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EXTRACTION AND IDENTIFICATION OF ESSENTIAL OIL FROM CINNAMOMUM ZEYLANICUM BARKS AND STUDY THE ANTIBACTERIAL ACTIVITY

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ABSTRACT

This study aimed to identify the essential oil compound from *Cinnamomum zeylanicum* barks by using GC-MS analysis and evaluate essential oil inhibition effects against *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas erogenous* and *Staphylococcus aureus* by using agar diffusion method and studied kill-time for this bacterium. The yield of *Cinnamomum zeylanicum* essential oil was 5%. Cinnamaldehyde 57.83% was the main compound in the essential oil extract, followed by cyclohexane carboxylic acid 9.29% and 6-octadecenoic acid 8.41%. Gram-positive and Gram-negative bacteria were inhibited growth after addition 6, 12 and 18 µL of essential oil. Zone diameter of inhibition ranged from 17.00-30.30 mm for bacterial test. A concentration of 10, 20 and 30 µL.mL^{-1} of the essential oil caused a rapid and steady decline in the number of viable cells from 2 to 5 Log. cycles of all strains during 24 hours.

Keywords: *Cinnamomum zeylanicum*, essential oil, antibacterial activity

INTRODUCTION

Cinnamon spice obtained from the inner bark of several trees from the genus *Cinnamomum* that is used in both sweet and savory foods. *Cinnamomum zeylanicum* is sometimes considered a "true cinnamon", and most of cinnamon in international trade is derived from related species, which are also referred to as "cassia" to distinguish it from the "true cinnamon" **(Mishra, 2016)**. Cinnamon has been used for many purposes since ancient times. Since the 16th century, it has been used as cooking spices, prevent food from being damaged and used cinnamon flavoring in cookies, biscuits and cakes. It is also used extensively in medicine, traditional, modern scents and perfumes **(Al-Sahlany, 2016; Anand *et al*., 2016).**

The essential oil of plants such as cinnamon oil has both antibacterial and antifungal compounds that can be used for the prevention of food spoilage due to microbial contamination **(Mahmoud, 2012; Al-Sahlany, 2017)**. Furthermore, it is also proven that cinnamon oil is effective against some species of toxigenic fungi **(Abdel Ghany *et al*., 2016)** and respiratory tract pathogens **(Viollon and Chaumont, 1994)**.

In *C. zeylanicum* there are many constituents such aslinalool, eugenol, cinnamic acid and cinnamaldehyde. The different types of extraction methods used to obtain essential oils, which are solvent extraction, ultrasonic extraction, hydro distillation, shaking and stirring with organic solvents **(Kamaliroosta *et al*., 2012).** The most popular physical way to isolate the essential oil is distillation. Before distillation, plants materials in most cases are dried and then grinding appropriate so that the division of oil vesicles is exposed maximum space for the release of the oil efficiency **(Niamah and Alali, 2016)**. Currently, essential oils from *C. zeylanicum* are widely used in medical field and in aromatherapy. It has antifungal properties **(de Castro and Lima, 2013)**, antibacterial properties (**Nabavi *et al*., 2015)** and antioxidant activity **(Ervina *et al*., 2016)**.

Therefore, this study aimed to essential oils extraction from *C. zeylanicum* by using hydro distillation method, and analyzed the chemical, compound present in the essential oil using Gas Chromatography Mass Spectrometer (GCMS). As well as, estimating antibacterial activity against some types of Gram-positive and Gram-negative bacteria that causes food spoilage.

MATERIAL AND METHODS

Plant sample

The cinnamon barks were bought from local market in Basrah city, Iraq. It was ground into powder by electric grinder and kept in plastic bags at room temperature until using.

Bacterial strains

Listeria monocytogenes ATCC 9525, *Pseudomonas erogenous* ATCC 10145, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 35029, and *Staphylococcus aureus* ATCC 25923 strains that used in this study were supplied by Biotechnology lab., College of Agriculture, University of Basrah, Iraq. Bacterial strains were grow and kept on nutrient agar (Himedia-India) slants at 4°C. 0.1mL of bacterial inoculation was obtained from overnight cultures grown on nutrient agar slants at 37°C and diluted in peptone water solution (0.1g of peptone in 1L distill water) to provide a final concentration of approximately 10^6 (CFU.mL^{-1}) adjusted according to the turbidity of 0.5 McFarland scale tube (**Niamah and Alali, 2016**).

