have bactericidal effect or growth-inhibitory effect on microorganisms. The essential oils of aromatic plants are commonly used in food preservation and flavoring (1), such as cinnamon (*Cinnamomum zeylanycum* Boiss.), a member of the *Lauracaea* family that grows in southern Asia (2). Several reports have shown the promising effect of essential oils against several species of bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Bacillus cereus*, and *Salmonella typhimurium* (3-5). It is well documented that compounds that have phenolic groups are the most effective; thus, the oils of cinnamon, thyme, and rosemary have been found to be most effective against foodborne microorganisms (6, 7). The antimicrobial effect of cinnamon essential oil against various bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus fecalis*, *S. aureus*, *Salmonella* sp., and *Vibrio parahaemolyticus* have been reported (8, 9). Moreover, antioxidant (10), acaricidal (11), and insecticidal (12) effects of this essential oil are well established. Nisin is a well-known bactericide produced by *Lactococcus lactis* that is active against several Gram-positive pathogens (13). Chemical components such as monolaurin and ethylenediaminetetraacetic acid (EDTA) have been used widely for food preservation. Monolaurin, a monoester of lauric acid, is used in food production because of its flavoring and emulsifying effect and has been found to have antimicrobial properties against Gram-positive bacteria (14, 15). EDTA is the most widely used chelating agent with strong activity against the lipopolysaccharide layer of Gram-negative cells, making them sensitive to other antimicrobials such as nisin and lactoferrin (13, 16). In this study, different concentrations of cinnamon essential oil, monolaurin, and nisin were used alone and in combination to determine the antibacterial effect of these components on *E. coli* and *S. aureus*.

2. Objectives

The present study was conducted in 2013 by Faculty of Veterinary Medicine of Urmia University. The objective

was evaluation of antibacterial effects of natural antimicrobials such as cinnamon essential oil, nisin, monolaurin, and EDTA when used alone and in combination (essential oil + monolaurin, essential oil + nisin and essential oil + EDTA).

3. Materials and Methods

3.1. Preparation of Antimicrobials

Nisin (NisaplineTM) was dissolved in sterile 0.02 N dilute HCL, filter-sterilized, and kept at -18°C. Monolaurin was dissolved in ethanol and then filtered and fresh stock solution of monolaurin was prepared for each experiment. A stock solution of EDTA was prepared by dissolving disodium EDTA in sterile distilled water.

3.2. Microbial Strains

The microorganisms were obtained from the culture collection of the Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran. The bacteria used in this experiment were *E. coli* ATCC43894 and *S. aureus* ATCC6538.

3.3. Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The bark of *C. zeylanycum* was purchased from local grocery store and authenticated at Faculty of Agriculture, Urmia University, Urmia, Iran. Essential oil was obtained by hydrodistillation (3 hours) using a Clevenger-type collector. The essential oil was dehydrated using sodium sulfate and then filtered by 0.22-μm filters. The filtrate was stored in sealed dark vials at 4°C. The gas chromatograph was equipped with DB5 capillary column (30 × 0.25 mm ID × 0.25 μm film thickness). The data were acquired under the following conditions: initial temperature 50°C, final temperature 250°C, and ionization energy of 70 eV. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/minutes.

3.4. Agar Disk Diffusion Test

Agar disk diffusion assay was used for determination of the antimicrobial activity of cinnamon essential oil, nisin, and monolaurin. For this purpose, 0.1 mL of bacterial suspensions (10^7 cells per mL) was spread on nutrient agar plates and then filter paper disks (6 mm in diameter) were impregnated with 10 μL of the cinnamon essential oil and incubated at 37°C for 24 hours for both tested bacteria. The diameters of the inhibition zones were measured in mm. The same procedure was used for the determination of the antibacterial effect of nisin and monolaurin. All experiments were performed in triplicate.

3.5. Estimation of the Minimum-Inhibitory Concentration (MIC) Value of Cinnamon Essential Oil, Monolaurin, and Nisin

The MIC was determined using broth microdilution susceptibility assay. Briefly, two-fold serial dilutions of the antimicrobials were prepared in brain-heart infusion (BHI) broth. Final concentrations of antimicrobials used in this study were as follows: essential oil (5000.00, 2500.00, 1250.00, 625.00, 312.50, and 156.25 μg/mL), monolaurin (500.00, 250.00, 125.00, 62.5.00, 31.25, and 15.62 μg/mL), nisin (125.00, 62.50, 31.25, and 15.62 μg/mL), and EDTA (250.00 μg/mL).

BHI broth (160 μL) and 20 μL of the inoculum were added to each well of a 96-well microplate. A 20-μL aliquot from the stock solutions of each antimicrobial was added into each well, with the last well in each strip containing 180 μL of broth and 20 μL of the inoculum without any antimicrobial as negative control. The microplates were incubated in standard conditions (temperature 37°C for 24 hours). Thereafter, the absorbance of each well was read by microplate reader spectrophotometry (Biotek Instrument Inc., USA). The MIC was defined as the lowest concentration of the antimicrobial at which the microorganism did not show visible growth. The combined effect of the antimicrobials was studied as follows: 140 μL of BHI broth, 20 μL of the inoculums, and 40 μL aliquot from the stock combination solutions (20 μL of essential oil + 20 μL of monolaurin, 20 μL of essential oil + 20 μL of nisin, and 20 μL of essential oil + 20 μL of EDTA) were added into wells and the MIC was determined as described above. All experiments were conducted in triplicate.

