

ORIGINAL ARTICLE

Evaluation of Antimicrobial Effect of *Cinnamomum verum* Methanolic Extract and Essential Oil: A Study on Bio-preservative in Ketchup Sauce

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KEYWORDS

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Bio-preservative;

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Methanolic extract

ABSTRACT: Use of natural antimicrobial compounds extracted from plants such as cinnamon as preservative to extend the shelf life has gain much attention. In this study, we evaluated the antimicrobial activity and bio- preservative potential of the methanolic extract of *Cinnamomum verum* bark and its oil against *Bacillus subtilis*, *Pseudomonas aeruginosae* and *Escherichia coli* in ketchup sauce. In order to evaluate the Minimum Inhibitory Concentration (MIC) of essential oil and methanolic extract from the bark of *C. verum*, agar dilution method was performed. Then the effective inhibitory concentrations were evaluated on growth of test bacteria in ketchup sauce at 4 °C and room temperature in different storage times (1, 7, 14 and 30 days). In addition, the sensory quality of treated ketchups was assessed. This study showed bacteriostatic effect of the essential oils and methanolic extract on all tested bacteria. The best treatment in ketchup sauce was obtained on days 14 at 4 °C in concentrations of 1500 µg/ml essential oil. In sensory evaluation, the sample containing 1000 µg/ml essential oil had higher score in odor, taste and overall acceptability than other treated samples ($P<0.05$). This study shows that cinnamon oil is a more potent antimicrobial agent than cinnamon extract and it has the potential to be used as food preservative and is recommended for quality attributes enhancement.

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INTRODUCTION

The microbial food safety is a fundamental concern to consumers, regulatory officials, food industries and public health agencies throughout the world. To retard the spoiling of foodstuffs by microorganisms, antimicrobial substances such as artificial preservatives are used which inhibit, delay or prevent the growth and proliferation of bacteria, yeasts and moulds [1].

Certain preservatives, including sulfite and sodium benzoate are applied to limit the growth of bacteria in different kinds of food and they are generally recognized as safe. In spite of fact that, they may cause adverse effects in a small population who are sensitive such as potentially deadly allergic reactions, asthma, hives, low blood pressure, diarrhea, and flushing, abdominal pains and also may increase the risk of leukemia and other types of cancer [2]. Therefore, the recent trend has focused on use of natural preservatives, which requires the exploration of alternative sources of safe, effective and economical. Plants provide a rich source of novel compounds with antimicrobial properties. The antimicrobial activity of plant oils and extracts has been proved [3-5]. Therefore, application of plant extracts and oils with known antimicrobial properties can pave the way for improving food safety.

Bioactive compounds with definite effect on microbiological, chemical and sensory quality of foods are the major value of some plants that have drawn attention toward application of them as bioreservative in food industry. The bark of various cinnamon species is one of the most important spices applied all over the world for cooking and in traditional and modern medicines. Approximately 250 different species have been identified among the cinnamon genus and have been found worldwide. *Cinnamomum verum* called "true cinnamon" is one of the several *Cinnamomum* species commercially considerable. Several studies on *C. verum* reported that cinnamon essential oils have inhibitory

activity against microorganisms such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella choleraesuis* [6-8].

The most important constituents of cinnamon essential oil are cinnamaldehyde and *trans*-cinnamaldehyde that have effective role in fragrance and various biological activities of cinnamon [9, 10]. In addition, the extract of cinnamon bark has been reported to have potent antimicrobial and antioxidant properties. Methanolic extract of cinnamon bark reduced the levels of *E.coli* in apple juice [11]. Moreover, the artificially inoculation of *L. monocytogenes* in pasteurized apple juice with 0.1–0.3% (w/v) of ground cinnamon resulted to bacterial count reduction after 1 h of incubation, and no further growth of bacteria during 7 days of storage [12].

Phytochemical analysis indicated that methanol extract of cinnamon is a rich source of alkaloids, flavonoids, tannins, phenols and saponins [13]. Due to the reported antimicrobial properties of cinnamon and its popularity and acceptable flavor in foods therefore, the present study was aimed to investigate potential antimicrobial activity of *C. verum* essential oil and methanolic extracts in inhibiting the microbial growth in ketchup sauce. The specific objectives of this study were 1) to test the antibacterial activity of *C. verum* against *Bacillus subtilis*, *Pseudomonas aeruginosae* and *Escherichia coli* and 2) to evaluate the bioreservative and organoleptic properties of cinnamon essential oil and extract were added to ketchup sauce inoculated with mentioned bacteria.

