



## A synergistic system based on *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils to control selected foodborne microorganisms

Mohamed Reda Zahi\*<sup>1</sup>, Smain Sabour<sup>1</sup>, Nacera Riad<sup>1</sup>, Meriem Abbas<sup>2</sup>, Ryma Nadjela Maouche<sup>2</sup>,  
Mohamed El Hattab<sup>1</sup>

<sup>1</sup>Laboratory of Natural Products Chemistry and Biomolecules, Faculty of Sciences, Saad Dahlab

University of Blida 1- POB 270, Soumaa Road, Blida, Algeria

<sup>2</sup>Department of Chemistry, Faculty of Sciences, Saad Dahlab University of Blida 1- POB 270, Soumaa  
Road, Blida, Algeria

\* Corresponding Author Email ID: [reda.zahi@univ-blida.dz](mailto:reda.zahi@univ-blida.dz) or [z-reda@hotmail.com](mailto:z-reda@hotmail.com)

---

**Abstract:** The present work was conducted to study the chemical composition, and the antibacterial activity of *Cuminum cyminum* (*C. cyminum*) and *Cinnamomum zeylanicum* (*C. zeylanicum*) essential oils (EOs), singly or in combination against selected foodborne microorganisms. The GC-MS analysis revealed that *C. cyminum* EO was governed by cuminaldehyde (44.8%), 2-carene-10-al (18.89%), 3-carene-10-al (15.95%) and  $\gamma$ -terpinene (14.29%). Meanwhile, the dominant components of *C. zeylanicum* EO were (*E*)-cinnamaldehyde (76.27%) and 1,8-cineole (11.27%). The antibacterial tests demonstrated that both EOs had a valuable inhibitory effect with growth inhibition zones extending from 19 to 44 mm, and MICs range from 0.14-1.13 mg/mL. Regarding the synergism assay, the combination of EOs displayed a strong synergism against *Escherichia coli* (*E. Coli*), *Enterobacter cloacea* (*E. cloacea*) and *Listeria monocytogenes* (*L. monocytogenes*) followed by an appreciable additive effect against *Enterococcus faecium* (*E. faecium*) and *Staphylococcus aureus* (*S. aureus*) with a significant decrease in their individual MICs. These results could be a good opportunity for the food industry to control undesired microorganisms.

---

**Keywords:** *Cuminum cyminum*, *Cinnamomum zeylanicum*, Essential oils, Synergism, GC-MS, Antibacterial activity

---

### 1. Introduction

Foodborne microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* among others, are recognized as a growing public health problem. Their

presence in food is related with infections, poisoning, spoilage, disease and the deterioration of its quality at any stage of production, storage, delivery or consumption [1]. Foodborne contamination cover wide number of illnesses from diarrhea to cancer, which is a global burden of disease and mortality, in this regard, it was reported in 2022 that about 600 million people almost 1 in 10, especially children became sick after eating contaminated food, and 420000 are dying every year because of diarrhea [2]. To overcome this global threat, many non-natural attempts were applied, for example, the use of synthetic preservatives. Unfortunately, their use is limited due to their adverse effects (tendinoapthy, ototoxicity, respiratory allergies and nephrotoxicity among others) [3,4]. In addition, the trend of green consumerism have pushed researchers to find out a suitable natural alternative such as EOs with a similar or enhanced preservative effect without influencing the organoleptic properties of food [5-7].

EOs are natural complex mixtures of highly lipophilic volatile secondary metabolism of plants [8,9]. They are generally found in the flower, bud, seed, leaves, herbs, fruits, bark and roots of aromatic plants [10]. EOs are endowed with many biological activities including the antimicrobial one with the ability to prolong the shelf-life of foods [11-14]. Regarding their alimentary ingestion, they are considered by the U.S. Food and Drug Administration (FDA) as safe ingredients [15]. Furthermore, food industries are paying more attention to their use as natural preservative agents to control the growth of microorganisms in food [16]. Unfortunately, EOs are volatile and very susceptible to conversion, polymerization, oxidation and degradation reactions [17], resulting in a loss of their quality. In this case, an elevated concentration is required to obtain a satisfactory preservative effect [18]. However, the use of higher concentrations of EOs may affect the taste of the food by exceeding the sensory threshold acceptability of consumers [3, 19]. To deal with this drawback, smart combinations between EOs with an interesting synergistic effect, could help to achieve a good preservative effect at low concentrations, and without influencing the taste and the color of the food [20, 21]. *C. cyminum* and *C. zeylanicum* EOs are well recognized for their wide spectrum of biological properties [22-25]. These latter occurs due to the presence of various bioactive terpenoids in their composition [26-29]. Both EOs are widely used in food preparation, beverages, perfumery and traditional medicines. As far as our literature survey could ascertain, the possible synergistic combination between *C. cyminum* and *C. zeylanicum* EOs against the tested microorganisms was not investigated so far. Thus, the aim of the current work was to investigate the chemical composition of the *C. cyminum* and *C. zeylanicum* EOs, as well as the evaluation of their antibacterial activities alone or in combination against five foodborne microorganisms, including *E. coli*, *E. faecium*, *E. cloacae*, *L. monocytogenes* and *S. aureus*.