3.6. Statistical Analysis

The data were analyzed using SPSS version 19 software and one way ANOVA was performed for analysis comparison between groups' variances. A P value less than 0.05 was considered statistically significant. The results are expressed as Mean ± Standard Deviations (SD) of triplicate measurements.

4. Results

4.1. Chemical Composition of the Essential Oil

The yield of the essential oil based on the dry weight of the cinnamon barks was determined to be 1%, and 14 components were identified in the essential oil representing 94.25% of the total oil. The results of GC-MS analysis of the cinnamon essential oil are presented in Table 1. The major component was cinnamaldehyde (79.74%). Other components such as trans-calamenene, borneol, benzaldehyde, and cinnamyl acetate were also found in low amounts.

4.2. Antimicrobial Activity of Essential Oil, Monolaurin, Nisin, and EDTA

The antimicrobial activity of essential oil, monolaurin, nisin, and EDTA was determined using broth microdilution susceptibility test and agar disk diffusion assay against *E.*

coli and *S. aureus* (Tables 2, 3, and 4). The MIC value of the essential oil for both *E. coli* and *S. aureus* was 2500.00 μg/mL while the MBC value of the essential oil against these bacteria was 625.00 μg/mL. As shown in Table 2, both the MIC and MBC values of monolaurin against *E. coli* were > 500 and those against *S. aureus* were 31.25 μg/mL. In the case of nisin, the MIC values of nisin against *E. coli* and *S. aureus* were > 125.00 and 15.62 μg/mL, respectively.

According to Table 3, using monolaurin, nisin and EDTA in combination with essential oil, reduced the MIC values of essential oil against both Gram-positive and negative bacteria. For instance, using 500.00 μg/mL of monolaurin together with essential oil, reduced the MIC values of essential oil two-fold for *E. coli* and *S. aureus*. Nisin also, reduced the MIC values of essential oil against both tested bacteria two fold.

Table 4 represents the results of agar disk diffusion assay for essential oil, monolaurin, nisin and combinations of essential oil + monolaurin, essential oil + nisin, and essential oil + EDTA. The inhibition zones of essential oil for *E. coli* and *S. aureus* were 21.7 ± 0.3 and 28.5 ± 0.6 mm. combination of essential oil with monolaurin had no significant difference with the results of essential oil alone but using nisin in combination with essential oil increased inhibition zones in comparison with using essential oil and nisin alone ($P < 0.05$). Essential oil alone had significantly higher ($P < 0.05$) effect on both Gram-positive and negative bacteria than nisin and monolaurin alone.

Table 1. Chemical Composition of *Cinnamomum Zeylanycum* Boiss. Essential Oil

Number	Compounds	KI [a]	%
1	Benzaldehyde	960	1.71
2	Borneol	1169	1.73
3	Cinnamaldehyde	1270	79.74
4	α copaene	1377	1.31
5	Cinnamyl acetate	1446	1.58
6	Gamma muurolene	1480	0.53
7	Curcumene	1481	0.45
8	α Muurolene	1500	1.62
9	Trans-calamenene	15.29	2.62
10	2-Propenal,3-2-methoxyphenyl	1550	1.21
11	(epi-α) Cadinol	1640	0.78
12	α-Muurolol	1646	0.47
13	Cadalene	1677	0.21
14	(epi-α) Bisabolol	1685	0.29
--	Total	--	94.25

[a] Kovats retention indices.

Table 2. Minimum Inhibition Concentrations (μg/mL) and Minimum Bactericidal Concentrations (μg/mL) for Cinnamon Essential Oil, Monolaurin, Nisin, and EDTA [a]

Bacteria	MIC				MBC			
	ML	EO	Nisin	EDTA	ML	EO	Nisin	EDTA
E. coli	> 500.00	2500.00	> 125.00	> 250.00	> 500.00	2500.00	> 125.00	> 250.00
S. aureus	31.25	625.00	15.62	250.00	31.25	625.00	31.25	> 250.00

[a] Abbreviations: ML: monolaurin, EO: Cinnamon essential oil, EDTA: Ethylenediaminetetraacetic acid.

Table 3. Minimum-Inhibitory Concentrations (μg/mL) and Minimum-Bactericidal Concentrations (μg/mL) of Combination Of Cinnamon Essential Oil, Monolaurin, Nisin, and EDTA [a]

Bacteria	MIC			MBC		
	EO + ML	EO + Nisin	EO + EDTA	EO + ML	EO + Nisin	EO + EDTA
E. coli	625.00 + 500.00	625.00 + 62.50	1250.00 + 250.00	1250.00 + 500.00	1250.00 + 62.50	1250.00 + 250.00
S. aureus	156.25 + 15.62	156.25 + 31.25	312.50 + 250.00	156.25 + 15.62	156.25 + 62.50	1250.00 + 250.00

[a] Abbreviations: ML: monolaurin, EO: Cinnamon essential oil, EDTA: Ethylenediaminetetraacetic acid.