MATERIALS AND METHODS

Plant preparation and extraction procedure

The fresh barks of *C. verum* were prepared from the local markets in Tehran, Iran and air dried for about one week and ground into fine powder using a mechanical

grinder. The essential oil was extracted by hydro-distillation method using a Clevenger apparatus. For this mean, 200 g of the ground barks was heated with 1 liter distilled water by Clevenger apparatus. The solution was subsequently shaken and was filtered using Whatman filter paper. The obtained essence oil was dehumidified using the sodium sulfate and it was stored at 4 °C in amber colored bottles [14]. For methanolic extracting, 200 g of dried ground barks weighted into 800 ml of methanol (95%) in conical flasks. The flask, covered with aluminum foil, was shaken every 30 mins, for 6 h and then allowed to stand for approximately 48 h. The residue was re-extracted under the same condition for three times. The result solution was subsequently shaken and was filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator at room temperature. The extract was stored at 4 °C in amber colored bottles [15].

Determining essential oil and methanolic extract yield

The essence and methanolic extract yield was calculated as weight /weight ratio (w/w) by using dry fresh barks of *C. verum* weight used in extraction process and the weight of pure essence and methanolic extract [16].

Bacterial inoculation and growth media

Three genera of bacteria, *Bacillus subtilis* PTCC1156, *P. aeruginosa* PTCC1430 and *E. coli* PTCC1533 obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran) are applied in this experiment. The bacterial cultures were maintained in CASO agar (Soybean Casein digest Agar) (Sigma-Aldrich, Missouri, USA) slants at 37 °C.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MICs) of essence oil and methanolic extract of this plant was determined using agar dilution method recommended by the CLSI with some modifications [17]. Briefly, serial dilutions of the essence oil (500, 1000, 2000, 4000, 8000, 16000 µg/ml) and methanolic extract (62.5, 125, 250, 500, 1000, 200, 4000 µg/ml) were prepared in CASO agar media at 45 °C in a ratio of 1:20. Plates containing only 5% (V/V) DMSO (Dimethyl sulfoxide) and methanol were used as control. Then 2-3 µL of test bacteria strains were spotted on the surface of agar plates containing different concentrations of plant extracts. The cell densities were adjusted to 0.5 McFarland standards ($1-1.5 \times 10^8$ cells/ml) at 530 nm wavelength using a spectrophotometric method. Agar plates were incubated at 37 °C and the MIC of bacteria was read in µg/ml after 24 h of incubation. The MIC was defined as the lowest concentration of essential oil and methanolic extract in a plate with no visible growth. All experiments were made in triplicate.

Treatment of ketchup sauce

Ketchup sauce was made under aseptic condition according to manufacturer formulation (Table 1) and it was divided into equal batches to test the antimicrobial efficiency of *C. verum* extract and essential oil. According to the results of previous step, MIC dilutions, as well as $1.5 \times \text{MIC}$, of plant extract and essential oil that had been shown more antimicrobial inhibition against test bacteria were prepared in ketchup sauce separately. Then a bacterial culture cocktail (*E. coli* and *P. aeruginosae*) was added to each dilution to the final cell densities of 1.5×10^8 cells/ml. The samples were stored at 4 °C and 25 °C for 30-day incubation period. They were checked daily for bacterial growth. The samples without extract and essential oil were kept as

control at the above-mentioned temperatures. Besides, for individually enumeration of test bacteria, *E.coli* and

P. aeruginosae, specific culture media, EMB agar and cetrimide agar have been considered, respectively.

Table 1. Manufacture formulation of ketchup sauce

Ingredients	wt (% w/w)
Tomato puree	20
Sucrose	13
Vinegar	8
Glucose syrup	7.5
Salt	2
Mixed spice	0.15
Modified starch	1.5
Xanthan	0.4
Guar	0.18
Total	100

Legend- wt% means weight percent which is sometimes written as w/w

Sensory analysis

Sensory evaluation of Ketchup sauce treated with MIC level and $1.5 \times$ MIC level of Cinnamomum essence and methanolic extract and control was carried out by a group of 10 semi trained panelists using 5- point hedonic scale within the range of 1 to 5 and asked to evaluate odor, taste and total acceptability of the Ketchup samples on a scale from 5 to 1 indicating decreasing taste. The data from the 10 panelists were pooled and the mean values and standard deviations were determined [18].

STATISTICAL ANALYSIS

Data were statistically analyzed using SPSS 23.0 software (Chicao, IL, USA). For this purpose, analysis of variance (ANOVA) was used and differences among the means were determined using least significant differences (LSD) test (by SAS software) at $P \leq 0.05$.

The data are presented as mean \pm standard deviation of the three determinations.