Essential oil extract

Essential oil of *Cinnamomum zeylanicum* was extracted from cinnamon barks using Clevenger apparatus. 100g of barks with 500mL of distilled water was transferred into oil distillation at 90°C for 1-2 hours. The essential oil was collected and determined by calibrated tube. It kept in the freezer until used **(Al-Sahlany, 2016).**

Gas Chromatography-Mass Spectrum Analysis (GC-MS)

GC-MS technique used to identify the chemical compound in the essential oil extracted from cinnamon barks *C. zeylanicum*. This technique was carried at the GC-mass Lab., College of Agriculture, University of Basrah, Iraq. GC-MS analysis of 1µL essential oils extract from cinnamon barks was performed using GC SHIMADZU QP2010 ultra and gas chromatograph, interfaced to a Mass Spectrometer (GC-MS) equipped with DbB5ms capillary column, split injection mode and 49.5kPa pressure. The relative percentage amount of each chemical compound was calculated by comparing its average peak area to the total areas.

Software adopted to handle mass spectra and chromatograms was a GC-MS solution ver.2.53.

Antibacterial activity assay

The antibacterial activity of essential oil extract from cinnamon barks was determined by wells in agar diffusion method. 0.1mL (Approximately 10^6 $CFU.mL^{-1}$) of bacteria test was streaked by L-shape on Mueller-Hinton agar (Hi media-India) and worked on three wells of 6mm diameter in agar. 6, 12 and 18µL of essential oil extract transferred to wells. Petri dishes kept in the refrigerator for 2 hours and incubated at 37°C for 24-48 hours. Antibacterial activity was estimated by measuring diameters of clear zones around wells **(Niamah, 2014; Al-Manhel and Niamah, 2015).**

Kill time assay

The kill time assay executed with the essential oil of *C. zeylanicum* at 10, 20 and $30\mu L.mL^{-1}$ and the viable cells of bacteria test count method was used. 5mLof nutrient broth (Hi media-India) was inoculated with 1mL (10^6 $CFU.mL^{-1}$) of the bacterial suspension. After that, 4mL of the essential oil solution was added to the system and followed by shaking for 1min using vortex. The system was incubated at 37°C. At different periods of time 0, 3, 6, 12, 18 and 24 hours of exposure, 1mL of the suspension was serially diluted 10^{-1}–10^{-5} in the peptone water and inoculated onto nutrient agar Petri dishes for 24 hours at 37°C **(Viljoen *et al*., 2003)**. In the control assay, the sterile distilled water replaced the essential oil solution. At the end of the incubation period, the mean number of the colonies was counted and compared with the control assay. The results were expressed in Log of $CFU.mL^{-1}$, all assays were implemented in three duplicate and the results expressed as average **(Trajano *et al*., 2010)**.

RESULTS AND DISCUSSION

Chemical composition of cinnamon oil

The yield of cinnamon essential oil was 5%. GC-MS technique revealed the existence of 30 compounds in essential oil of *C. zeylanicum.* The major compound in cinnamon essential oil was cinnamaldehyde. It was 57.83% from total area of other components. The cyclohexane carboxylic acid and 6-octadecenoic acid were 9.29 and 8.41% respectively, while others were 0.15% for cinnamic alcohol to 2.97% for 3-phenyl, acetate from total area (Figure. 1) and (Table 1). The essential oils compound may variant based on the differences of agriculture dates, origin, vegetable state, the growing season for plants and storage conditions in markets **(Trajano *et al*., 2010)**.

