# Systematic analysis of antimicrobial activity, phytochemistry, and *in silico* molecular interaction of selected essential oils and their formulations from different Indian spices against foodborne bacteria

Neha Gupta [a], Soham Bhattacharya [b], Klára Urbanová [c], Adrish Dutta [a], Alok Kumar Hazra [d], Eloy Fernández-Cusimamani [a,*], Olga Leuner [a]

[a] *Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, Suchdol, 165 00 Prague 6, Czech Republic*
[b] *Department of Agroecology and Crop Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6 – Suchdol, 165 00, Czech Republic*
[c] *Department of Sustainable Technologies, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic*
[d] *Analytical Research Division, Chromogen, Howrah, India*

A R T I C L E   I N F O

A B S T R A C T

Essential oils (EOs) from Indian spices like *Elettaria cardamomum* (L.) Maton (small green cardamom), *Syzygium aromaticum* (L.) Merr. & L.M. Perry (clove), *Cinnamomum zeylanicum* Blume (cinnamon quills), and *Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm (Indian bay leaves) exhibit a broad spectrum range of biological activity including antibacterial and anti-fungal activity. Yet, there is a lack of data regarding the antimicrobial activity of their formulations. Also, the link between the antimicrobial effect of individual EO with their chemical composition and molecular interaction with bacterial pathogens has not been systematically explored. Therefore, the objectives of the current study were to evaluate the antimicrobial activity and phytochemical characterization of EOs and to bridge the gap between them through in-silico molecular interactions. The antibacterial activity of EOs of four different spices and their formulations against foodborne pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was evaluated using the disc volatilization method. The chemical profile of the individual EO was determined through GC-MS analysis and molecular interactions of identified major components with bacterial proteins were carried out through molecular docking studies. All EOs and their formulations exhibited antibacterial activity ranging from 5.92 to 24.55 mm and 11–23.52 mm, respectively. Among all EOs, cinnamon and formulation C (cardamom: cinnamon- 2:1) exhibited the highest antibacterial activity. The composition of the EOs included sesquiterpenes, monoterpenoids, monoterpenes, and, phenylpropanoids such as (E)-cinnamaldehyde, δ-cadinene, α-copaene, eugenol, caryophyllene, eugenol acetate, methyl eugenol, menthadiene, eucalyptol, α-terpinyl acetate, and sabinene. Furthermore, docking study revealed that the abundant compounds from cinnamon EO mainly α-copaene and δ-cadinene had a high binding affinity towards the bacterial essential proteins which increases the bacterial susceptibility towards cinnamon EO. The selected EOs and their formulations were systematically

---

\* Corresponding author.
*E-mail address:* eloy@ftz.czu.cz (E. Fernández-Cusimamani).

analysed and they were effective against foodborne pathogens. The current findings suggest the application of these EOs against food pathogens with further research.

## 1. Introduction

Foodborne diseases due to bacterial transmissions such as anorexia, nausea, vomiting, and diarrhea, with or without fever, are leading causes of death, especially among children and elderly people. The complex interaction between humans and foodborne diseases depends on various factors like consumption of contaminated food such as meat, dairy products, vegetables, and so on, emphasizing the interdependence of human, animal, and environmental health [1]. The outbreaks caused due to foodborne diseases lead to high morbidity and mortality as well as pose a huge negative impact on the economy worldwide [2]. According to the United Nations, about 600 million cases of foodborne diseases and up to 4,20,000 deaths have been reported across the world annually [3]. Some of the major foodborne disease outbreaks may take place in massive proportions. For instance, due to the *Escherichia coli* outbreak in 2011, more than 12,600 people were infected, and a total of 4321 cases and 50 deaths were reported in Germany [4]. According to the World Health Organization (WHO) between the years 2007 and 2015, the number of infected victims due to multidrug-resistant *Pseudomonas aeruginosa* increased from 23,575 to 68,278, and the number of deaths went up to 4564 from 1573 in the European Union region [5]. Back in 2019, an epidemiological investigation revealed a staphylococcal food poisoning outbreak in Italy where the victims consumed contaminated chicken salad [6].

To circumvent these foodborne bacterial pathogens, there are different antibiotics available in the market. Due to over-reliance on antibiotics and chemical preservatives in the past consecutive years, the bacterial strains have become multi-drug resistant and have had serious side effects on consumers [1,7]. Moreover, if no actions are taken against the spread of antibiotic resistance, it is estimated that the death rate will rise approximately to 10 million with a huge economic loss of more than $100 trillion by 2050 [8,9]. There is a worldwide trend of "green" consumerism, aiming to minimize the use of synthetic additives in foods with enhanced nutrition, safety, quality, and shelf-life. The food industries must emphasize a way to estimate the risks to human health from the consumption of contaminated food and identify, select, and implement mitigation strategies to control and reduce these risks by exploring the trend of "green" consumerism [10]. EOs are one of the promising natural alternatives which are aromatic, volatile, and concentrated hydrophobic liquids with complex blends of a ubiquitous range of biologically active compounds that can be used to counter the spread of pathogenic microbes that cause severe and life-threatening infections. EOs are recognized analyzingsafe by the Food and Drug Administration of the United States and are widely used for their biodegradability and low or non-toxic effects against vertebrates [11]. Nowadays, food preservatives containing EOs are commercially available and are being widely consumed by society. For example, "DMC Base Natural" is a food preservative that comprises about 50 % of EOs from rosemary, sage, and citrus [12]. "Protecta One" and "Protecta Two" are fused herbal extracts consisting of one or more EOs [13]. Also, Indian companies like FabIndia, Nutriorg, RAS luxury oils, and SoulFlower are using EOs extracted from basil, lemongrass, and peppermint as flavoring agents in beverages, desserts, or culinary creations.

India is well renowned for its rich culinary spice heritage which plays a significant role in Indian cuisine. Indian spices are highly valued for their aromatic flavors, vibrant colors, and medicinal properties. Some well-known Indian spices like *Elettaria cardamomum* (small green cardamom), *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum* (cinnamon quills), and *Cinnamomum tamala* (Indian bay leaves) have been used to thwart spoilage of food items and as flavoring agents since time immemorial. The compounds isolated from the EOs of different Indian spices have shown antibacterial, antiviral, antifungal, and antioxidant activities [14–16], and are potential agents used commercially in food industries. Even though there are several pieces of literature that reported antimicrobial activity of the EOs against foodborne pathogens [17,18], it is observed that the chemical composition and biological activity of these EOs vary on the basis of geographical and agro-climatic locations due to several edaphic factors along with environmental and genetic factors [19,20]. Regardless of the previously reported study on EOs of these spices, their antimicrobial effect along with their chemical composition and molecular interaction with bacterial pathogens has not been systematically studied, thereby leaving an existing knowledge gap between the molecular interaction of phytochemicals with antimicrobial competency. Also, none of them have been reported against their formulations by broadening the concern about antimicrobial activity. Therefore, our research aims to examine the antimicrobial activity of these selected EOs and their formulations against foodborne pathogens along with characterization of their phytochemical compositions and bridging the gap between antimicrobial activity and chemical compositions through molecular docking study. Our current findings not only fill the existing knowledge gap in this field but also provide the basis for further research to establish other EOs as potent food preservative agents.

## 2. Materials and methods

### 2.1. Collection of materials and sample preparation

Dried spices of *Cinnamomum zeylanicum* (cinnamon quills), *Cinnamomum tamala* (bay leaf), *Elettaria cardamom* (cardamom), and *Syzygium aromaticum* (clove) were purchased from a local spice store (supplier- S.R. Das grocery store, Kolkata, India). All spices were authenticated at the Quality Testing Laboratory of RKMVERI, Narendrapur. Spice materials were grounded and homogenized by the Grindomix apparatus.

## 2.2. Preparation of EOs and their formulations

EOs from spices were obtained by hydrodistillation from ground materials. 100 g of ground spice materials were placed in 1L of distilled water and hydrodistilled for 3 h using a Clevenger-type apparatus. Extracted EOs were collected and dried over anhydrous sodium sulfate and stored in glass vials at 4 °C until further use. The concentration of EOs suspension was kept at 90 % along with 10 % of acetone for antibacterial assay. All the obtained EOs were combined in different proportions to investigate their additive combinatory effects (Table 1).