RESULTS

Minimum Inhibitory Concentration (MIC) of C. verum methanolic extract and essential oil

Table 2 and 3 show the MIC of *C. verum* extract and essential oil, respectively, for the test bacteria. Both extract and essential oil have inhibitory effect against three tested bacteria, *B. subtilis* PTCC1156, *P. aeruginosa* PTCC1430 and *E. coli* PTCC1533. The MIC of *C. verum* extract for *B. subtilis*, *P. aeruginosae* and *E. coli* was 500, 100, 250 $\mu\text{g/ml}$, respectively. Therefore, the lowest MIC was for *E. coli* with 200 $\mu\text{g/ml}$ and highest MIC was for *P. aeruginosae* with 1000 $\mu\text{g/ml}$ (Table 2). The MIC of *C. verum* essence for *E. coli*, 4000 $\mu\text{g/ml}$ and for *B. subtilis*, *P. aeruginosae* was determined 8000 $\mu\text{g/ml}$ (Table 3).

Table 2. Antimicrobial activity of different concentrations ($\mu\text{g/ml}$) of *C. verum* essential oil against *E. coli*, *P. aeruginosae*, *B. subtilis* through agar dilution method

Microorganisms	62.5 $\mu\text{g/ml}$	125 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	2000 $\mu\text{g/ml}$	4000 $\mu\text{g/ml}$	Negative control (DMSO)	Control of microbial growth
<i>E. coli</i>	+	+	-	-	-	-	-	+	+
<i>P. aeruginosa</i>	+	+	+	+	-	-	-	+	+
<i>B. subtilis</i>	+	+	+	-	-	-	-	+	+

Legend- + No inhibition, - Inhibition, DMSO: Dimethyl sulfoxide

Table 3. Antimicrobial activity of different concentrations ($\mu\text{g/ml}$) of *C. verum* methanolic extract against *E. coli*, *P. aeruginosae*, *B. subtilis* through agar dilution method

Microorganisms	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	2000 $\mu\text{g/ml}$	4000 $\mu\text{g/ml}$	8000 $\mu\text{g/ml}$	16000 $\mu\text{g/ml}$	Negative control (Methanol)	Control of microbial growth
<i>E. coli</i>	+	+	+	-	-	-	+	+
<i>P. aeruginosa</i>	+	+	+	+	-	-	+	+
<i>B. subtilis</i>	+	+	+	+	-	-	+	+

Legend-- + No inhibition, - Inhibition

Evaluation of antimicrobial activity of C. verum methanolic extract and essential oil in ketchup sauce

The \log_{10} cfu/g of bacterial total counts as individual culture and mixed culture in treated ketchup samples are showed in Table 4, 5, respectively. The antimicrobial effect of different concentrations of *C. verum* methanolic extract and essential oil on total count of test bacteria is depicted in Figure 1 (A-F). Small decrease of bacterial counts with the prolongation of the storage period was observed in control sample with no significant difference. In contrast, the \log cfu/g of mixed culture count in all treated ketchup samples compared to control showed a progressively decrease ($P < 0.05$) in microbial population during days 1, 7, 14, 30 at 4°C and 25°C. In sample treated with 1000 $\mu\text{g/ml}$ essential oil kept at 25°C, bacterial count (8.17 \log cfu/g) decreased to 3.61 \log cfu/g after one day and on day 30 the number of bacteria reached to less than 0.9 \log cfu/g. There were significant difference ($P < 0.05$) of bacterial total counts

among days 1, 7, 14 and 30 of storage. In sample containing 1500 $\mu\text{g/ml}$ essential oil microbial population was 2.86 \log cfu/g on day1 and it reached to less than 0.95 \log cfu/g after 14 days? No significant difference ($P > 0.05$) in bacterial count was observed from day14 to day 30. Therefore, the sample contained 1500 $\mu\text{g/ml}$ of essential oil was found to be more effective and statistically higher than the other treatment. Similar results were recorded by treatment of ketchup sauce with 8000 $\mu\text{g/ml}$ and 12000 $\mu\text{g/ml}$ methanolic extract during storage time at 25°C, but *C. verum* essential oil was more effective than its methanolic extract in reducing bacterial total.

Count under mentioned condition in treated samples. The same treatments were conducted at 4 °C. Although according to statistical analysis no significant different was observed between treatments in different storage temperatures (4 °C, 25 °C), antimicrobial activity of essential oils and methanolic extract were benefited by decreasing temperature.

Table 4. Antimicrobial effect of different concentrations ($\mu\text{g/ml}$) of *C.verum* essential oil and methanolic extract on total count of bacterial culture cocktail (*E. coli* and *P. aeruginosae*) in ketchup sauce on days 1, 7, 14, 30 at 4 °C and 25 °C