## **2. Materials and methods**

### **2.1 Chemicals**

Anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), and slants of nutrient agar were purchased from Sigma Aldrich (Algeria)

### **2.2 Extraction of *C. cyminum* and *C. zeylanicum* essential oils**

The seeds of *C. cyminum* and the barks of *C. zeylanicum* were subjected to hydrodistillation at atmospheric pressure, using a Clevenger type apparatus for 3 h according to the European pharmacopeia method [30]. The obtained EOs were separated from the aqueous phase, then dried with anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated using a rotary-evaporator. The obtained EOs were recovered, weighed and stored at 4 °C in hermetically sealed brown vials for further analysis and use.

### **2.3 Chemical composition of the essential oils**

The EOs were analyzed by gas chromatography coupled with mass spectrometry on a Perkin Elmer GC 680- MS-SQ8T, using a fused silica capillary column Rtx-5MS: (5% diphenyl: 95% methylsiloxane) 30 m, 0.25 mm i.d, 0.25  $\mu\text{m}$  film thickness. The column oven temperature was maintained at 80 °C for 5 min and up at a rate of 3°C /min to 250 °C (10 min hold). The flow rate of the carrier gas (He) was set at 30 mL/min, the ion source and interface temperature were set at 250 °C and 280 °C, respectively. The

ionization energy was 70 eV. 1 µl of solution at 10% (v/v) of EOs in hexane was injected using a split ratio of 1:50. The composition of the EOs were evaluated as the (%) of the relative amount of individual compounds and expressed as (%) peak area relative to the total peak area from MS analysis, and by NIST library according to a similarity greater than 90%.

## 2.4 Antibacterial activity Experiments

### 2.4.1 Microbial strains and culture conditions

Five foodborne microorganisms from the American Type Culture Collection (ATCC) were used for the evaluation of the antibacterial activity of both *C. cyminum* and *C. zeylanicum* EOs including Gram (–) bacteria: *E. coli* (ATCC 25922), *E. cloacae* (ATCC 23355), *E. faecium* (ATCC 51559) and Gram (+) bacteria: *L. monocytogenes* (ATCC 13932) and *S. aureus* (ATCC 44300). All the above microorganisms were kindly provided from Pasteur institute of Algiers (Algeria), and were stored at 4 °C into their respective slants. Active bacterial cultures were prepared by transposing a single colony of cells from the solid nutrient agar plate to a test tube previously filled with a nutrient agar solution. These later were then incubated at 37 °C overnight. The bacterial suspensions were adjusted at a turbidity equivalent to 0.5 McFarland standard ( $1 \times 10^8$  CFU.mL<sup>-1</sup>) using a UV–visible spectrophotometer (RAYLEIGH UV-2601) at 600 nm [31,32].

### 2.4.2 Determination of the inhibition zones diameters

*C. cyminum* and *C. zeylanicum* EOs were evaluated for their qualitative antibacterial activity using the paper disc method according to the method of Yazgan et al. [33], with minor modifications. Nutrient agar was sterilized in an autoclave (30 min, 120 °C) cooled to 40-45 °C and poured into sterilized petri dishes. After solidification, the Agar plates were seeded by spreading of 100 µl of fresh inoculum suspensions. Then, discs (9 mm) filled with *C. cyminum* or *C. zeylanicum* EOs were disposed on the surfaces of the plates. Kanamycin and Chloramphenicol (30 µg/mL) were used as a positive control. The inoculated plates were incubated for 18-24 h at 37 °C. The diameters of the inhibition zones were measured and recorded in millimeters. The qualitative antibacterial efficiency was classified into three levels: weak activity (inhibition growth zone ≤ 12 mm), moderate activity (12 mm < inhibition growth zone < 20 mm) and strong activity (inhibition growth zone ≥ 20 mm) [34].

### 2.4.3 Determination of the minimal inhibitory concentration

10 mL of sterile test tubes were used for the preparation of two-fold serially diluted *C. cyminum* and *C. zeylanicum* EOs in the nutrient. Subsequently, the freshly prepared bacterial suspensions ( $1 \times 10^8$  CFU.mL<sup>-1</sup>) were added to each testing tube. These later were incubated for 18-24 h at 37 °C. Negative control test tubes filled with bacterial suspensions alone with nutrient agar and positive control containing Kanamycin and Chloramphenicol were also prepared for eventual comparison. The MIC was recorded as the lowest concentration of *C. cyminum* and *C. zeylanicum* EOs in the serial dilution tubes with no visible growth of the microorganisms after incubation [21,35].

### 2.4.4 Synergistic interaction

The interactive inhibition of *C. cyminum* and *C. zeylanicum* EOs against the five tested foodborne microorganisms was evaluated using a 2-D checkerboard experiment according to the methods of Mangoni et al. [36] with minor modifications. Briefly, serial two-fold dilution of *C. cyminum* and *C. zeylanicum* EOs were prepared according to their respective MICs values. And then, ( $1 \times 10^8$  CFU.mL<sup>-1</sup>) of fresh bacterial suspensions were added to each testing tube. These latter were incubated over night at 37 °C. The interactive combination effect of the EOs was obtained by the calculation of the fractional inhibitory concentration index (FICI) using the following formula:

$$\sum \text{FICI} = \text{FIC (A)} + \text{FIC (B)}.$$

Where:

FIC (A) = MIC (A) in combination/MIC (A) alone.