Table 4. Antibacterial Properties of Cinnamon Essential Oil, Monolaurin, and Nisin, and Their Combinations Using Agar Disk Diffusion Method [a,b,c]

Bacteria	EO	ML	Nisin	EO + ML	EO + Nisin	EO + EDTA
	10 μL (5000 μg/mL)	10 μL (1000 μg/mL)	10 μL (500 μg/mL)	10 μL (2500 μg/mL + 500 μg/mL)	10 μL (2500 μg/mL + 250 μg/mL)	10 μL (2500 μg/mL + 250 μg/mL)
E. coli	21.7 ± 0.3A	6.6 ± 0.6B	8.3 ± 0.2B	22.3 ± 0.9A	26.5 ± 0.4C	18.2 ± 0.4D
S. aureus	28.5 ± 0.6A	11.8 ± 0.3B	15.2 ± 0.5C	32.8 ± 0.8D	33.8 ± 0.4D	23.4 ± 0.3E

[a] Diameter of inhibition zone.
[b] Different letter subscripts on the same row are significantly different ($P < 0.05$).
[c] Abbreviations: ML: monolaurin, EO: Cinnamon essential oil, EDTA: Ethylenediaminetetraacetic acid.

5. Discussion

The results of the GC/MS analysis of the cinnamon essential oil showed that cinnamaldehyde was the major component of this essential oil. According to studies carried out by several investigators, the major component of cinnamon essential oil was cinnamaldehyde in the range of 44% - 97% (7, 17, 18). Marongiu et al. (2007) (19) and Fei et al. (2011) (2) reported that the main component of cinnamon essential oil was trans-cinnamaldehyde (77.1% and 77.3%, respectively). These findings are in agreement with the results of our study, since the concentration of cinnamaldehyde was found to be 79.74% in our study.

Results of MIC determination in this study revealed that cinnamon essential oil had antibacterial effect against both *E. coli* and *S. aureus*. The antimicrobial activity of cinnamon essential oil may be due to the presence of high concentration of cinnamaldehyde but in our opinion, low amount components such as Benzaldehyde and Borneol had moderate activity on bacteria tested too. As shown in Table 2, cinnamon essential oil showed higher antimicrobial activity against *S. aureus* than *E. coli*. The higher resistance of Gram-negative bacteria than Gram-positive bacteria to essential oils is possibly owing to differential membrane structure of these bacteria. Gram-negative bacteria have an outer phospholipid membrane that acts as a barrier and renders the membrane impermeable to lipophilic constituents (19, 20). In Gram-positive bacteria, direct contact between hydrophobic components and the phospholipid layer of the cell membrane occurs owing to lack of an outer phospholipid membrane. This results in increased ion permeability, deterioration of protein components such as enzymes, and excretion of the intracellular constituents (20-22). Our results showed that individual administration of nisin and monolaurin alone had a limited effect on *E. coli* but *S. aureus* was more susceptible. Antimicrobials, such as nisin and monolaurin showed higher antimicrobial activity against Gram-positive bacteria than that against Gram-negative bacteria; we believe this is because in Gram-positive bacteria, the outer membrane is made of peptidoglycan but in Gram-negative bacteria, the peptidoglycan layer lies between the plasma membrane and a lipopolysaccharide outer membrane. Hence, antimicrobials cannot pass through the outer layer of Gram-negative bacteria easily (13); Therefore, using these antimicrobials in combination with cinnamon essential oil showed better effects on tested bacteria especially *E. coli*. EDTA is known as a chelating agent and using it in combination with cinnamon essential oil enhanced antimicrobial property of essential oil because EDTA chelated Ca and Mg salts required for binding LPS to the cell wall and consequently released LPS from the membrane of Gram-negative bacteria (23).

The inhibitory effect of cinnamon essential oil, nisin, monolaurin, and EDTA, as evaluated by agar disk diffusion method is presented in Table 4. The results obtained from this test confirmed the results of broth microdilution susceptibility tests. Gram-positive bacteria were found to be more sensitive than Gram-negative bacteria and *S. aureus* had larger inhibition zone than *E. coli* in all conditions of experiments. In general, results obtained from this study showed that cinnamon essential oil, monolaurin, and nisin were more effective against *S. aureus* than that against *E. coli*.

In conclusion, cinnamon essential oil, monolaurin and nisin alone showed proper effect on *S. aureus*. Results obtained from our study demonstrated that combination of nisin or monolaurin with cinnamon essential oil could increase the effects of these antimicrobials against both *S. aureus* and *E. coli*, reducing undesirable organoleptic effects of essential oils in food products.

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Authors' Contributions

Study concept and design: Hossein Tajik and Mojtaba Raeisi; data analysis and interpretation: Mojtaba Raeisi and Sirvan Sanginabadi; manuscript drafting: Mojtaba Raeisi and Arman Yarahmadi; revisions of the manuscript Hossein Tajik.

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