Temperature	Concentration	Bacterial count (\log_{10} CFU/g \pm SD) in ketchup samples ^A			
		After 1 day	After 7 days	After 14 days	After 30 days
25 °C	Control	7.00 \pm 0.04 ^a	6.89 \pm 0.11 ^a	6.80 \pm 0.14 ^a	6.56 \pm 0.24 ^a
	1000(MIC)	3.61 \pm 0.18 ^{bc}	2.95 \pm 0.05 ^{bc}	2.19 \pm 0.20 ^{bc}	0.90 \pm 0.05 ^{bc}
	1500(1.5 \times MIC)	2.86 \pm 0.24 ^{bd}	1.76 \pm 0.15 ^{bd}	0.95 \pm 0.10 ^{bd}	0.76 \pm 0.15 ^{bd}
	8000(MIC)	3.77 \pm 0.07 ^{bc}	3.16 \pm 0.15 ^{bc}	2.56 \pm 0.24 ^{bc}	1.90 \pm 0.05 ^{bc}
	12000(1.5 \times MIC)	3.00 \pm 0.04 ^{bd}	2.56 \pm 0.09 ^{bd}	1.63 \pm 0.31 ^{bd}	0.83 \pm 0.13 ^{bd}
4 °C	Control	6.92 \pm 0.08 ^a	6.84 \pm 0.03 ^a	6.69 \pm 0.09 ^a	6.26 \pm 0.24 ^a
	1000(MIC)	3.39 \pm 0.36 ^{bc}	2.86 \pm 0.12 ^{bc}	1.96 \pm 0.24 ^{bc}	0.95 \pm 0.10 ^{bc}
	1500(1.5 \times MIC)	2.81 \pm 0.07 ^{bd}	1.67 \pm 0.19 ^{bd}	0.95 \pm 0.10 ^{bd}	0.90 \pm 0.05 ^{bd}
	8000(MIC)	3.59 \pm 0.11 ^{bc}	2.96 \pm 0.24 ^{bc}	2.39 \pm 0.09 ^{bc}	1.67 \pm 0.19 ^{bc}
	12000(1.5 \times MIC)	2.85 \pm 0.00 ^{bd}	1.90 \pm 0.00 ^{bd}	0.95 \pm 0.05 ^{bd}	0.90 \pm 0.05 ^{bd}

Legends- ^{a-d}Means in the same column for each concentration followed by different superscript letters (a-d) differ significantly ($P < 0.05$). ^A Results are expressed as log CFU/ml (mean \pm SD). MIC: Minimum Inhibitory Concentration

Table 5. Antimicrobial effect of different concentrations ($\mu\text{g/ml}$) of *C.verum* essential oil and methanolic extract on total count of *E.coli* and *P.aeruginosae*, individually, in ketchup sauce on days 1, 7, 14, 30 at 4°C and 25°C

Temperature	Concentration	Bacterial count (\log_{10} CFU/g \pm SD) in ketchup samples ^A							
		After 1 day		After 7 days		After 14 days		After 30 days	
		<i>E. coli</i>	<i>P. aeruginosae</i>	<i>E. coli</i>	<i>P. aeruginosae</i>	<i>E. coli</i>	<i>P. aeruginosae</i>	<i>E. coli</i>	<i>P. aeruginosae</i>
25 °C	Control	5.74 \pm 0.04 ^a	4.90 \pm 0.05 ^a	5.46 \pm 0.15 ^a	4.74 \pm 0.04 ^a	5.11 \pm 0.10 ^a	4.47 \pm 0.07 ^a	4.84 \pm 0.06 ^a	3.99 \pm 0.09 ^a
	1000 (MIC)	2.14 \pm 0.06 ^{bc}	2.63 \pm 0.01 ^{bc}	1.77 \pm 0.07 ^{bc}	1.90 \pm 0.08 ^{bc}	0.90 \pm 0.05 ^{bc}	0.90 \pm 0.05 ^{bc}	0.90 \pm 0.05 ^{bc}	0.90 \pm 0.05 ^{bc}
	1500 (1.5 \times MIC)	1.74 \pm 0.04 ^{bd}	2.00 \pm 0.04 ^{bd}	0.84 \pm 0.06 ^{bd}	0.84 \pm 0.06 ^{bd}	0.85 \pm 0.00 ^{bc}	0.80 \pm 0.08 ^{bc}	0.85 \pm 0.00 ^{bc}	0.85 \pm 0.00 ^{bc}
	8000(MIC)	2.39 \pm 0.36 ^{bc}	2.77 \pm 0.11 ^{bc}	1.89 \pm 0.11 ^{bc}	2.17 \pm 0.06 ^{bc}	0.85 \pm 0.00 ^{bc}	0.85 \pm 0.00 ^{bc}	0.85 \pm 0.00 ^{bc}	0.85 \pm 0.00 ^{bc}
	12000(1.5 \times MIC)	1.84 \pm 0.09 ^{bd}	2.08 \pm 0.07 ^{bd}	0.90 \pm 0.05 ^{bd}	0.78 \pm 0.00 ^{bd}	0.78 \pm 0.00 ^{bc}	0.78 \pm 0.00 ^{bc}	0.78 \pm 0.00 ^{bd}	0.75 \pm 0.05 ^{bd}
4 °C	Control	5.29 \pm 0.11 ^a	4.86 \pm 0.15 ^a	4.89 \pm 0.11 ^a	4.59 \pm 0.11 ^a	4.64 \pm 0.15 ^a	4.30 \pm 0.24 ^a	4.37 \pm 0.19 ^a	3.95 \pm 0.05 ^a
	1000(MIC)	2.00 \pm 0.04 ^{bc}	2.47 \pm 0.07 ^{bc}	1.44 \pm 0.24 ^{bc}	1.82 \pm 0.20 ^{bc}	0.90 \pm 0.05 ^{bc}	0.95 \pm 0.00 ^{bc}	0.84 \pm 0.06 ^{bc}	0.90 \pm 0.00 ^{bc}
	1500(1.5 \times MIC)	1.51 \pm 0.20 ^{bd}	1.76 \pm 0.15 ^{bd}	0.84 \pm 0.06 ^{bd}	0.77 \pm 0.07 ^{bd}	0.78 \pm 0.00 ^{bc}	0.82 \pm 0.04 ^{bc}	0.78 \pm 0.00 ^{bc}	0.70 \pm 0.00 ^{bd}
	8000(MIC)	2.43 \pm 0.05 ^{bc}	2.69 \pm 0.09 ^{bc}	1.67 \pm 0.19 ^{bc}	1.89 \pm 0.11 ^{bc}	0.90 \pm 0.05 ^{bc}	0.90 \pm 0.00 ^{bc}	0.90 \pm 0.05 ^{bc}	0.85 \pm 0.00 ^{bc}
	12000(1.5 \times MIC)	1.67 \pm 0.19 ^{bd}	1.99 \pm 0.09 ^{bd}	0.78 \pm 0.00 ^{bd}	0.85 \pm 0.00 ^{bd}	0.78 \pm 0.00 ^{bc}	0.80 \pm 0.04 ^{bc}	0.78 \pm 0.00 ^{bc}	0.78 \pm 0.00 ^{bd}