## 2.3. Bacterial strains and culture media

In this study, the antibacterial activity was performed against two Gram-positive and two Gram-negative foodborne bacteria. Grampositive: *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96). Gram-negative: *Escherichia coli* (MTCC 1687) and *Pseudomonas aeruginosa* (MTCC 741). Nutrient media was used for both cultivation and assay (broth/agar). All media were purchased from Himedia (Mumbai, Maharashtra, India).

Stock cultures of all bacterial strains were cultivated in nutrient media at 37 °C for 24 h before testing and turbidity of bacterial suspension was adjusted ranging from 0.08 to 0.1 according to 0.5 McFarland constant using a spectrophotometer. Ampicillin and acetone were used as the positive and negative controls (purchased from Himedia).

## 2.4. Antimicrobial assay

The in-vitro antibacterial activity of EOs from all four spices and their formulations was evaluated by Kirby Bauer's disc diffusion method. The experiment was performed using plastic petri plates (90 mm × 15 mm). Initially, the plates were subsequently inoculated with a bacterial suspension using a glass spreader by spread plate technique. The inoculated and non-inoculated plates were prepared simultaneously to confirm growth and purity controls. Sterilized Whatman filter paper discs of 6 mm diameter were placed on the inoculated plates in which the inoculum was spread and with the help of a micropipette 6 μl of EO samples was added to each disc, respectively. Similarly, Ampicillin (100 μg/ml) and acetone were added separately on other discs as positive and negative controls, respectively. All plates were incubated at 37 °C for 24 h. The zone of inhibition (diameter) was recorded in each case. All antibacterial experiments were done in triplicates in three independent measurements and the mean was taken for the final value calculation.

## 2.5. Chemical analysis of individual EOs

For characterization of the individual EOs, GC-MS analysis was performed using a gas chromatograph Agilent GC-7890B system (Agilent Technologies, Santa Clara, CA, USA) coupled with a single quadrupole mass selective detector Agilent MSD- 5977B. Column used was a fused-silica HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent 19091s-433). For carrier gas, helium was used at a flow rate of 1 ml/min. The separation of components was executed by 1 μl injection onto capillary column HP-5ms. The injector temperature was 240 °C. The oven was programmed with an initial temperature of 45 °C for 1 min and then increased to 240 °C at a rate of 3 °C/min. The transfer line temperature was kept at 250 °C. EOs were diluted in n-hexane for GC-MS analysis at 20 μl/mL concentration. 1 μl of the solution was injected at the split ratio of 50:1. The mass spectra were taken in electron impact ionization (EI) mode at 70 eV and the ion source temperature was 230 °C. The mass range scanned was 30–550 *m/z*. The identification of chemical components was obtained from the comparison of their retention indices (RI), retention times (RT), and spectra with the National Institute of Standards and Technology Library (NIST 2.0.f) and the available literature [21]. The relative percentage content was expressed as the ratio of individual peak area to the total area of all peaks. The RI of the separated compounds were calculated using the retention times of the n-alkanes series ranging from C8 to C40 (Sigma-Aldrich, Prague, Czech Republic).

## 2.6. Molecular docking studies

To understand the interaction of the identified compounds from the individual EOs with bacteria, molecular docking studies were performed. Based on previously published studies, seven universal bacterial proteins were selected. The crystal structure of isoleucyl-tRNA synthetase (PDB ID: 1JZQ), DNA gyrase (PDB ID: 1KZN), dihydropteroate synthase (PDB ID: 2VEG), D-alanine: D-alanine ligase (PDB ID: 2ZDQ), topoisomerase IV (PDB ID: 3RAE), dihydrofolate reductase (PDB ID: 3SRW), and penicillin-binding protein 1a (PDB

**Table 1**
Different formulations of EOs, sample codes, and their ratios.

| Formulation (Code) | EOs Used | Ratios (v/v) |
| --- | --- | --- |
| A | Clove: Cardamom | 1:1 |
| B | Cardamom: Cinnamon | 1:1 |
| C | Cardamom: Cinnamon | 2:1 |
| D | Cardamom: Cinnamon: Bay leaf | 1:1:1 |
| E | Cardamom: Cinnamon: Bay leaf | 1:1:4 |
| F | Cardamom: Cinnamon: Clove | 1:1:1 |
| G | Cardamom: Cinnamon: Clove: Bay Leaf | 1:1:1:1 |

ID: 3UDI) were obtained from Protein Data Bank (https://www.rcsb.org/, accessed on June 18, 2023) [22]. It was prepared as a receptor by removing unwanted chains and residues using UCSF Chimera ver. 1.16; removing water molecules; adding polar hydrogens and Kollman charges using AutoDock Tools software version 1.5.7; and saved as a pdbqt file. Meanwhile, the 3D structures of the ligands were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed on June 18, 2023) [23] as SDF file and converted to a PDB file using OpenBabel Tool version 2.4.1. and was optimized by using MMFF94 force field by Avogadro Software ver. 1.2.0. Some compounds whose 3D structures were not available, were converted from 2D structures downloaded from PubChem using OpenBabel Tool version 2.4.1. Later, it was imported to AutoDock Tools and saved as pdbqt file. Blind docking was performed using a web-based program called CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/php/blinddock.php, accessed on June 18, 2023) [24,25]. After submission of the receptors and ligands, CB-DOCK2 checks the input files, predicts the cavities of the protein, and calculates the centers and sizes of the top 5 cavities. Each center and size were submitted to AutoDock Tools for docking, using the interface of the command prompt. The final results were displayed after the computation of the rounds and the best pose was selected. The interaction and visualization were performed for the best-docked complexes using PyMOL ver. 2.5 and LigPlot + ver. 2.2.

### 2.7. Statistical analysis

The chemical analysis of the EO samples was carried out in one replication only. Antibacterial assay was performed in triplicate and the zone of inhibition was expressed as the mean average with standard deviation. All antimicrobial activity data were analysed using one-way ANOVA (analysis of variance) followed by Tukey's honest significant difference post hoc comparison test ($p < 0.05$).

## 3. Results and discussion

### 3.1. Evaluation of the antibacterial activity of individual EOs

A qualitative disc diffusion method was performed to evaluate the antimicrobial activity of EOs against selected bacterial strains. The growth-inhibiting effect of EOs was shown by evaluating the clear zones on inoculated nutrient agar plates (Fig. S1). The zone of inhibition was an indication of the effectiveness and sensitivity of the EOs against the tested microorganisms. The effect of EOs was recorded (diameter) in each case and the results were summarized in Table 2. It was observed that individual EOs were effective against all selected bacterial strains as the zone of inhibition lies in the region of 5.92–24.55 mm. The zone of inhibition for positive control ranged from 25 to 35 mm, which confirmed that the commercial antibiotic has better effectiveness against all the bacterial species examined in this study. All the EOs exhibited more efficacy against Gram-positive bacteria rather than Gram-negative bacteria. This weak antibacterial activity against Gram-negative bacteria was imputed to the hydrophilic polysaccharide chain present in the outer membrane of the cell wall which prevents the hydrophobic EOs from entering the bacterial cell membrane [26]. In the present study, all the tested bacterial strains were highly sensitive to cinnamon EO (Fig. S2) followed by clove, cardamom, and bay leaf. So, the antibacterial activity of EOs determined in this study can be arranged as cinnamon EO > clove EO > cardamom EO > bay leaf EO. Bay leaf EO showed lower antibacterial efficacy against all tested bacterial strains compared to other EOs. Similar research on bay leaf EO reported a higher antibacterial activity against both Gram-positive and Gram-negative bacteria [27] and a higher antibacterial activity for clove EO was also mentioned [28,29]. It might be possible to explain variations in antibacterial activity by analyzing chemical composition based on geographic location and agro-climatic conditions [19]. It is widely acknowledged that the chemical composition and antimicrobial activities of EOs from different regions depicted that humidity, height, luminosity, circadian cycle, pluviometry, soil, nutritional conditions, method of collection, drying, the portion of the plant, temperature, and seasonality influence the chemical diversity in EOs [19,20]. Furthermore, a study also mentioned a similar range of results against *E. coli* and *S. aureus* [30] which was equivalent to our findings where cinnamon EO was highly effective against both types of bacterial strains and also less effectivity against *P. aeruginosa* was observed. In this study, they found no antibacterial activity of cardamom EO against *E. coli* and *S. aureus* and a higher antibacterial activity against *P. aeruginosa* in contrast to our findings when the EO was used as neat. The discrepancy in this result could be explained by the quality of samples as well as the chemical composition of EOs and most importantly the disparate bacterial strains and diverse methods used in the microbial assay [27]. Similarly, another study has shown a very similar result where cinnamon EO was highly effective on Gram-positive and Gram-negative bacteria [31], and these results establish the fact that it could be used as an effective microbial agent against foodborne bacteria.