FIC (B) = MIC (B) in combination/MIC (B) alone.

The data was interpreted as: synergy ( $FICI \leq 0.5$ ); addition ( $0.5 \leq FICI \leq 1$ ); indifference or non-interactive ( $1 \leq FICI \leq 4$ ); antagonism ( $FICI \geq 4$ ).

### 2.5 Statistical analysis

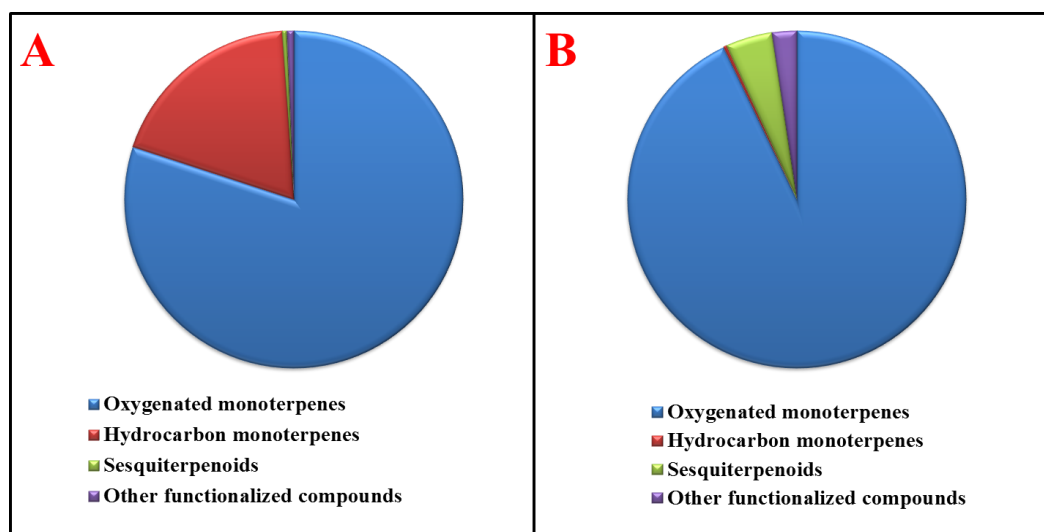
All measurements were performed in triplicate on the freshly prepared samples. The results are recorded as mean  $\pm$  standard deviation for the measurements.

## 3. Results and discussion

### 3.1 Identification of the chemical composition of *C. cyminum* and *C. zeylanicum* EOs

The extraction yields of *C. cyminum* and *C. zeylanicum* EOs were 1.67% and 1.72% (w/w), respectively on a dry weight basis. In fact, the extraction yield and the chemical composition of EOs are governed by many parameters, including the nature and origin of the plant, type of cultivar, soil composition, harvest time, storage conditions and the extraction method. In general, the antibacterial properties of EOs are related to their respective composition, especially their major components [37]. Thus, it is very important to determine the chemical composition of the tested EOs. The GC-MS analysis of both EOs (Table 1) revealed the presence of 12 components in *C. cyminum* EO and 21 compounds in *C. zeylanicum* EO representing 99.69% and 98.79% of the total EOs composition respectively.

According to the obtained results, *C. cyminum* EO was composed of oxygenated (79.78%) and hydrocarbon (18.84%) monoterpenes, sesquiterpenoids (0.43%) and other functionalized components (0.64%) fractions (Fig. 1A).



**Fig 1.** Percentage of the chemical classes found in both *C. cyminum* (A) and *C. zeylanicum* (B) EOs

The principal compounds of *C. cyminum* EO were cuminaldehyde (44.8%), 2-carene-10-al (18.89%), 3-carene-10-al (15.95%) and  $\gamma$ -terpinene (14.29%) followed by *p*-cymene (4.55%). It is not surprising to find that cuminaldehyde was the predominant compound of the EO, this compound was reported by many researchers [26, 27] as the major component in the *C. cyminum* EO. Our GC-MS results are in agreement with those of Ghasemi et al. [38] the authors reported similar major components with different contents. In addition, a fraction of sesquiterpenoids including,  $\alpha$ -elemene (0.12 %), (*Z*)-caryophyllene, (0.06%), (*E*)- $\beta$ -farnesene, (0.09 %),  $\beta$ -acoradiene (0.09%) and carotol (0.07%) was present in the *C. cyminum* EO composition. In addition, *p*-mentha-1,4-dien-7-al (0.64%) was also detected in the EO (Table 1).

selected foodborne microorganisms

Concerning *C. zeylanicum* EO, it was composed of oxygenated (91.76%) and hydrocarbon (0.32%) monoterpenes, sesquiterpenoids (4.40%) and other functionalized compounds (2.31%) (Fig.1B).