Legends- ^{a-d}Means in the same column for each concentration followed by different superscript letters (a-d) differ significantly ($P < 0.05$).

^A Results are expressed as log CFU/ml (mean \pm SD). MIC: Minimum Inhibitory Concentration

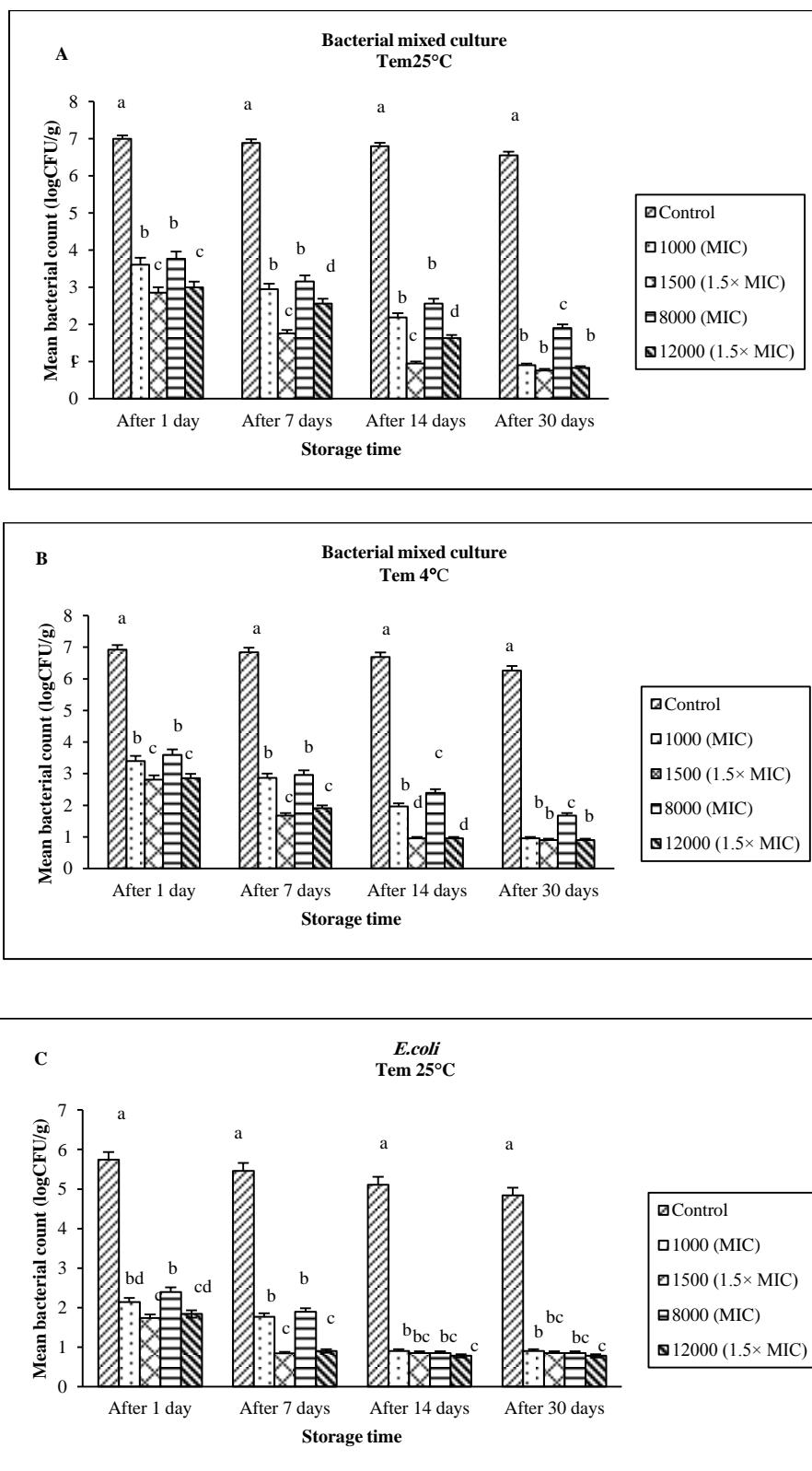


Figure 1 (A-F). The antimicrobial effect of different concentrations ($\mu\text{g/ml}$) of *C. verum* methanolic extract and essential oil on total count