**Table 2**
Mean zone of inhibition (mm) produced by individual spice EOs against selected microorganisms.

| Microorganisms | Kirby Bauer's disc diffusion method | | | | |
| --- | --- | --- | --- | --- | --- |
| | Zone of inhibition (mm)* of EOs | | | | Antibiotic (positive control) |
| | Bay leaf | Cardamom | Clove | Cinnamon | Ampicillin |
| *Bacillus subtilis* | 12.05 ± 0.08 d | 10.03 ± 0.08 c | 15.5 ± 0.06 ab | 24.08 ± 0.13 b | 25.32 ± 0.07 a |
| *Staphylococcus aureus* | 12.58 ± 0.08 bc | 10.5 ± 0.06 d | 14.98 ± 0.04 c | 22.02 ± 0.04 b | 35.32 ± 0.07 a |
| *Escherichia coli* | 6.07 ± 0.08 c | 6.05 ± 0.08 c | 14.47 ± 0.24 d | 24.55 ± 0.08 b | 33.34 ± 0.14 a |
| *Pseudomonas aeruginosa* | 5.92 ± 0.08 d | 7.02 ± 0.10 c | 16 ± 0.71 b | 23.03 ± 0.10 ab | 36.22 ± 0.85 a |

*Zone of inhibition <5 mm is considered as not detected i.e., ND. Negative control is acetone which did not show any inhibition zone in all the experiments. Different superscript letters within the same row differ significantly (Tukey HSD Test, $p < 0.05$).

## 3.2. Evaluation of the antibacterial activity of EOs formulations

Since individual EOs showed antibacterial activity against tested microbes, it was worth studying the effectiveness of different EO formulations against the same microorganisms. Qualitative disc diffusion method was used to evaluate the antimicrobial activity of different EO formulations. The growth inhibitory effect of all formulations against tested microbes was recorded by evaluating the zone of inhibition (diameter) and summarized in Table 3. It was observed that all EO formulations were comparatively effective against all tested bacterial strains as the zone of inhibition ranged between 11 and 23.52 mm. Based on the zone of inhibition, it was observed that the overall range of antibacterial activity had increased in comparison to individual EOs as the zone of inhibition of individual EOs ranged between 5.92 and 24.55 mm. This increasing efficacy of EO combinations was due to the synergistic and additive effect of individual components [32]. Also, an interesting observation was that all the formulations showed similar activity against both Gram-positive and Gram-negative bacterial strains whereas the individual EOs were more effective against Gram-positive bacteria. Among all formulations, sample C (cardamom: cinnamon- 2:1) showed the highest activity against all tested microbes (Fig. S3) followed by sample E (cardamom: cinnamon: bay leaf- 1:1:4) and sample D (cardamom: cinnamon: bay leaf- 1:1:1). A study has observed similar increased additive effect of EO combination against both Gram-positive and negative bacteria in comparison to individual EOs [33]. Another similar result was also reported where they observed an increased combinatory effect of EOs against *S. aureus* [34]. Antimicrobial activity is closely related to the interaction of chemical compounds of EOs with bacterial receptors. So, these findings require a more in-depth investigation into the effect of EO formulations on antimicrobial activity.

## 3.3. Chemical analysis of EOs

In this investigation, EOs from four different spices (cardamom, bay leaf, clove, and cinnamon) were extracted with respective yield values of 2.06, 0.92, 1.34, and 0.41 %. There was a strong peculiar fragrance associated with all EOs and their colors ranged from colorless to pale yellow. After the GC-MS analysis, we identified 18 compounds for cardamom, 35 compounds for bay leaf, 6 compounds for clove, and 23 compounds for cinnamon were identified which represented 99.42, 98.15, 99.27, and 99.88 % of their corresponding total constituents, respectively. The complete chemical analysis of all EOs is presented in Table 4. In bay leaf EO, phenylpropanoids represented by eugenol, methyleugenol, and menthadiene were found as the most abundant compounds with 22.30, 16.39, and 11.85 % of the total composition, respectively. The rest of the compounds were detected in amounts lower than 2.35 %, except spathulenol, β-cyclogermacrane, caryophyllene, and *m*-cymene. In cardamom EO, eucalyptol (monoterpenoid) was found as the most abundant compound with 43.46 % of the total composition followed by α-terpinyl acetate (monoterpenoid), and sabinene (monoterpene) with 40.33, and 4.89 %, respectively. The rest of the components did not exceed 2.47 %. In cinnamon EO, sesquiterpenes represented by (E)-cinnamaldehyde, δ-cadinene, and α-copaene were found as the most abundant compounds with 87.04, 2.51, and 2.41 % of the total composition, respectively. The rest of the compounds were detected below the amount of 2.28 %. Also, in clove EO, eugenol was the most abundant compound with 69.83 % followed by caryophyllene (sesquiterpenes), and eugenol acetate (phenylpropanoid) with a percentage of 23.29 and 3.58 %, respectively. The other compounds did not exceed 1 % except for humulene.

The antibacterial properties of EOs mainly depend on their chemical compositions, which have been already established extensively. The major constituents of these EOs were mainly sesquiterpenes, monoterpenoids, monoterpene, and phenylpropanoids. Our findings of abundant compounds of all EOs through chemical analysis depicted similar results in accordance with previously published studies [35–38]. Also, the variation of EO yields and concentration of chemical constituents depend on various factors such as the growing condition of plants, harvesting, and storage conditions [26]. However, different sources indicate the extent of variability in composition for bay leaf EO [39]. According to the antimicrobial activity, cinnamon EO showed the highest activity and (E)-cinnamaldehyde was found to be the most abundant compound in our study followed by α-copaene, and δ-cadinene mainly contributed to its antimicrobial activity. For clove EO, eugenol, caryophyllene, and eugenol acetate were the abundant compounds that were possibly responsible for antimicrobial activity. Also, for cardamom and bay leaf EOs eucalyptol, α-terpinyl acetate, and sabinene; and eugenol, methyleugenol, and menthadiene were abundant compounds that played major roles in their antibacterial activity. However,

**Table 3**

Mean zone of inhibition (mm) produced by EO formulations against selected microorganisms.

| Sample code | Kirby Bauer's disc diffusion method | | | |
| --- | --- | --- | --- | --- |
| | Zone of inhibition (diameter in mm)* | | | |
| | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* |
| A | 13.43 ± 0.12 cd | 11 ± 0.13 e | 12 ± 0.63 ab | 11.07 ± 0.10 [bc] |
| B | 15.13 ± 0.15 e | 14.7 ± 0.63 ac | 15.03 ± 0.10 e | 14.95 ± 0.08 ab |
| C | 23.05 ± 0.08 a | 22.38 ± 0.04 a | 23.52 ± 0.04 a | 22.02 ± 0.04 b |
| D | 19.98 ± 0.04 b | 16 ± 1.26 c | 18.17 ± 0.08 c | 23 ± 0.63 a |
| E | 21 ± 0.13 c | 17.23 ± 0.05 b | 18 ± 0.63 d | 22 ± 0.38 b |
| F | 18.13 ± 0.14 [bc] | 15.03 ± 0.05 d | 14.03 ± 0.08 [bc] | 16.07 ± 0.10 d |
| G | 19.15 ± 0.08 ab | 15.1 ± 0.15 d | 18.98 ± 0.04 b | 14.62 ± 0.04 e |

*Zone of inhibition <5 mm is considered as not detected i.e., ND. Negative control is acetone which did not show any inhibition zone in all the experiments and the positive control is ampicillin. Different superscript letters within the same column differ significantly (Tukey HSD Test, p < 0.05).