*C. zeylanicum* EO was particularly rich on (*E*)-cinnamaldehyde (76.27%), responsible of the distinct flavor and odor of cinnamon barks [39], 1,8-cineole (11.27%) followed by (*Z*)-cinnamaldehyde (2.07%). Our results are in agreement with Zhang et al. [28], they have reported that *trans*-cinnamaldehyde was the major components of *C. zeylanicum* EO. In addition, 1,8-cineole was also found by Alizadeh Behbahani et al. [29] among the main constituents of *C. zeylanicum* EO. Furthermore, other oxygenated monoterpenes including linalool (0.09%), terpinen-4-ol (0.33%),  $\alpha$ -terpineol (0.56%), bornyl acetate (1.17%), and hydrocarbon monoterpenes, such as *p*-cymene (0.19%) and  $\gamma$ -terpinene (0.13%) were found in the *C. zeylanicum* EO composition (Table 1).

**Table 1:** Chemical composition of *C. cyminum* and *C. zeylanicum* EOs

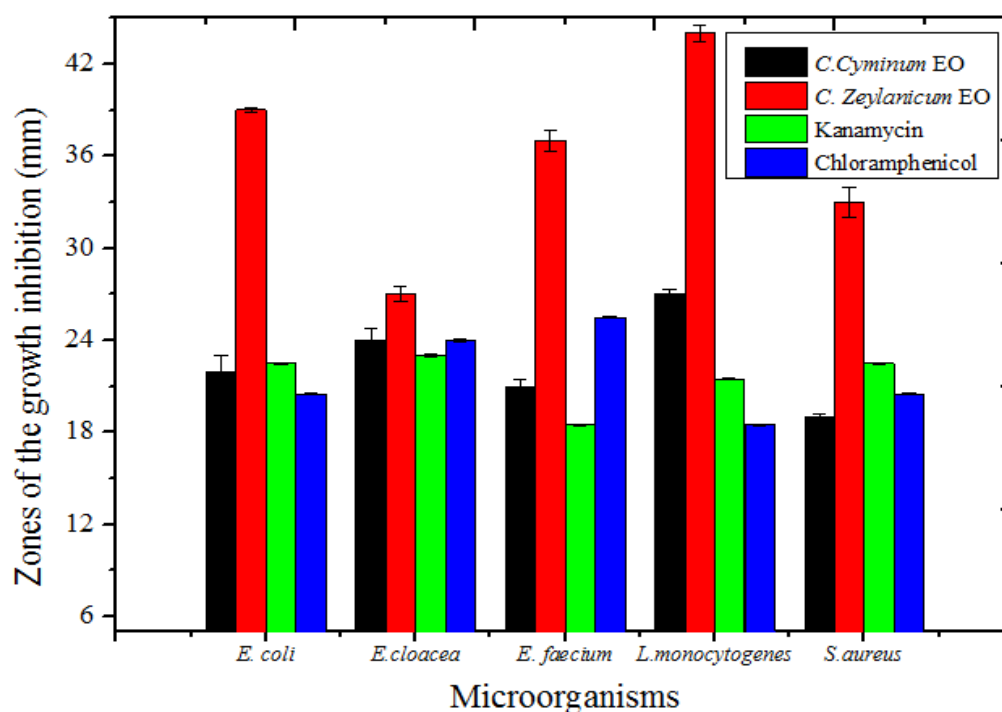
N°	Compound Name	Rt (min)	Relative %	
			<i>C. cyminum</i> EO	<i>C. zeylanicum</i> EO
1	<i>p</i> -Cymene	05.12	04.55	0.19
2	<b>1,8-Cineole</b>	<b>05.36</b>	-	<b>11.27</b>
3	<b><math>\gamma</math>-Terpinene</b>	<b>06.16</b>	<b>14.29</b>	0.13
4	Linalool	07.52	-	0.09
5	Benzenpropanal	09.87	-	1.76
6	Terpinen-4-ol	10.67	0.14	0.33
7	$\alpha$ -Terpineol	11.26	-	0.56
8	( <i>Z</i> )-Cinnamaldehyde	12.16	-	2.07
9	<b>Cuminaldehyde</b>	<b>13.31</b>	<b>44.8</b>	-
10	<b>(E)-Cinnamaldehyde</b>	<b>14.70</b>	-	<b>76.27</b>
11	Bornyl acetate	15.06	-	1.17
12	<b>2-Caren-10-al</b>	<b>15.12</b>	<b>18.89</b>	-
13	<b>3-Caren-10-al</b>	<b>15.34</b>	<b>15.95</b>	-
14	<i>p</i> -Mentha-1,4-dien-7-al	16.92	0.64	-
15	$\alpha$ -Copaene	18.90	-	1.83
16	$\alpha$ -Elemene	19.04	0.12	-
17	( <i>Z</i> )-Caryophyllene	20.66	0.06	0.89
18	Coumarin	21.12	-	0.38
19	( <i>E</i> )-Cinnamyl acetate	21.78	-	0.17
20	$\alpha$ -Humulene	22.14	-	0.10
21	( <i>E</i> )- $\beta$ -Farnesene,	22.21	0.09	-
22	$\beta$ -Acoradiene	22.94	0.09	-
23	$\gamma$ -Muurolene	23.00	-	0.06
24	$\alpha$ -Muurolene	23.97	-	0.40
25	$\delta$ -Cadinene	24.76	-	0.73
26	Carotol	27.90	0.07	-
27	Cubenol	28.91	-	0.13
28	$\alpha$ -Cadinol	29.53	-	0.18
29	Cadalene	30.56	-	0.08
Hydrocarbons monoterpene (%)			18.84	0.32
Oxygenated monoterpene (%)			79.78	91.76
Sesquiterpenoids (%)			0.43	4.40
Other functions (%)			0.64	2.31
Total identified (%)			99.69	98.79