of a bacterial mixed culture and *E.coli* and *P. aeruginosae* individually in Ketchup sauce on days 1, 7, 14, 30 at 4°C and 25 °C. MIC: Minimum Inhibitory Concentration. a-d Means in the same column for each concentration followed by different superscript letters (a-d) differ significantly ($P < 0.05$).

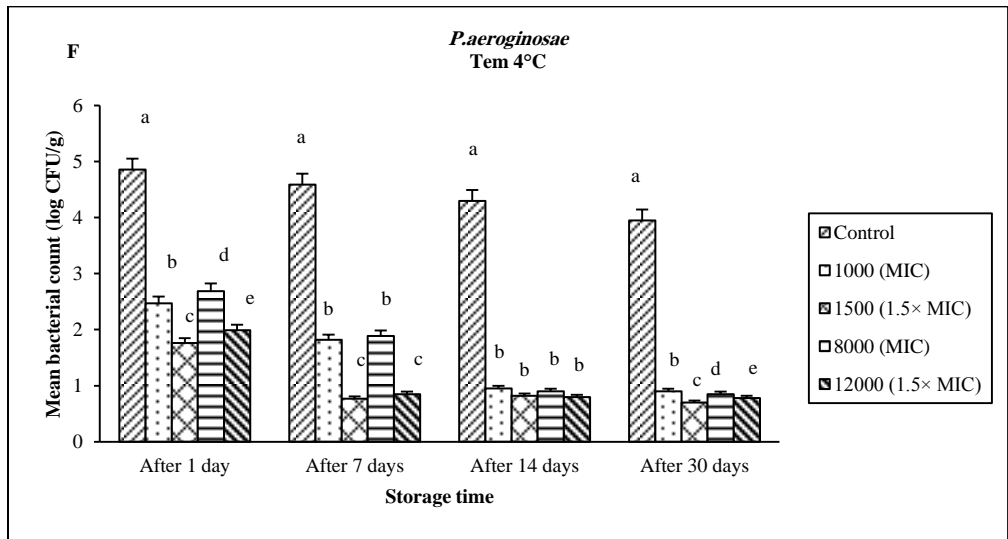
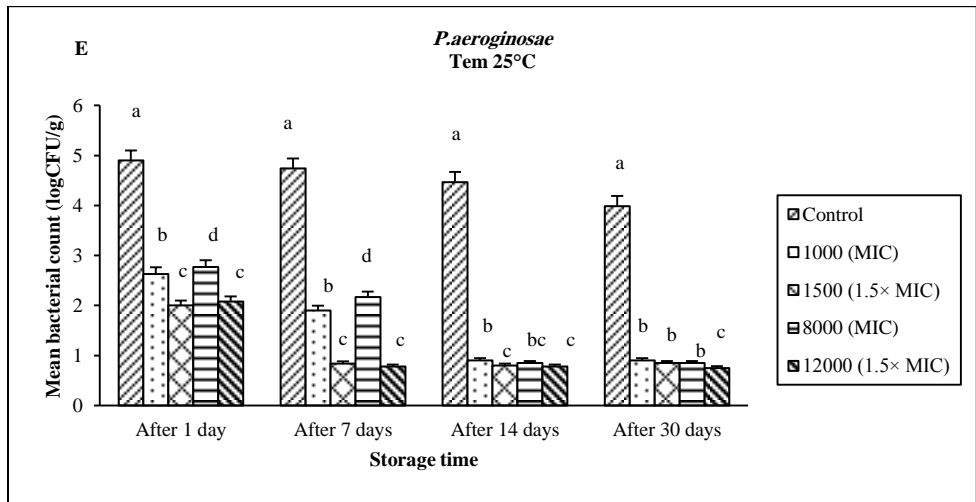
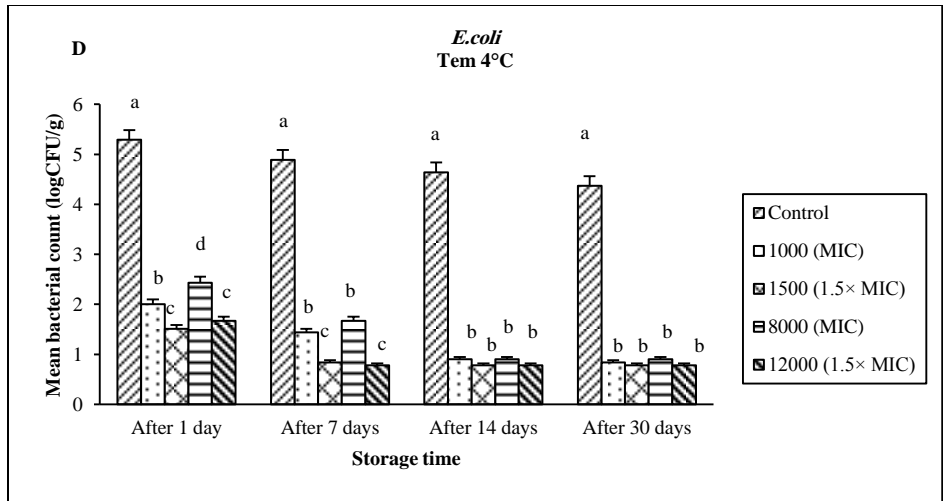