**Table 4**
Chemical composition of four individual spice EOs.

Content [%][c]

| RI[a] | RI (lit)[b] | Compounds | Bay leaf | Cardamom | Cinnamon | Clove |
|---|---|---|---|---|---|---|
| 926 | 931 | 3-Thujene | 0.68 | 0.17 | – | – |
| 933 | 937 | α-Pinene | 1.68 | 1.29 | 0.09 | – |
| 946 | 953 | Fenchene | tr | – | – | – |
| 966 | 961 | Benzaldehyde | – | – | 0.24 | – |
| 974 | 976 | Sabinene | 0.32 | 4.89 | – | – |
| 978 | 980 | Nopinene | 0.43 | 0.55 | – | – |
| 991 | 991 | Myrcene | 0.43 | 2.47 | – | – |
| 1007 | 1005 | Menthadiene | 11.85 | – | – | – |
| 1012 | 1011 | 3-Carene | 0.79 | – | – | – |
| 1018 | 1004 | (+)-4-Carene | 0.2 | 0.45 | – | – |
| 1026 | 1026 | *m*-Cymene | 5.21 | – | – | – |
| 1030 | 1027 | *m*-Mentha-6,8-diene | 0.87 | – | – | – |
| 1035 | 1035 | Eucalyptol | 1.81 | 43.46 | – | – |
| 1048 | 1050 | Ocimene | 0.41 | – | – | – |
| 1059 | 1062 | γ-Terpinene | 0.35 | 0.62 | – | 0.08 |
| 1070 | 1070 | cis-Sabinene hydrate | – | 0.21 | – | – |
| 1090 | 1088 | Terpinolene | 0.96 | 0.44 | – | – |
| 1104 | 1098 | Linanool | – | 0.9 | – | – |
| 1117 | 1116 | (E)-4,8-Dimethylnona-1,3,7-triene | – | 0.1 | – | – |
| 1173 | 1165 | Camphol | – | – | 0.2 | – |
| 1182 | 1182 | L-Terpinen-4-ol | 0.69 | 1.81 | – | – |
| 1198 | 1189 | ʟ-α-Terpineol | 2.35 | 1.24 | – | – |
| 1219 | 1214 | (E)-Cinnamaldehyde | – | – | 87.04 | – |
| 1257 | 1256 | Linalyl formate | – | 0.27 | – | – |
| 1270 | 1266 | (Z)-Cinnamaldehyde | – | – | 0.53 | – |
| 1293 | 1283 | Anethole | 0.32 | – | – | – |
| 1327 | 1319 | Methylgeranate | – | 0.08 | – | – |
| 1352 | 1339 | δ-Elemene | 0.11 | – | – | – |
| 1357 | 1354 | α-Terpinyl acetate | – | 40.33 | – | – |
| 1370 | 1368 | Cyclosativene | – | – | 0.28 | – |
| 1376 | 1356 | Eugenol | 22.3 | – | – | 69.83 |
| 1381 | 1376 | α-Copaene | – | – | 2.41 | – |
| 1395 | 1396 | (+)-Sativen | – | – | 0.31 | – |
| 1412 | 1401 | Methyleugenol | 16.39 | – | – | – |
| 1416 | 1417 | Isosativene | – | – | 0.14 | – |
| 1425 | 1418 | Caryophyllene | 6.19 | – | 0.11 | 23.29 |
| 1432 | 1451 | Methyl isoeugenol | 0.23 | – | – | – |
| 1444 | 1447 | Aromandendrene | 0.52 | – | – | – |
| 1459 | 1455 | Humulene | 0.62 | – | – | 2.26 |
| 1481 | 1477 | γ-Muurolene | 0.15 | – | 0.65 | – |
| 1486 | 1480 | ᴅ-Germacrene | 1.9 | – | 0.13 | – |
| 1492 | 1491 | Valencene | 0.92 | – | – | – |
| 1466 | 1485 | β-Selinene | 0.15 | 0.14 | – | – |
| 1502 | 1499 | β-Cyclogermacrane | 9.77 | – | – | – |
| 1506 | 1499 | α-Muurolene | – | – | 2.28 | – |
| 1511 | 1508 | Farnesene | – | – | – | 0.23 |
| 1520 | 1513 | γ-Cadinene | – | – | 0.09 | – |
| 1528 | 1524 | δ-Cadinene | 0.54 | – | 2.51 | – |
| 1535 | 1524 | Eugenol acetate | 0.37 | – | – | 3.58 |
| 1538 | 1533 | Cubenene | – | – | 0.31 | – |
| 1546 | 1544 | 4,5,9,10-Dehydro-isolongifolene | – | – | 0.25 | – |
| 1550 | 1566 | β-Calacorene | – | – | 0.81 | – |
| 1563 | 1561 | Germacrene B | 1.42 | – | – | – |
| 1587 | 1576 | Spathulenol | 6.36 | – | – | – |
| 1636 | 1627 | Epicubenol | – | – | 0.23 | – |
| 1648 | 1644 | β-Spathulenol | 0.83 | – | – | – |
| 1652 | 1640 | T-Muurolol | – | – | 0.49 | – |
| 1655 | 1654 | α-Muurolol | – | – | 0.57 | – |
| 1665 | 1645 | T-Cadinol | – | – | 0.12 | – |
| 1687 | 1676 | Mustakone | – | – | 0.09 | – |
| | | **Total identified %** | **98.12** | **99.42** | **99.88** | **99.27** |

[a] Kovats' retention indices measured on HP-5MS column.

[b] retention indices from literature.

[c] relative percentage content based on the total area of all peaks; tr: trace amount (<0.05 %); -: not detected.

according to previous reports, eugenol was the most abundant compound in cinnamon EO [40,41]. This variation in chemical constituents of EOs could be attributed to various factors mainly based on geographical and agro-climatic differences [19]. The quality and quantity of a plant's secondary metabolites could be significantly influenced by various factors including climate conditions, air humidity, altitude, wind speed, solar radiation, and edaphic factors. All of these factors were involved in the activation mechanisms of certain enzymes which are essential for specific biosynthetic pathways. In addition, environmental stress can affect gene expression, specific enzyme production, and the transformation of various chemical compounds into volatile compounds. Also, soil nutrition and ecological factors influence plant's vegetative growth which can enhance EO yield quality and quantity [42,43]. These factors not only affect the chemical composition of the EO but also the antimicrobial activity that is associated with it. For instance, *O. dubium* plants grown at higher altitudes gave lower EO yield with high carvacrol content whereas at lower altitudes EO yield was higher with lower carvacrol content, hence higher antimicrobial activity was observed against *S. sclerotiorum* by the EO obtained from the higher altitudes *O. dubium* plants [44]. However, cinnamaldehyde compound has been elucidated to possess antibacterial activity against Gram-positive and Gram-negative bacteria including *E. coli, B. subtilis, Staphylococcus* spp., and *Pseudomonas* spp. [45,46]. Furthermore, molecular interactions of these abundant compounds with bacterial receptors can fully confirm the whole bioactivity scenario.

### 3.4. Molecular interaction of abundant compounds

In this study, an *in-silico* approach employing molecular docking was used to understand the molecular interaction between major volatile components of EOs of four spices (*E. cardamomum, S. aromaticum, C. zeylanicum, and C. tamala*) and the vital enzymes involved in biosynthesis and repair of cell walls, nucleic acids, and proteins in bacteria such as isoleucyl-tRNA synthetase, DNA gyrase, dihydropteroate synthase, D-alanine: D-alanine ligase, topoisomerase 4, dihydrofolate reductase, and penicillin-binding protein. The binding affinity of all molecular interactions has been summarized in Table 5.