The fraction of sesquiterpenoids in the *C. zeylanicum* EO was represented by  $\alpha$ -copaene (1.83%), (Z)-caryophyllene (0.89%),  $\alpha$ -humulene (0.10%),  $\gamma$ -muurolene (0.06%),  $\alpha$ -muurolene (0.40%),  $\delta$ -cadinene (0.73%), cubenol (0.13%),  $\alpha$ -cadinol (0.18%) and cadalene (0.08%). Finally, the others variously functionalized components of *C. zeylanicum* EO were benzenpropanal (1.76%), coumarin (0.38%) and (*E*)-cinnamyl acetate (0.17%). From the chemical composition of *C. cyminum* and *C. zeylanicum* EOs we can clearly conclude that both EOs are rich with oxygenated monoterpenes. These latter, are famous with their wide spectrum of biological activities, including antibacterial, antifungal, antioxidant, anticancer among others. Thus, these EOs could be valuable sources of natural oxygenated monoterpenes.

### 3.2 Antibacterial activity of *C. cyminum* and *C. zeylanicum* EOs

#### 3.2.1 Determination of the inhibition zone diameter

The antibacterial activity of both *C. cyminum* and *C. zeylanicum* EOs as well as Kanamycin and Chloramphenicol, was evaluated qualitatively against *E. coli*, *E. faecium*, *E. cloacae*, *L. monocytogenes* and *S. aureus* by the presence or the absence of the microorganisms' growth around the sterile discs. Very interesting results were obtained with both EOs (Fig.2). Accordingly, *C. cyminum* and *C. zeylanicum* EOs inhibited moderately to strongly the growth of the all tested microorganisms with zones of the growth inhibition being in the range of 19-44 mm.



**Fig 2.** *In vitro* qualitative antibacterial activity of *C. cyminum* EO, and *C. zeylanicum* EO, Kanamycin and Chloramphenicol against the tested microorganisms

The relative sensitivity of the tested microorganisms to the *C. cyminum* EO had the following order: *L. monocytogenes* ( $27 \pm 0.33$  mm)  $\geq$  *E. cloacae* ( $24 \pm 0.83$  mm)  $\geq$  *E. coli* ( $22 \pm 1$  mm)  $\geq$  *E. Faecium* ( $21 \pm 0.5$  mm)  $\geq$  *S. aureus* ( $19 \pm 0.16$  mm). *L. monocytogenes* was the most sensitive microorganism. However, *S. aureus* was the most resistant one. Our results are in agreement with those of Khalil et al. [40] they reported the higher inhibition zones were obtained with *C. cyminum* EO against some microorganisms such as *E. coli*, *S. aureus* and *Micrococcus lotus* among others.

**A synergistic system based on *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils to control selected foodborne microorganisms**

Section A-Research paper

In addition, the sensitivity towards *C. zeylanicum* EO was as follows: *L. monocytogenes* ( $44 \pm 0.5$  mm)  $\geq$  *E. coli* ( $39 \pm 0.16$  mm)  $\geq$  *E. Faecium* ( $37 \pm 0.66$  mm)  $\geq$  *S. aureus* ( $33 \pm 1$  mm)  $\geq$  *E. cloacea* ( $27 \pm 0.5$  mm). In this case, *L. monocytogenes* was the most resistant bacteria whereas; *E. cloacea* was the most resistant microorganism. Alizadeh Behbahani et al. [29] have reported that the essential oil of *C. zeylanicum* had an antibacterial activity with the inhibition zone diameter being in the range of 18 to 27 mm against a panel of Gram (+) and Gram(−) bacteria. In contrast, our EO displayed a greater antibacterial effect against *E. coli* and *S. aureus* compared to their EO. From the above data, it's obviously seen that all the tested microorganisms were more sensitive to *C. zeylanicum* EO as compared to *C. cyminum* EO. Finally, both antibiotics have shown a moderate to strong antibacterial activity against the tested microorganisms, the zones of inhibition growth were in the range of 18.5 to 23 for Kanamycin, and 18.5 to 25.5 for Chloramphenicol.

**3.2.2 Evaluation of the minimum inhibitory concentration**

The quantitative antibacterial activity of both *C. cyminum* and *C. zeylanicum* EOs and two antibiotics (kanamycin and chloramphenicol), tested against the bacterial strains was evaluated by the determination of their respective MIC using conventional dilution method.