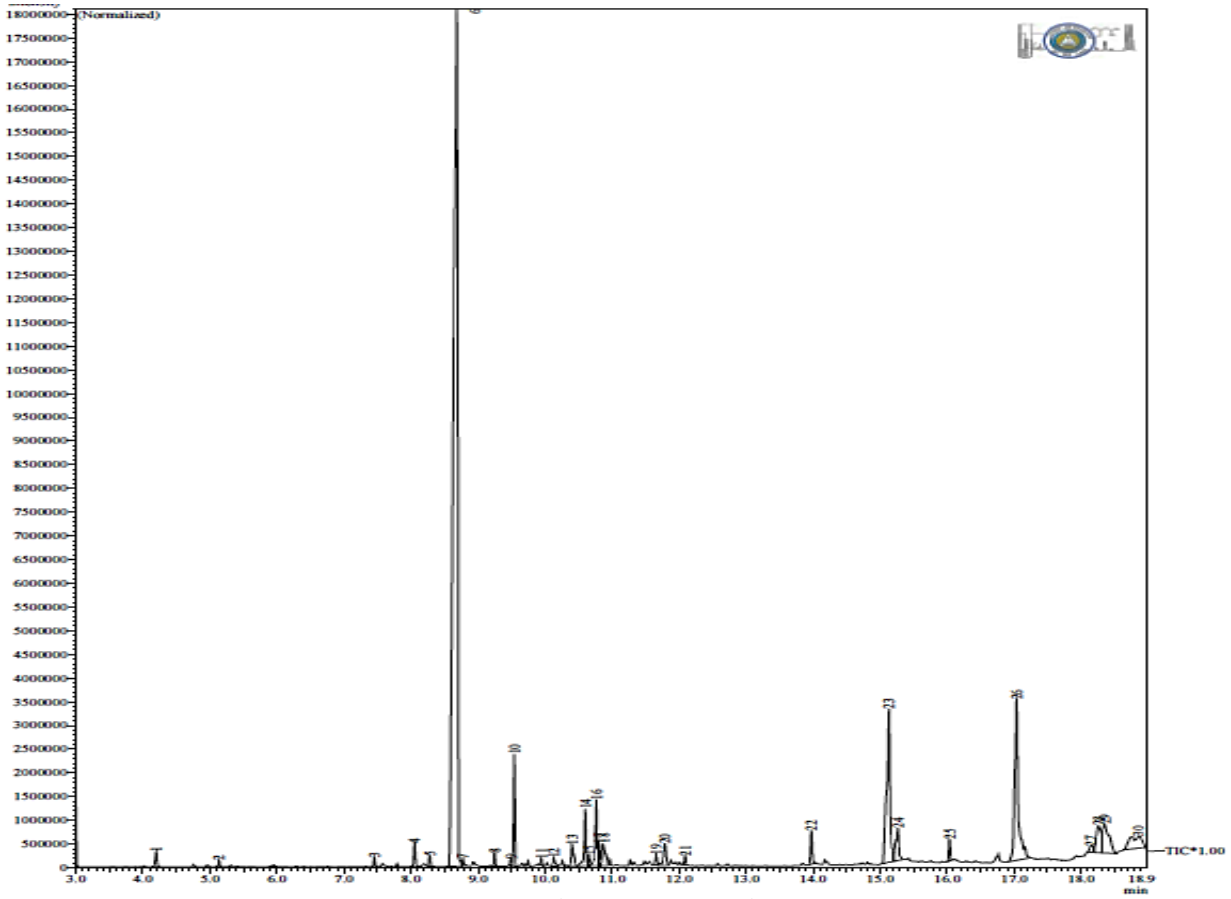

Figure 1 the chromatogram of essential oils extract from cinnamon barks

Table 1 the composition of essential oils extract from cinnamon barks analyzed by GC-MS

Peak	Name of compound	Real time	Area%
1	Benzenepropanal	4.197	0.44
2	Cinnamic alcohol	5.134	0.15
3	1,2,3,4,5,8-Hexahydronaphthalene	7.453	0.23
4	Cinnamaldehyde	8.053	57.83
5	(E)-Cinnamaldehyde	8.279	0.27
6	Benzylidenemalonaldehyde	8.683	0.60
7	Propanal	8.775	0.19
8	Benzaldehyde, p-isopropyl	9.247	0.32
9	3-Isopropylbenzaldehyde	9.489	0.17
10	Copaene	9.542	2.61
11	Caryophyllene	9.943	0.16
12	2-Propenal	10.132	0.28
13	(E)-3-Phenylpropenal	10.404	0.85
14	Benzalmalonicdialdehyde	10.601	1.67
15	4-chloro-2-methyl-1-phenyl	10.655	0.29
16	Mandelic acid	10.765	1.93
17	2-Methyl-1-phenylbut-3-en-1-ol	10.808	0.70
18	Cyclohexene-1-methanol	10.868	1.09
19	3-Cyclohexene-1-methanol	11.660	0.34
20	gamma.-Terpineol	11.788	0.96
21	1,2,4-Metheno-1H-indene	12.091	0.23
22	Cycloisosativene	13.976	0.95
23	6-Octadecenoic acid	15.133	8.41
24	Copaene	15.266	1.59
25	alpha.-Cubebene	16.036	0.56
26	Cyclo hexanecarboxylic acid	17.038	9.29
27	Caryophyllene	18.142	0.46
28	Bicyclo	18.260	2.09
29	3-phenyl-, acetate	18.352	2.97
30	Neronine	18.869	2.37