Aminoacyl-tRNA synthetases have been previously identified as possible drug targets for several infectious diseases responsible for charging specific tRNA with amino acid which is essential for protein synthesis [47]. The major volatile compounds found in the EOs namely α-copaene (−7.6 kcal/mol), δ-cadinene (−6.8 kcal/mol), and β-caryophyllene (−6.6 kcal/mol) had the highest binding affinity towards isoleucyl-tRNA synthetase (PDB ID: 1JZQ). DNA gyrase is another essential target for antibacterial agents as it controls the topology of DNA during transcription and replication by introducing breaks to both DNA strands which is very important for bacterial survival. So, molecular docking was carried out with the pocket of DNA gyrase (PDB ID: 1KZN) to study their interactions with the major volatile compounds. The compounds displayed binding energy ranging from −4.4 to −7.2 kcal/mol for all the major volatiles with δ-cadinene (−7.2 kcal/mol) from cinnamon EO showing the highest binding energy. The dihydropteroate synthetase (PDB ID: 2VEG) is responsible for the synthesis of dihydrofolic acid which is essential for DNA synthesis. Our study found that the binding affinity ranged from −4.4 to −6.2 kcal/mol for all the major volatiles with β-caryophyllene (−6.2 kcal/mol) from clove EO being the highest followed by α-copaene (−6.0 kcal/mol) from cinnamon. The enzyme D-alanine: D-alanine ligase (PDB ID: 2ZDQ) catalyzes the condensation of two D-Ala molecules using ATP to produce D-Ala-D-Ala, the terminal peptide of a peptidoglycan monomer for the cell wall peptidoglycan polymer [48]. The results reported that α-terpinyl acetate (−7.7 kcal/mol) from cardamom EO and δ-cadinene (−7.7 kcal/mol) from cinnamon EO showed the highest binding affinity and were able to inhibit the growth of microbes, and α-copaene (−7.1 kcal/mol) also contributed to increased activity of cinnamon EO towards various microbes [49]. Another target enzyme topoisomerase 4 (PDB ID: 3RAE) in Gram-positive bacteria catalyzes the separation of daughter strands after replication. The molecular-docking analysis of the present study revealed that binding affinity ranged from −5.3 to −6.5 kcal/mol with sesquiterpenes showing the highest binding affinity. Dihydrofolate reductase (PDB ID: 3SRW) is an enzyme involved in the thymidine synthesis pathway that is crucial for DNA synthesis. It was identified that sesquiterpenes like δ-cadinene (−7.7 kcal/mol), β-caryophyllene (−7.6 kcal/mol), and α-copaene (−7.4 kcal/mol) had a higher binding affinity towards 3SRW enzyme. The Penicillin-binding proteins

**Table 5**
Binding free-energy values of major volatile components of *E. cardamomum, S. aromaticum, C. zeylanicum,* and *C. tamala* EOs as ligands calculated through molecular docking and bacterial metabolic enzymes as receptors.

| Ligands[b] | Binding Free Energy ΔG (kcal/mol) | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | 1JZQ[a] | 1KZN | 2VEG | 2ZDQ | 3RAE | 3SRW | 3UDI |
| Eugenol | −5.8 | −5.6 | −4.6 | −7.1 | −5.5 | −4.8 | −5.3 |
| Menthadiene | −5.7 | −6.0 | −4.8 | −7.5 | −5.9 | −4.6 | −5.0 |
| Methyleugenol | −5.8 | −4.4 | −4.9 | −7.2 | −5.2 | −5.7 | −5.5 |
| α-terpinyl acetate | −6.4 | −5.0 | −5.3 | −7.7 | −6.1 | −6.8 | −5.9 |
| Eucalyptol | −5.6 | −4.2 | −4.4 | −6.3 | −6.1 | −4.6 | −4.4 |
| Sabinene | −5.2 | −4.4 | −4.6 | −6.0 | −6.0 | −5.6 | −4.8 |
| α-copaene | −7.6 | −6.6 | −6.0 | −7.1 | −6.5 | −7.4 | −6.2 |
| (E)-cinnamaldehyde | −5.3 | −4.4 | −4.7 | −4.1 | −5.3 | −4.4 | −4.5 |
| δ-Cadinene | −6.8 | −7.2 | −5.2 | −5.6 | −6.3 | −7.6 | −5.2 |
| β-Caryophyllene | −6.6 | −5.3 | −6.2 | −4.8 | −6.3 | −7.6 | −5.9 |
| Eugenol | −5.8 | −5.6 | −4.6 | −7.1 | −5.5 | −4.8 | −5.3 |
| Eugenol acetate | −6.2 | −5.9 | −5.5 | −7.1 | −5.3 | −5.0 | −5.4 |

[a] Protein PDB ID:1JZQ-isoleucyl-tRNA synthetase, 1KZN- DNA gyrase, 2VEG-dihydropteroate synthase, 2ZDQ-D-alanine:D-alanine ligase, 3RAE-topoisomerase 4, 3SRW-dihydrofolate reductase, and 3UDI-penicillin-binding protein 1a.
[b] Major volatiles of *C. tamala, E. cardamomum, C. zeylanicum,* and *S. aromaticum* EO.

(PDB ID: 3UDI) are responsible for the development of the bacterial cell wall. Therefore, the inactivation of 3UDI proteins limits the development of cell walls eventually causing inhibition of bacterial growth [50]. Our docking study revealed the highest binding with α-copaene (−6.2 kcal/mol) of cinnamon EO.

Since α-terpinyl acetate (−7.7 kcal/mol) and α-copaene (−7.6 kcal/mol) had the best docking scores towards 2ZDQ and 1JZQ, respectively among all the other volatile compounds examined, therefore, binding analysis was performed to reveal the interaction between ligands and protein-binding sites (Fig. 1). There was a hydrogen bond of bond length 2.80 Å between the hydroxyl group of α-terpinyl acetate and LYS A:228 of 2ZDQ. In addition, there were hydrophobic interactions involving 'A chains' of GLU197, ILE163, LEU192, LYS153, PHE151, PHE222, PHE272, SER160, TYR223, and VAL195 (Fig. 1B). On the other hand, α-copaene had hydrophobic interactions involving amino acids GLU135, LEU18, LYS22, PHE27, THR134, TRP21, TRP140, TYR139, and VAL141 (Fig. 1A). According to the antimicrobial study, cinnamon EO showed the highest activity that was associated with our study. Docking results showed α-copaene as the best-docked compound majorly contributing to its antimicrobial activity which states that the contribution of the less abundant compound to antimicrobial activity is huge [51]. For clove EO, β-caryophyllene, eugenol, and eugenol acetate showed good ligand activity with 3SRW and 2ZDQ, respectively that also mainly contributed towards antimicrobial activity. Cardamom EO showed the highest binding affinity of α-terpinyl acetate with the 2ZDQ but the binding affinity with other targeted proteins was found to moderate and also, the docking results of other abundant compounds were less which may have affected its antimicrobial properties. For bay leaf EO all the abundant compounds showed a good binding affinity with 2ZDQ but a weak binding affinity towards other targeted proteins which may have been the reason for its comparatively low antimicrobial activity. Through molecular docking studies, identification of potential targets and prediction of interactions between volatile compounds and bacterial targets with atomic-level precision can be made possible which may validate the potential antimicrobial effects of these EOs in real-world settings. For instance, 5,7-Dihydroxy-3-phenylcoumarin was found as the potential binding ligand towards tyrosyl-tRNA synthetase and topoisomerase II DNA gyrase contributing to the antibacterial activity against Gram-positive bacteria [52]. This could be explained by the number of hydrophobic bond interactions between ligands and proteins where EO components bind to the cell surface forming a monolayer around the cell that modifies the electrostatic potential and hydrophobicity and therefore, destabilizes the membrane integrity that resulted in the release of internal cellular components [53]. Here the number of hydrophobic bonds were less for compounds with low dock score compared to compounds with high dock score which explained the binding affinity and antimicrobial activity of the respective EOs. However, there are many hydrophobic atoms in drugs available in the market which defines the importance of these interactions in drug designing [54].
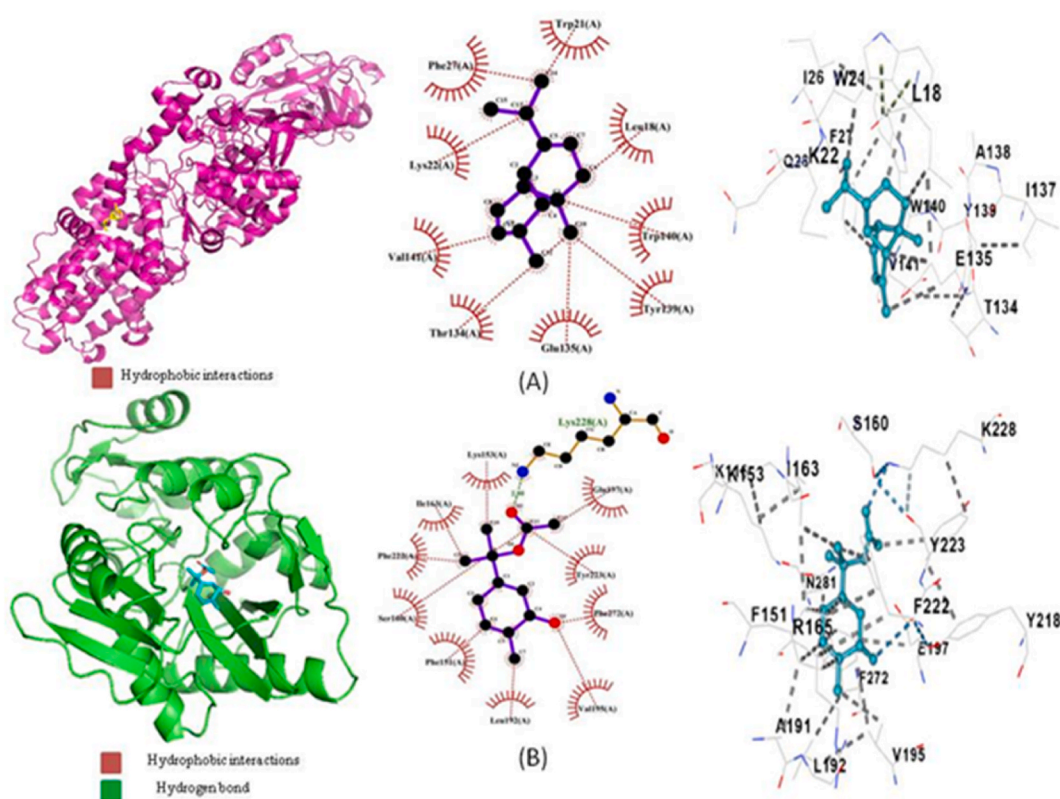