**Table 2:** Minimum inhibitory concentration of both *C. cyminum* and *C. zeylanicum* EOs

Microorganisms	MIC <sup>a</sup>			
	<i>C. cyminum</i> EO	<i>C. zeylanicum</i> EO	Kanamycin	Chloramphenicol
<b>Gram (−) Bacteria</b>				
<i>E. coli</i>	1.13	0.65	0.20	0.02
<i>E. cloacea</i>	0.56	0.16	0.008	0.004
<i>E. Faecium</i>	0.13	0.65	0.06	0.004
<b>Gram (+) Bacteria</b>				
<i>L. monocytogenes</i>	1.13	0.65	0.01	0.008
<i>S. aureus</i>	1.13	0.32	0.06	0.02

MIC<sup>a</sup>: Minimum inhibitory concentration in mg/mL.

From the obtained results (Table 2) it's obviously perceived that both *C. cyminum* and *C. zeylanicum* EOs had an important inhibitory effect where the MICs values were in the range of 0.14-1.13 mg/mL.

*C. cyminum* EOs, displayed a strong inhibitory effect against *E. Faecium* and *E. cloacea*, concentrations of 0.14 and 0.56 mg/mL respectively, were quite sufficient to inhibit the growth of these microorganisms. However, *L. monocytogenes*, *E. coli* and *S. aureus* were less sensitive where their MIC value was 1.13 mg/mL. Our results are in agreement with Jardak et al. [27], the authors have reported the MIC values of *C. cyminum* EO were in the range of 0.31-2.25 µl/mL against a wide number of Gram (+) and Gram (−)

**A synergistic system based on *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils to control selected foodborne microorganisms**

Section A-Research paper

bacteria.

In the case of *C. zeylanicum* EO, the highest antibacterial activity was found with respect to the growth of *E. cloacea* showing an MIC of 0.16 mg/mL, followed by *S. aureus* (MIC= 0.32 mg/mL), for the rest of microorganisms, the MIC value was the same 0.65 mg/mL. As compared to the MIC results of Alizadeh Behbahani et al. [29] our *C. zeylanicum* EO has shown a better inhibitory effect against *E. coli* and *S. aureus*.

The obtained data weren't unexpected, and they seem to match well with the results from the above qualitative antibacterial test, as in this experience we have found that *C. zeylanicum* EO demonstrated a stronger antibacterial effect as compared to *C. cyminum* EO (section 3.2.1). This difference could be explained by the nature and the content of bioactive components constituting both EOs [41]. According to the GC-MS data (section 3. 1), the total amount of oxygenated monoterpene in *C. zeylanicum* EO was higher than that of *C. cyminum* EO. Furthermore, the sesquiterpenoids fraction was 10-folds higher in *C. zeylanicum* EO than in the *C. cyminum* EO. These compounds are well known for their wide array of antibacterial activities among others.

**3.2.3 Synergistic interactions**

In the aim to reduce undesired side effects of synthetic antibacterial agents and replace them with natural ones, the synergy between *C. zeylanicum* and *C. cyminum* EOs was investigated by the calculation of the FICI. The obtained results are provided in the Table 3.

**Table 3:** FIC (FICI) of *C. cyminum* and *C. zeylanicum* EOs against the tested microorganisms<sup>a</sup>

EOs combination	Microorganisms									
	<i>E. coli</i>		<i>E. cloacea</i>		<i>E. Faecium</i>		<i>L. monocytogenes</i>		<i>S. aureus</i>	
	FIC	FICI	FIC	FICI	FIC	FICI	FIC	FICI	FIC	FICI
<i>C. cyminum</i>	0.24		0.24		0.49		0.12		0.24	
and		0.36 (S)		0.35 (S)		0.98 (A)		0.24 (S)		0.74 (A)
<i>C. zeylanicum</i>	0.12		0.11		0.49		0.12		0.50	

<sup>a</sup>: The results were interpreted as: synergy (S, FICI <0.5), addition (A, 0.5 <FICI <1), indifference (I, < FICI<4) and antagonism (An, FICI >4)

Accordingly, nor indifference, neither antagonism effects were found in this synergism testing, which could be a positive indication and encouraging outcomes. Furthermore, an appreciated additive effect was obtained with regards to both *E. Faecium* (FICI= 0.98) and *S. aureus* (FICI= 0.74). Fascinatingly, this combination exhibited a strong synergistic effect against *E. coli*, *E. cloacea* and *L. monocytogenes* showing FICI values of 0.36, 0.35 and 0.24 respectively. In addition, this synergistic combination reduced the MIC values of *C. zeylanicum* EO by 8-folds against *E. coli*, *E. cloacea* and *L. monocytogenes*. However, for *C. cyminum* EOs the MIC value was decreased by 8-times against *L. monocytogenes* and by 4-times against *E. coli* and *E. cloacea*. Unfortunately, no reports on the possible interaction between *C. cyminum* and *C. zeylanicum* EOs against the tested microorganisms was found in the bibliography, for this reason we couldn't compare our findings with others.