Figure 1 .Continued

Sensory attributes of treated ketchup sauce with *C.verum* essential oil and methanolic extract (Organoleptic Characters)

Organoleptic evaluation values of ketchup sauce containing essential oil and methanolic extract of *C. verus* in terms of odor, taste and overall acceptance were carried out by 10 panelists and the mean scores of sensory analysis were illustrated in Table 6 and Figure 2. Results indicated that the ketchup sauce containing 1000 µg/ml *C. verus* essential oil had better taste, odor and total acceptability in comparison with sample containing 1500 µg/ml essential oil and showed significant difference ($P \leq 0.05$) with control. With

respect to the samples containing methanolic extract, data showed that the 8000 µg/ml methanolic extract resulted in the enhancement of sensory quality (taste, odor and total acceptance) in treated ketchup sauce but on the contrary the sample with 12000 µg/ml methanolic extract did not significantly affect odor, taste and overall acceptance and did not show significant difference with control. Therefore, regarding the sensory attributes of treated ketchup sauces with *C. verum* essential oil and methanolic extract, the highest scores of panelists, for the individual attribute could be generally observed in ketchups treated with 1000 µg/ml essential oil and showed statically significant differences ($P \leq 0.05$) with control.

Table 6. Effect of *C.verum* essential oil and methanolic extract different levels on organoleptic character of ketchup sauce

Items	Treatments				
	Control	Level of <i>C. verum</i> essential oil (µg/ml) ^A			
		1000 (MIC)	1500 (1.5× MIC)	8000 (MIC)	12000 (1.5× MIC)
Flavor	4± 0.4714 ^b	4.9± 0.3162 ^a	3.8± 0.6325 ^a	3.5± 0.5270 ^b	2.3± 0.4830 ^c
Odor	3.4± 0.5164 ^b	4.8± 0.4216 ^a	4± 0.8165 ^b	3.6± 0.5164 ^b	2± 0.6667 ^c
Total acceptability	3.8± 0.6325 ^b	4.8± 0.4216 ^a	3.9 ± 0.5676 ^b	3.6± 0.5164 ^b	2.2± 0.4216 ^b

Legends^A Results are expressed as mean±SD

^{a-c}Means in the same row for each concentration followed by different superscript letters (a-c) differ significantly ($P < 0.05$).MIC: Minimum Inhibitory Concentration