**Fig. 1.** 3D structures and 2D and 3D Interactions of (A) α-copaene with isoleucyl-tRNA synthetase 1JZQ and (B) α-terpinyl acetate with D-alanine:D-alanine ligase 2ZDQ.

## 4. Conclusions

To summarize, this study evaluated the antibacterial activity of EOs from four different spices and their formulations against four standard bacterial strains associated with foodborne diseases namely *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*. While all bacterial strains were sensitive against all EOs to a certain range, Gram-positive strains of *B. subtilis*, and *S. aureus* showed the highest susceptibility. While comparing the antimicrobial activities among the four EOs, cinnamon EO showed the highest activity against all bacterial strains tested in this study. The comparative antibacterial activity of different EO combinations revealed that formulation sample C (cardamom: cinnamon- 2:1) showed the highest activity against all tested microbes. The GC-MS analysis of individual EO samples identified phenylpropanoids represented by eugenol, methyleugenol, and menthadiene as the most abundant compounds in bay leaf EO; eucalyptol, α-Terpinyl acetate, and sabinene in cardamom EO and sesquiterpenes represented by (E)-cinnamaldehyde, δ-cadinene, and α-copaene in cinnamon EO; eugenol, caryophyllene, and eugenol acetate in clove EO. Furthermore, the molecular interactions of these major compounds with essential bacterial proteins through docking studies indicated that α-copaene and δ-cadinene from cinnamon EO had a high binding affinity towards all bacterial proteins which made cinnamon EO capable against all tested microbes in comparison to other EOs. This study systemically analysed the EOs of different Indian spices and their formulations which can be used as a potential antimicrobial agent in food and pharmaceutical industries contributing to green consumerism. The in-silico study helped to make a link between antimicrobial activity and chemical composition and also laid the foundation for the systematic study of EOs that could be effective in analyzing other EOs in the future. Further research based on the antimicrobial activity of these EOs in vapor and liquid phases and the molecular simulation study can shed light to better access the applications of these volatile EOs and their formulations in the healthcare and food industries. Additionally, this study suggests the potential of EOs derived from these four Indian spices to combat foodborne pathogens. However, pharmacological evaluation and quality assessment are needed to verify their potential practical use.

## Data availability statement

Data will be made available on request.

## Additional information

No additional information is available for this paper.

## CRediT authorship contribution statement

**Neha Gupta:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Soham Bhattacharya:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Klára Urbanová:** Writing – review & editing, Investigation, Formal analysis. **Adrish Dutta:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Alok Kumar Hazra:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Eloy Fernández-Cusimamani:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Olga Leuner:** Writing – review & editing, Funding acquisition, Formal analysis.

## Declaration of competing interest

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

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22480.

# References

[1] H. Cui, C. Zhang, C. Li, L. Lin, Antimicrobial mechanism of clove oil on *Listeria monocytogenes*, Food Control 94 (2018) 140–146, https://doi.org/10.1016/j. foodcont.2018.07.007.

[2] W. Hu, C. Li, J. Dai, H. Cui, L. Lin, Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin-resistant Staphylococcus aureus (MRSA), Ind. Crops Prod. 130 (2019) 34–41, https://doi.org/10.1016/j.indcrop.2018.12.078.

[3] H. Lee, Y. Yoon, Etiological agents implicated in foodborne illness world wide, Food Sci. Anim. Resour. 41 (2021) 1–7, https://doi.org/10.5851/kosfa.2020.e75.

[4] U. Buchholz, H. Bernard, D. Werber, M.M. Böhmer, C. Remschmidt, H. Wilking, Y. Deleré, M. an der Heiden, C. Adlhoch, J. Dreesman, J. Ehlers, S. Ethelberg, M. Faber, C. Frank, G. Fricke, M. Greiner, M. Höhle, S. Ivarsson, U. Jark, M. Kirchner, J. Koch, G. Krause, P. Luber, B. Rosner, K. Stark, M. Kühne, German outbreak of *Escherichia coli* O104:H4 associated with sprouts, N. Engl. J. Med. 365 (2011) 1763–1770, https://doi.org/10.1056/NEJMoa1106482.

[5] L.D. Cassini, D. Högberg, A. Plachouras, A. Quattrocchi, G.S. Hoxha, M. Simonsen, M.E. Colomb-Cotinat, B. Kretzschmar, M. Devleesschauwer, D.A. Cecchini, T. C. Ouakrim, M.J. Oliveira, C. Struelens, D.L. Suetens, R. Monnet, K. Strauss, T. Mertens, B. Struyf, K. Catry, I.N. Latour, E.G. Ivanov, A.T. Dobreva, S. Andraševic, A. Soprek, N. Budimir, H. Paphitou, S.S. Žemličková, U.W. Olsen, P. Sönksen, M. Märtin, O. Ivanova, J. Lyytikäinen, B. Jalava, T. Coignard, M.A. Eckmanns, S. Sin, G.L. Haller, A. Daikos, S. Gikas, F. Tsiodras, Á. Kontopidou, Á. Tóth, Ó. Hajdu, K.G. Guólaugsson, S. Kristinsson, K. Murchan, P. Burns, C. Pezzotti, U. Gagliotti, A. Dumpis, M. Liuimiene, M.A. Perrin, S.C. Borg, J.C. de Greeff, M.B. Monen, P. Koek, D. Elstrøm, A. Zabicka, W. Deptula, M. Hryniewicz, P. J. Caniça, P.A. Nogueira, V. Fernandes, G.A. Manageiro, R.I. Popescu, E. Serban, S. Schréterová, M. Litvová, J. Štefkovicová, I. Kolman, A. Klavs, B. Korošec, A. Aracil, M. Asensio, H. Pérez-Vázquez, S. Billström, J.S. Larsson, A. Reilly, S. Johnson, Hopkins, Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis, Lancet Infect. Dis. 19 (2019) 56–66, https://doi.org/10.1016/S1473-3099(18)30605-4.