The advantages of combinations with strong synergism propriety like (*C. cyminum* and *C. zeylanicum*



# A synergistic system based on *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils to control selected foodborne microorganisms

Section A-Research paper

EOs) are the replacement of synthetic undesired antibacterial agents by the natural ones. It contributes also on the increase of the sensitivity of foodborne microorganisms with respect to the tested EOs where the high resistance of microorganisms against antibacterial agents is increasing exponentially. Besides that, it reduces the high volatility and the intense aroma of these EOs when they are used as natural preservative agents by the food industry.

## 4. Conclusion

In this study *C. cyminum* and *C. zeylanicum* EOs were successfully extracted using a Clevenger type apparatus, their GC-MS analysis revealed the presence of 12 and 21 components accounting for 99.69% and 98.79% of their respective total composition. In addition, both EOs were characterized by the dominance of oxygenated monoterpenes (79.78 % for *C. cyminum* and 91.76% for *C. zeylanicum* EOs) compounds known for their wide array of antibacterial activities among others. The qualitative antibacterial assay has shown that all the tested bacterial strains were sensitive to both EOs especially for *C. zeylanicum* EOs showing the highest zones of the growth inhibition. Furthermore, the same ascertainment was found in the quantitative antibacterial test, where *C. zeylanicum* EO displayed the lowest MICs values as compared *C. cyminum* EO. Finally, it should be pointed that the combination of *C. cyminum* and *C. zeylanicum* EOs exhibited appreciated additive and robust synergetic effects against the tested microorganisms, resulting on the decrease of their individual MICs values. Giving the above-described results, the combination of *C. cyminum* and *C. zeylanicum* EOs could be considered as a potential alternative to control the growth of undesired foodborne microorganisms in food and beverage.

## Acknowledgment

The authors are thankful to Pasteur institute of Algiers (Algeria) for providing the microorganisms.

## Conflict of interest

We declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

- [1] Jacob, C., Mathiasen, L., & Powell, D. (2010). Designing effective messages for microbial food safety hazards. *Food Control*, 21(1), 1-6.
- [2] WHO (2022). Food safety: Key fact. Geneva: World Health Organization.
- [3] Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interactions with food components. *Food Microbiology*, 26(2), 142-150.
- [4] Fleming-Jones, M. E., & Smith, R. E. (2003). Volatile organic compounds in foods: A five year study. *Journal of agricultural and food chemistry*, 51(27), 8120-8127.
- [5] Kalghatgi, S., Spina, C. S., Costello, J. C., Liesa, M., Morones-Ramirez, J. R., Slomovic, S., ... & Collins, J. J. (2013). Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. *Science translational medicine*, 5(192), 192ra85-192ra85.
- [6] Singh, S., Chaurasia, P. K., & Bharati, S. L. (2022). Functional roles of Essential oils as an effective alternative of synthetic food preservatives: A review. *Journal of Food Processing and Preservation*, 46(8), e16804.
- [7] Arena, P., Rigano, F., Guarnaccia, P., Dugo, P., Mondello, L., & Trovato, E. (2022). Elucidation of the Lipid Composition of Hemp (*Cannabis sativa* L.) Products by Means of Gas Chromatography and Ultra-High Performance Liquid Chromatography Coupled to Mass Spectrometry Detection. *Molecules*, 27(10), 3358.
- [8] Deans, S. G., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International*

*journal of food microbiology*, 5(2), 165-180.

[9] Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—a review. *Food and chemical toxicology*, 46(2), 446-475.

[10] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, 94(3), 223-253.

[11] Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., & Yildirim, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of agricultural and food chemistry*, 53(24), 9452-9458.

[12] DeMartino, L., De Feo, V., Formisano, C., Mignola, E., & Senatore, F. (2009). Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart growing wild in Campania (Southern Italy). *Molecules*, 14(8), 2735-2746.

[13] Rahman, A., & Kang, S. C. (2009). *In vitro* control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. *Food Chemistry*, 116(3), 670-675.

[14] Gao, C., Tian, C., Lu, Y., Xu, J., Luo, J., & Guo, X. (2011). Essential oil composition and antimicrobial activity of *Sphallerocarpus gracilis* seeds against selected food-related bacteria. *Food Control*, 22(3-4), 517-522.

[15] Smith, R. L., Cohen, S. M., Doull, J., Feron, V. J., Goodman, J. I., Marnett, L. J., ... & Adams, T. B. (2005). A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: essential oils. *Food and chemical toxicology*, 43(3), 345-363.

[16] Du Plooy, W., Regnier, T., & Combrinck, S. (2009). Essential oil amended coatings as alternatives to synthetic fungicides in citrus postharvest management. *Postharvest Biology and Technology*, 53(3), 117-122.

[17] Turek, C., & Stintzing, F. C. (2013). Stability of essential oils: a review. *Comprehensive reviews in food science and food safety*, 12(1), 40-53.

[18] Shelef, L. A., Jyothi, E. K., & Bulgarellii, M. A. (1984). Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. *Journal of Food Science*, 49(3), 737-740.