Cinnamaldehyde is volatile aldehyde compound, which is naturally synthesized in plants by the shikimate pathway. The results agreed with the **Singh *et al.* (2007)** recalling, the essential oil of cinnamon barks is about 49.9% cinnamaldehyde. Cinnamon bark contains up to 4% of essential oil mainly composed of 60-75% cinnamaldehyde **(Wang *et al*., 2009)**. The cinnamaldehyde percentage in extracted essential oil from *C. zeylanicum* by steam distillation method was 90% while 62-73% by Soxhlet method when determination of bioactive compound using HPLC **(Wong *et al*., 2014)**.

The analysis of the essential oil of *C. zeylanicum* by GC-MS is described and found to be 52.3% cinnamic aldehyde, which was the major compound in essential oil extract **(Kazemi and Mokhtariniya, 2014)**. While **Pooja *et al.* (2013)** found, the main ingredient in cinnamon oil extract was 91.82% cinnamaldehyde with eight compounds have small percentage.

Bacterial inhibition

The antibacterial activity of cinnamon essential oils against six bacterial species shown in (Table 2). The inhibition effect was increased with increasing concentration of essential oil. Add 18µL of essential oils cinnamon lead to the formation of large inhibition zone for both Gram-positive and Gram-negative bacteria. They were 30.30, 29.86, 28.23, 27.20 and 26.56 mm of *S. aureus*, *L. monocytogenes*, *E. coli*, *E. aerogenes* and *P. erogenous* respectively.

Essential oils are a potential source of new compound antimicrobial, especially against some types of bacteria that causes food spoilage. The demonstration of antibacterial activity of cinnamon against both Gram-positive and Gram-negative bacteria may be indicated by the presence of broad spectrum antibiotic compound **(Nabavi *et al*., 2015)**. These results are similar with the previous studies in this field, which it reported that cinnamon bark oil fully inhibited the growth of some Gram-positive and Gram-negative bacteria **(Salem *et al.*, 2013; Hadri *et al.*, 2014; Saleem *et al.*, 2015).**

Table 2 inhibition zones for some type of bacteria by concentrations of essential oil cinnamon barks (SD±: Standard division).

Concentrations of essential oil cinnamon	Bacteria isolates inhibition zones (mm)				
	Escherichia coli ATCC 25922	*Enterobacter aerogenes* ATCC 35029	*Listeria monocytogenes* ATCC 9525	*Pseudomonas erogenous* ATCC10145	*Staphylococcus aureus* ATCC 25923
6µL	18.00±0.30	19.00±0.10	21.30±0.31	17.00±0.10	20.50±0.20
12µL	22.20±0.39	22.10±0.48	26.70±0.52	22.23±0.25	27.03±0.22
18µL	28.23±0.31	27.20±0.39	29.86±0.40	26.56±0.21	30.30±0.61

Kill time of bacteria strains

The results of the essential oil effect from *C. zeylanicum* barks at 10, 20 and 30µL.mL^{-1} on the cell viability (kill time) of pathogenic bacteria are shown in (Figure 2, 3, 4, 5 and 6). The essential oil reduced of viability of bacteria test compared with the control sample. All strains of bacteria, essential oil provided by killing characterized static concentration exposure to depend antibacterial effect. The essential oil of cinnamon was caused decrease in the viable cell count of bacteria ranging from 2 to 5 Log. cycles along the evaluated times in comparison to the control sample. The essential oil of *C. zeylanicum* are maybe to accumulate in the bacterial cell wall and cause a damage of the membrane cells, infiltration of cytoplasmic content, eliminate of the proton motive force, cell lysis, and cell death (**Zengin and Baysal, 2014; Zouheyr *et al*., 2014)**. Some studies have shown that, it is necessary added the whole essential oil usually have antibacterial activity higher than mixture of the major components, which indicates that the simple ingredients are crucial to the collaborative activity and antioxidant activity **(Bassolé and Juliani, 2012)**.