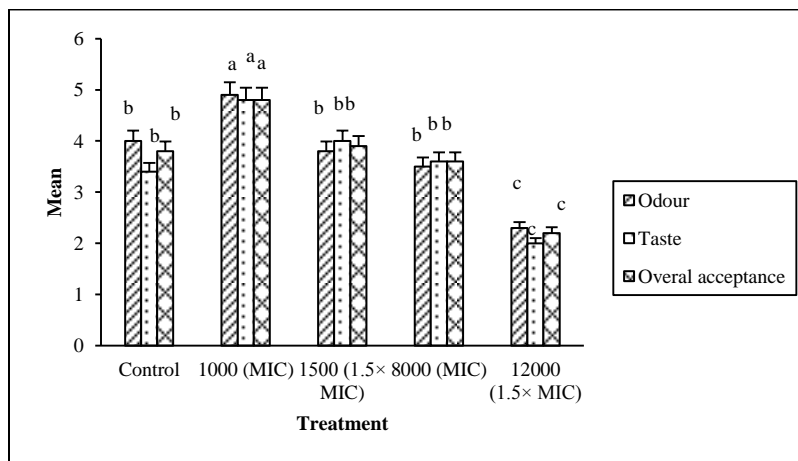


Figure 2. Effect of *C.verum* essential oil and methanolic extract different levels on organoleptic character of ketchup sauce. ^{a-}

^cMeans different superscript letters (a-c) differ significantly ($P < 0.05$). MIC: Minimum Inhibitory Concentration

DISCUSSION

To date, several studies have proven the antimicrobial properties of essential oil and methanolic extract of the cinnamon [19-21]. A study on the barks and leaves has identified cinnamaldehyde and eugenol as the most important components of the essential oil [22]. The bark aqueous extract had antibacterial activity against food borne pathogens, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecali*, *E. coli* and *Proteus mirabilis* [23]. In the present study, we carried out the same experiment. The essence and methanolic extract of this plant inhibited the growth of test isolates. It indicates that they possess substances that can inhibit the growth of some microorganisms. Present results are consistent with other studies [24, 25, and 26]. They indicated that methanol was an effective extractive solvent for *C. verum* extraction as it extracted higher concentration of phenolics that exhibited better antioxidant activities. In addition, they showed antibacterial activity of methanol extract of Cinnamon bark against Gram positive (*S. aureus*, *B. cereus*, *E. faecali*) and Gram negative (*E. coli*, *P. mirabilis*) bacterial strains and introduced it as potential sources of new antioxidant and antimicrobial agents.

Another study has reported the effects of cinnamon essential oil on different bacteria (*Pediococcus halophilus* and *Staphylococcus aureus*), indicating that cinnamon is a natural antimicrobial agent [27]. According to the results of current investigation and previous findings from related studies on antimicrobial activities of cinnamon, routine flavoring of food with cinnamon spice could help to prolong food shelf life. For example, the previously studies reported antimicrobial properties of cinnamon and its acceptable flavor with apple juice and application of cinnamon-derived natural antimicrobial agents for fresh-cut apple and to control microbes in meat [11, 28]. These findings encouraged us for the investigation of potential

innovative of essential oil and methanolic extraction of cinnamon barks as flavor and preservative in ketchup sauce. For this purpose, the effect of essential oil and methanolic extraction of *C. verum* fresh barks on total viable count (*E. coli* and *P. aeruginosae*) in ketchup sauce was evaluated at 4°C and 25°C during a 30-day storage time.

According to the results, the total bacterial counts for both *E. coli* and *P. aeruginosae* showed minor decrease with the prolongation of the storage period in the untreated samples. The reduction of total bacterial population in the control samples during storage time may have been caused by several reasons such as stable chilling condition and the presence of ingredients such as spices, vinegar, another antimicrobial compounds and inadequate growth condition of ketchup sauce. All ketchup samples treated with different concentrations of essential oil and plant extracts led to continuing significant reduction in bacterial cell number (log cfu/g) from the beginning of experiments to the end of the display time in comparison with untreated samples and bacterial viability declined by reduction of storage temperature. In addition, the results showed that the number of bacteria significantly decreased as the concentration of essential oil and methanolic extract increased, however the essential oil showed higher activities against bacterial tests. The bacterial counts for *E. coli*, in treated samples, were lower than those of *P. aeruginosae*, after the same essential oil and plant extract treatments. Therefore, *E. coli* was more sensitive to the essential oil and methanolic extract than *P. aeruginosae*. The most effective treatment on reduction of a bacterial culture cocktail (*E. coli* and *P. aeruginosae*) in ketchup sauce obtained on days 14 at 4°C in concentrations of 1500 µg/ml essential oil that reduced significantly bacterial population compared with control samples.

The other factor that was evaluated in this study was the organoleptic characteristic of treated ketchup sauce. Sensory evaluation is an important factor in judging about foodstuffs quality. It should be mentioned that when essential oils are used in food production systems as an antimicrobial agent amounts required are high and as such they are often higher in quality [29]. As our literature review revealed, this is the first report to evaluate the antimicrobial nature of cinnamon bark extract and essential oil in ketchup sauce.

CONCLUSIONS

Treatment of ketchup sauce with *C. verum* essential oil resulted in elevated biological quality and good sensory attributes of the product. From the viewpoints of health, quality, and economics, the bio-preservation of such products using plant oil could be proposed as the perfect approach to improve shelf life and flavor of the food.

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