[6] F. Savini, A. Romano, F. Giacometti, V. Indio, M. Pitti, L. Decastelli, P.L. Devalle, I.S.R. Gorrasi, S. Miaglia, A. Serraino, Investigation of a *Staphylococcus aureus* sequence type 72 food poisoning outbreak associated with food-handler contamination in Italy, Zoonoses Public Health 70 (2023) 411–419, https://doi.org/ 10.1111/zph.13046.

[7] I.I.K. Kashoma Yosra A. Helmy, P. Isaac, The emergence of antibiotic resistance in poultry farms Gireesh Rajashekara, the Ohio State University, in: Achiev. Sustain. Prod. Poult. Meat, vol. 1, Burleigh Dodds Science Publishing, USA, 2016.

[8] C.J.L. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G.R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S.C. Johnson, A.J. Browne, M.G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B.H.K. Hamadani, E.A.P. Kumaran, B. McManigal, S. Achalapong, R. Agarwal, S. Akech, S. Albertson, J. Amusai, J. Andrews, A. Aravkin, E. Ashley, F.-X. Babin, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, J.A. Berkley, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalheiro, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, R.C. Donatien, A.J. Cook, B. Cooper, T.R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N.P.J. Day, M.D. Luca, K. Dokova, A. Dramowski, S.J. Dunachie, T.D. Bich, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, C. Garcia, D. Garrett, P. Gastmeier, A.Z. Giref, R.C. Greer, V. Gupta, S. Haller, A. Haselbeck, S.I. Hay, M. Holm, S. Hopkins, Y. Hsia, K.C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, A.W.J. Jenney, M. Khorana, S. Khusuwan, N. Kissoon, E. Kobeissi, T. Kostyanev, F. Krapp, R. Krumkamp, A. Kumar, H.H. Kyu, C. Lim, K. Lim, D. Limmathurotsakul, M.J. Loftus, M. Lunn, J. Ma, A. Manoharan, F. Marks, J. May, M. Mayxay, N. Mturi, T. Munera-Huertas, P. Musicha, L.A. Musila, M.M. Mussi-Pinhata, R.N. Naidu, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C.W. Obiero, T.J. Ochoa, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, P. Ounchanum, G.D. Pak, J.L. Paredes, A. Y. Peleg, C. Perrone, T. Phe, K. Phommasone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, S. Rattanavong, A. Riddell, T. Roberts, J.V. Robotham, A. Roca, V.D. Rosenthal, K.E. Rudd, N. Russell, H.S. Sader, W. Saengchan, J. Schnall, J.A.G. Scott, S. Seekaew, M. Sharland, M. Shivamallappa, J. Sifuentes-Osornio, A. J. Simpson, N. Steenkeste, A.J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Tigoi, C. Turner, P. Turner, H.R. van Doorn, S. Velaphi, A. Vongpradith, M. Vongsouvath, H. Vu, T. Walsh, J.L. Walson, S. Waner, T. Wangrangsimakul, P. Wannapinij, T. Wozniak, T.E.M.W.Y. Sharma, K.C. Yu, P. Zheng, B. Sartorius, A.D. Lopez, A. Stergachis, C. Moore, C. Dolecek, M. Naghavi, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet 399 (2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.

[9] J. O'Neill, Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, Government of the United Kingdom, 2016. https://apo.org.au/ node/63983. (Accessed 12 July 2023).

[10] S. Bautista-Baños, A.N. Hernández-Lauzardo, M.G. Velázquez-del Valle, M. Hernández-López, E. Ait Barka, E. Bosquez-Molina, C.L. Wilson, Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities, Crop Protect. 25 (2006) 108–118, https://doi.org/10.1016/j. cropro.2005.03.010.

[11] D. Kuhn, R. Ziem, T. Scheibel, B. Buhl, G. Vettorello, L.A. Pacheco, D. Heidrich, C. Kauffmann, E.M. de Freitas, E.M. Ethur, L. Hoehne, Antibiofilm activity of the essential oil of *Campomanesia aurea* O. Berg against microorganisms causing food borne diseases, LWT 108 (2019) 247–252, https://doi.org/10.1016/j. lwt.2019.03.079.

[12] M.J. Mendoza-Yepes, L.E. Sanchez-Hidalgo, G. Maertens, F. Marin-Iniesta, Inhibition of *Listeria monocytogenes* and other bacteria by a plant essential oil (dmc) in Spanish soft cheese, J. Food Saf. 17 (1997) 47–55, https://doi.org/10.1111/j.1745-4565.1997.tb00175.x.

[13] C.N. Cutter, Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef†, J. Food Prot. 63 (2000) 601–607, https://doi.org/10.4315/0362-028X-63.5.601.

[14] H. Cui, X. Zhang, H. Zhou, C. Zhao, L. Lin, Antimicrobial activity and mechanisms of *Salvia sclarea* essential oil, Bot. Stud. 56 (2015) 16, https://doi.org/ 10.1186/s40529-015-0096-4.

[15] J. Hu, Y. Zhang, Z. Xiao, X. Wang, Preparation and properties of cinnamon-thyme-ginger composite essential oil nanocapsules, Ind. Crops Prod. 122 (2018) 85–92, https://doi.org/10.1016/j.indcrop.2018.05.058.

[16] Y. Wang, Y. Xia, P. Zhang, L. Ye, L. Wu, S. He, Physical characterization and pork packaging application of chitosan films incorporated with combined essential oils of cinnamon and ginger, Food Bioprocess Technol. 3 (2016) 503–511, https://doi.org/10.1007/s11947-016-1833-8.

[17] B.C. Nzeako, Z.S.N. Al-Kharousi, Z. Al-Mahrooqui, Antimicrobial activities of clove and thyme extracts, Sultan Qaboos Univ. Med. J. 6 (2006) 33–39. https:// www.ncbi.nlm.nih.gov/pmc/articles/PMC3074903/. (Accessed 12 July 2023).

[18] M. Anam, M. Tuseef, T. Gull, A. Saleem, A. Jamil, M.Y. Khan, J.Z. Ashraf, Addition of sweet potato powder and olive oil as fat replacer in chicken sausages coated with carom essential oil, RADS J. Food Biosci. 2 (2023) 19–27. https://jfbs.juw.edu.pk/index.php/jfbs/article/view/16. (Accessed 12 July 2023).

[19] A.F. Gomes, M. Ganzera, S. Schwaiger, H. Stuppner, M. Halabalaki, M.P. Almeida, M.F. Leite, J.G. Amaral, J.M. David, Simultaneous determination of iridoids, phenylpropanoids and flavonoids in *Lippia alba* extracts by micellar electrokinetic capillary chromatography, Microchem. J. 138 (2018) 494–500, https://doi. org/10.1016/j.microc.2018.02.003.

[20] A. Guimarães, A.C. Vinhas, A. Gomes, L. Souza, P. Krepsky, ESSENTIAL OIL of *Curcuma longa* L. RHIZOMES CHEMICAL COMPOSITION, YIELD VARIATION and STABILITY, Quím. Nova., 2020, https://doi.org/10.21577/0100-4042.20170547.

[21] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/mass- Spectrometry, Allured Publishing Co. Carol Stream, Illinois, 2007.

[22] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein Data Bank, Nucleic Acids Res. 28 (2000) 235–242, https://doi.org/10.1093/nar/28.1.235.

[23] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem 2023 update, Nucleic Acids Res. 51 (2023) D1373–D1380, https://doi.org/10.1093/nar/gkac956.

[24] Y. Liu, X. Yang, J. Gan, S. Chen, Z.-X. Xiao, Y. Cao, CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting, Nucleic Acids Res. 50 (2022) W159–W164, https://doi.org/10.1093/nar/gkac394.

[25] X. Yang, Y. Liu, J. Gan, Z.-X. Xiao, Y. Cao, FitDock: protein–ligand docking by template fitting, Brief, Bioinform 23 (2022), https://doi.org/10.1093/bib/ bbac087 bbac087.

[26] J. Antih, M. Houdkova, K. Urbanova, L. Kokoska, Antibacterial activity of *Thymus vulgaris* L. Essential oil vapours and their GC/MS analysis using solid-phase microextraction and syringe headspace sampling techniques, Molecules 26 (2021) 6553, https://doi.org/10.3390/molecules26216553.