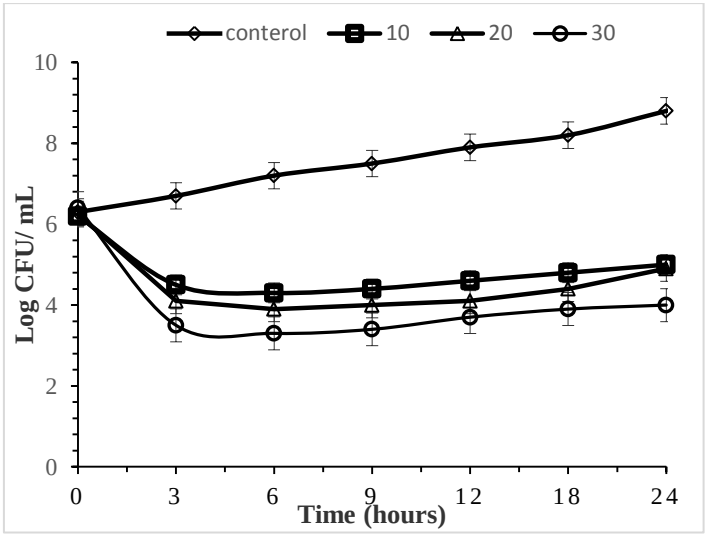

Figure 2 the viability cell of *E. coli* ATCC 25922 in nutrient broth at different concentration of *C. zeylanicum* barks essential oil at 37 °C.

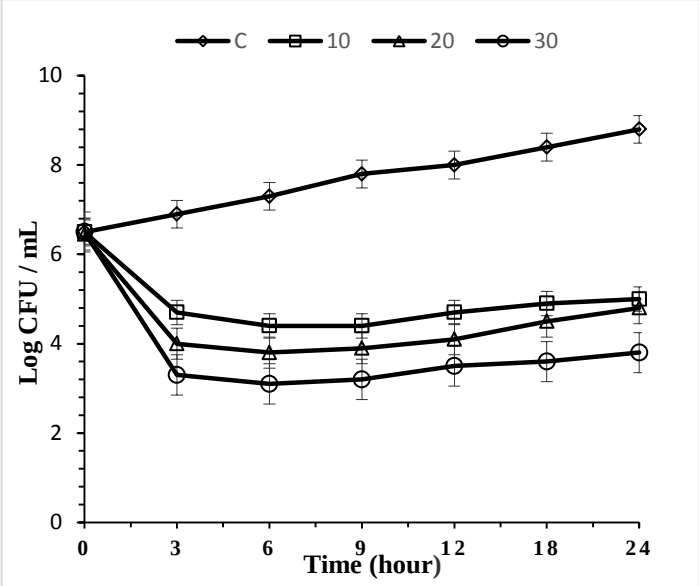

Figure 3 the viability cell of *E. aerogenes* ATCC 35029 in nutrient broth at different concentration of *C. zeylanicum* barks essential oil at 37°C.

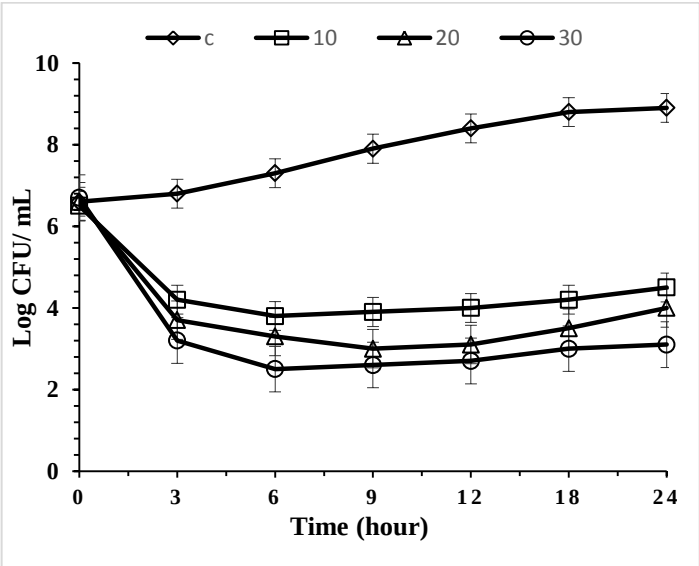

Figure 4 the viability cell of *L. monocytogenes* ATCC 9525 in nutrient broth at different concentration of *C. zeylanicum* barks essential oil at 37°C.