[19] Nazer, A. I., Kobilinsky, A., Tholozan, J. L., & Dubois-Brissonnet, F. (2005). Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella* sv. *Typhimurium*: a synergistic effect?. *Food microbiology*, 22(5), 391-398.

[20] Zhang, Z., Vriesekoop, F., Yuan, Q., & Liang, H. (2014). Effects of nisin on the antimicrobial activity of D-limonene and its nanoemulsion. *Food chemistry*, 150, 307-312.

[21] Zahi, M. R., El Hattab, M., Liang, H., & Yuan, Q. (2017). Enhancing the antimicrobial activity of d-limonene nanoemulsion with the inclusion of  $\epsilon$ -polylysine. *Food Chemistry*, 221, 18-23.

[22] Hajlaoui, H., Mighri, H., Noumi, E., Snoussi, M., Trabelsi, N., Ksouri, R., & Bakhrouf, A. (2010). Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against *Vibrio* spp. strains. *Food and Chemical Toxicology*, 48(8-9), 2186-2192.

[23] Kedia, A., Prakash, B., Mishra, P. K., Dwivedy, A. K., & Dubey, N. K. (2015). Biological activities of *Cuminum cyminum* seed oil and its major components against *Callosobruchus chinensis* and *Sitophilus oryzae*. *Journal of Asia-Pacific Entomology*, 18(3), 383-388.

[24] Jayaprakasha, G. K., & Rao, L. J. M. (2011). Chemistry, biogenesis, and biological activities of *Cinnamomum zeylanicum*. *Critical reviews in food science and nutrition*, 51(6), 547-562.

[25] Phu, H. H., Pham Van, K., Tran, T. H., & Pham, D. T. N. (2022). Extraction, Chemical Compositions and Biological Activities of Essential Oils of *Cinnamomum verum* Cultivated in

Vietnam. *Processes*, 10(9), 1713.

[26] Petretto, G. L., Fancello, F., Bakhy, K., Faiz, C. A., Sibawayh, Z., Chessa, M., ... & Pintore, G. (2018). Chemical composition and antimicrobial activity of essential oils from *Cuminum cyminum* L. collected in different areas of Morocco. *Food bioscience*, 22, 50-58.

[27] Jardak, M., Mnif, S., Ayed, R. B., Rezgui, F., & Aifa, S. (2021). Chemical composition, antibiofilm activities of Tunisian spices essential oils and combinatorial effect against *Staphylococcus epidermidis* biofilm. *Lwt*, 140, 110691.

[28] Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. (2016). Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Food Control*, 59, 282-289.

[29] Alizadeh Behbahani, B., Falah, F., Lavi Arab, F., Vasiee, M., & Tabatabaee Yazdi, F. (2020). Chemical composition and antioxidant, antimicrobial, and antiproliferative activities of *Cinnamomum zeylanicum* bark essential oil. *Evidence-based complementary and alternative medicine*, 2020.

[30] Council of Europe. (2007). European Directorate for the Quality of Medicines. *European Pharmacopoeia*, 6th ed. Council of Europe, Strasbourg, France.

[31] Firuzi, O., Asadollahi, M., Gholami, M., & Javidnia, K. (2010). Composition and biological activities of essential oils from four *Heracleum* species. *Food chemistry*, 122(1), 117-122.

[32] Zahi, M. R., Liang, H., & Yuan, Q. (2015). Improving the antimicrobial activity of D-limonene using a novel organogel-based nanoemulsion. *Food Control*, 50, 554-559.

[33] Yazgan, H., Ozogul, Y., & Kuley, E. (2019). Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on food-borne pathogens and fish spoilage bacteria. *International journal of food microbiology*, 306, 108266.

[34] Rota, M. C., Herrera, A., Martínez, R. M., Sotomayor, J. A., & Jordán, M. J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food control*, 19(7), 681-687.

[35] Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*, 44(9), 3057-3064.

[36] Mangoni, M. L., Epand, R. F., Rosenfeld, Y., Peleg, A., Barra, D., Epand, R. M., & Shai, Y. (2008). Lipopolysaccharide, a key molecule involved in the synergism between temporins in inhibiting bacterial growth and in endotoxin neutralization. *Journal of Biological Chemistry*, 283(34), 22907-22917.

[37] Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, 4(3), 58.

[38] Ghasemi, G., Fattahi, M., & Alirezalu, A. (2020). A new source of oxygenated monoterpenes with phytotoxic activity: essential oil of *Cuminum Cyminum* L. from Iran. *Natural product research*, 34(6), 843-846.

[39] Radi, M., Ahmadi, H., & Amiri, S. (2022). Effect of cinnamon essential oil-loaded nanostructured lipid carriers (NLC) against *Penicillium citrinum* and *Penicillium expansum* involved in tangerine decay. *Food and Bioprocess Technology*, 15(2), 306-318.

[40] Khalil, N., Ashour, M., Fikry, S., Singab, A. N., & Salama, O. (2018). Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits. *Future Journal of Pharmaceutical Sciences*, 4(1), 88-92.

[41] Bozin, B., Mimica-Dukic, N., Simin, N., & Anackov, G. (2006). Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of agricultural and food chemistry*, 54(5), 1822-1828.