[27] S.G. Deans, G. Ritchie, Antibacterial properties of plant essential oils, Int. J. Food Microbiol. 5 (1987) 165–180, https://doi.org/10.1016/0168-1605(87)90034-1.

[28] G. Ayoola, F. Lawore, T. Adelowotan, I. Aibinu, E. Adenipekun, H. Coker, T. Odugbemi, Chemical analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum* (clove), Afr. J. Microbiol. Res. 2 (2007).

[29] S. Sharma, S. Singh, J. Bond, A. Rustagi, Evaluation of antibacterial properties of essential oils from clove and eucalyptus, Asian J. Pharm. Clin. Res. 7 (2014).

[30] D. Pattnaik, D. Padhan, G. Jana, Evaluation of Cinnamon oil, Peppermint oil, Cardamom oil and Orange oil as antimicrobial agent, J. Pharm. Res. 3 (2010).

[31] C. Gupta, A.P. Garg, R.C. Uniyal, A. Kumari, Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes, Afr. J. Microbiol. Res. 2 (2008).

[32] I.H.N. Bassolé, H.R. Juliani, Essential oils in combination and their antimicrobial properties, Molecules 17 (2012) 3989–4006, https://doi.org/10.3390/molecules17043989.

[33] M. Ghabraie, K.D. Vu, L. Tata, S. Salmieri, M. Lacroix, Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat, LWT - Food Sci. Technol. 66 (2016) 332–339, https://doi.org/10.1016/j.lwt.2015.10.055.

[34] W. Ouedrhiri, B. Mounyr, E.H. Harki, S. Moja, H. Greche, Synergistic antimicrobial activity of two binary combinations of marjoram, lavender, and wild thyme essential oils, Int. J. Food Prop. 20 (2017) 3149–3158, https://doi.org/10.1080/10942912.2017.1280504.

[35] V.S. Rana, ChB. Devi, M. Verdeguer, M.A. Blázquez, Variation of terpenoids constituents in natural population of *Cinnamomum tamala* (L.) leaves, J. Essent. Oil Res. 21 (2009) 531–534, https://doi.org/10.1080/10412905.2009.9700237.

[36] S. Jena, A. Ray, A. Sahoo, B.B. Champati, B.M. Padhiari, B. Dash, S. Nayak, P.C. Panda, Chemical composition and antioxidant activities of essential oil from leaf and stem of *Elettaria cardamomum* from eastern India, J. Essent. Oil Bear. Plants. 24 (2021) 538–546, https://doi.org/10.1080/0972060X.2021.1937335.

[37] Y. Li, D. Kong, H. Wu, Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy, Ind. Crops Prod. 41 (2013) 269–278, https://doi.org/10.1016/j.indcrop.2012.04.056.

[38] J.-G. Xu, T. Liu, Q.-P. Hu, X.-M. Cao, Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against *Staphylococcus aureus*, Mol. Basel Switz. 21 (2016) 1194, https://doi.org/10.3390/molecules21091194.

[39] V. Sharma, L.J.M. Rao, An overview on chemical composition, bioactivity and processing of leaves of *Cinnamomum tamala*, Crit. Rev. Food Sci. Nutr. 54 (2014) 433–448, https://doi.org/10.1080/10408398.2011.587615.

[40] C.-W. Lin, C.-W. Yu, S.-C. Wu, K.-H. Yih, DPPH free-radical scavenging activity, total phenolic contents and chemical composition analysis of forty-two kinds of essential oils, J. Food Drug Anal. 17 (2020), https://doi.org/10.38212/2224-6614.2594.

[41] A. Tomaino, F. Cimino, V. Zimbalatti, V. Venuti, V. Sulfaro, A. De Pasquale, A. Saija, Influence of heating on antioxidant activity and the chemical composition of some spice essential oils, Food Chem. 89 (2005) 549–554, https://doi.org/10.1016/j.foodchem.2004.03.011.

[42] B. Patra, C. Schluttenhofer, Y. Wu, S. Pattanaik, L. Yuan, Transcriptional regulation of secondary metabolite biosynthesis in plants, Biochim. Biophys. Acta BBA - Gene Regul. Mech. 1829 (2013) 1236–1247, https://doi.org/10.1016/j.bbagrm.2013.09.006.

[43] P. Broun, Y. Liu, E. Queen, Y. Schwarz, M.L. Abenes, M. Leibman, Importance of transcription factors in the regulation of plant secondary metabolism and their relevance to the control of terpenoid accumulation, Phytochem. Rev. 5 (2006) 27–38, https://doi.org/10.1007/s11101-006-9000-x.

[44] M. Türkmen, M. Kara, H. Maral, S. Soylu, Determination of chemical component of essential oil of Origanum dubium plants grown at different altitudes and antifungal activity against Sclerotinia sclerotiorum, J. Food Process. Preserv. 46 (2022), e15787, https://doi.org/10.1111/jfpp.15787.

[45] H. Ye, S. Shen, J. Xu, S. Lin, Y. Yuan, G.S. Jones, Synergistic interactions of cinnamaldehyde in combination with carvacrol against food-borne bacteria, Food Control 34 (2013) 619–623, https://doi.org/10.1016/j.foodcont.2013.05.032.

[46] N.G. Vasconcelos, J. Croda, S. Simionatto, Antibacterial mechanisms of cinnamon and its constituents: a review, Microb. Pathog. 120 (2018) 198–203, https://doi.org/10.1016/j.micpath.2018.04.036.

[47] J.M. Ho, E. Bakkalbasi, D. Söll, C.A. Miller, Drugging tRNA aminoacylation, RNA Biol. 15 (2018) 667–677, https://doi.org/10.1080/15476286.2018.1429879.

[48] Y. Kitamura, A. Ebihara, Y. Agari, A. Shinkai, K. Hirotsu, S. Kuramitsu, Structure of d-alanine-d-alanine ligase from *Thermus thermophilus* HB8: cumulative conformational change and enzyme–ligand interactions, Acta Crystallogr. D Biol. Crystallogr. 65 (2009) 1098–1106, https://doi.org/10.1107/S0907444909029710.

[49] C.-L. Moo, S.-K. Yang, M.-A. Osman, M.H. Yuswan, J.-Y. Loh, W.-M. Lim, S.-H.-E. Lim, K.-S. Lai, Antibacterial activity and mode of action of β-caryophyllene on *Bacillus cereus*, Pol. J. Microbiol. 69 (2020) 1–6, https://doi.org/10.33073/pjm-2020-007.

[50] J. Basu, R. Chattopadhyay, M. Kundu, P. Chakrabarti, Purification and partial characterization of a penicillin-binding protein from *Mycobacterium smegmatis*, J. Bacteriol. 174 (1992) 4829–4832, https://doi.org/10.1128/jb.174.14.4829-4832.1992.

[51] Z.-H. Zhang, Oil yield, components and antimicrobial activity of volatile oils of Cinnamomi Cortex from different growth environments and growth years, Chin. Tradit. Herb. Drugs (2019) 2990–2996, https://doi.org/10.7501/j.issn.0253-2670.2019.12.036. (Accessed 9 October 2023).

[52] M.B. Pisano, A. Kumar, R. Medda, G. Gatto, R. Pal, A. Fais, B. Era, S. Cosentino, E. Uriarte, L. Santana, F. Pintus, M.J. Matos, Antibacterial activity and molecular docking studies of a selected series of hydroxy-3-arylcoumarins, Molecules 24 (2019) 2815, https://doi.org/10.3390/molecules24152815.

[53] G. Donadio, F. Mensitieri, V. Santoro, V. Parisi, M.L. Bellone, N. De Tommasi, V. Izzo, F. Dal Piaz, Interactions with microbial proteins driving the antibacterial activity of flavonoids, Pharmaceutics 13 (2021) 660, https://doi.org/10.3390/pharmaceutics13050660.

[54] A.M. Davis, S.J. Teague, Hydrogen bonding, hydrophobic interactions, and failure of the rigid receptor hypothesis, Angew. Chem. Int. Ed. 38 (1999) 736–749, https://doi.org/10.1002/(SICI)1521-3773(19990315)38:6<736::AID-ANIE736>3.0.CO;2-R.