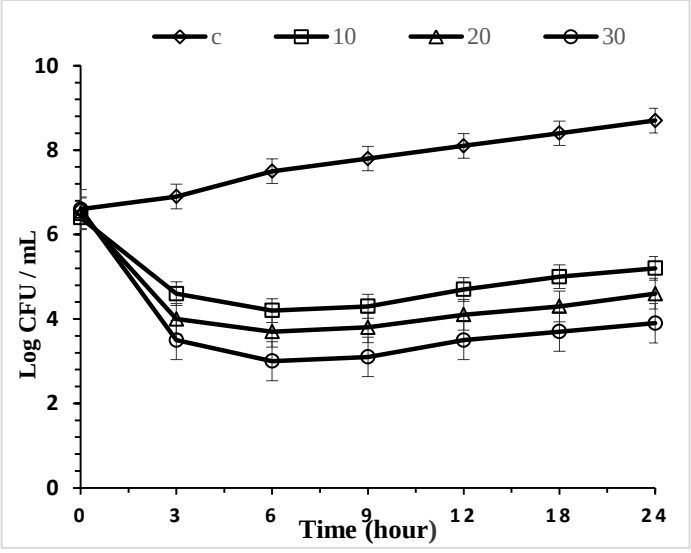

Figure 5 the viability cell of *P. erogenous* ATCC 10145 in nutrient broth at different concentration of *C. zeylanicum* barks essential oil at 37°C.

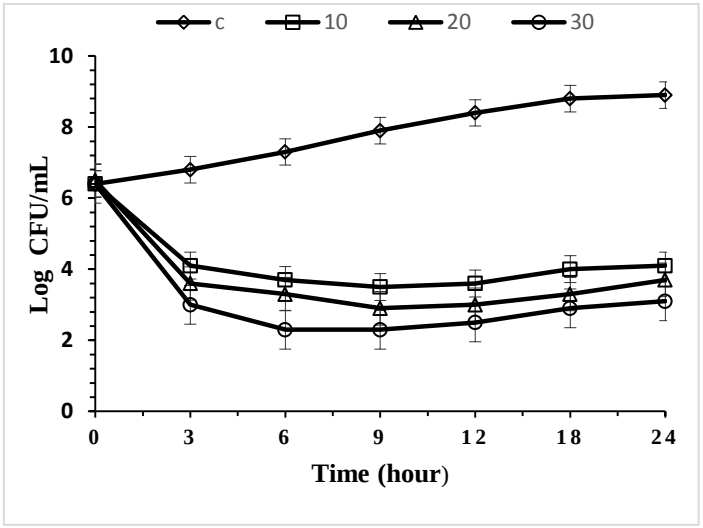

Figure 6 the viability cell of *S. aureus* ATCC 25923 in nutrient broth at different concentration of *C. zeylanicum* barks essential oil at 37°C.

The action mode of essential oil cinnamon caused breakdown bacterial membrane structure and denaturation of proteins. Cinnamaldehyde is an organic compound with the formula C_6H_5CH=CHCHO as the major compound of the essential oil and believed to show antibacterial activity by the inhibition of cell enzymes synthesis, disruption of the cell wall structure resulting in shortage in the cytoplasm, cytoplasmic granulation, cytoplasm acidity, and depletionof intracellular ATP collect **(Trajano *et al*., 2010)**.

CONCLUSIONS

The results of the present study showed, the thirty compounds found in essential oil of *Cinnamomum zeylanicum* barks. These compounds were identified by GC-MS technique. The essential oil of cinnamon has antibacterial activity against both Gram-positive and Gram-negative bacteria might be indicated by the presence of broad-spectrum antibiotic compound. These results point to the possibility with respect to the essential oil of *C. zeylanicum* barks as alternative sources of antibacterial compound to be applied in food preservation systems.

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