RNA-seq analysis of antibacterial mechanism of *Cinnamomum camphora* essential oil against *Escherichia coli*

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**ABSTRACT**

**Background.** Transcriptome analysis plays a central role in elucidating the complexity of gene expression regulation in *Escherichia coli*. In recent years, the overuse of antibiotics has led to an increase in antimicrobial resistance, which greatly reduces the efficacy of antibacterial drugs and affects people’s health. Therefore, several researchers are focused on finding other materials, which could replace or supplement antibiotic treatment.

**Methods.** *E. coli* was treated with water, acetone and *Cinnamomum camphora* essential oils, respectively. The antibacterial activity was assessed using the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), the dry weight and the wet weight of the cells. To explore the antibacterial mechanism of the oil, the RNA-Seq analysis was adopted under three different treatments. Finally, the expression of related genes was verified by Quantitative PCR.

**Results.** In this study, we showed that the *C. Camphora* essential oil exerted a strong antibacterial effect. Our results showed that the inhibitory efficiency increased with increasing of the concentration of essential oil. RNA-seq analysis indicated that the essential oil inhibited the growth of *E. coli* by inhibiting the metabolism, chemotaxis, and adhesion, meanwhile, life activities were maintained by enhancing *E. coli* resistance reactions. These results are contributed to uncover the antimicrobial mechanisms of essential oils against *E. coli*, and the *C. Camphora* essential oil could be applied as an antibacterial agent to replace or ally with antibiotic.

**INTRODUCTION**

*Escherichia coli*, discovered in 1884 by a German biologist, is classified as a rod-shaped, Gram-negative bacterium in the family Enterobacteriaceae. The bacterium mainly inhabits the lower intestinal tract of warm-blooded animals, including humans, and is often discharged into the environment through faeces or wastewater effluent. Many gene manipulation systems have been developed using *E. coli* as the host bacterium, producing countless enzymes and other industrial products. Genome sequence analysis of *E. coli* was first reported in 1997. Since then, more than 4800 *E. coli* genomes have been sequenced.
E. coli, paradoxically, is also one of the main pathogens, being responsible for both intraintestinal and extraintestinal infections. In some cases, some E. coli strains multiply and become pathogenic pathogens, and cause diseases such as diarrhea, peritonitis, colitis, bacteremia, urinary tract infections, and in severe cases, kidney failure and cancer (Welch et al., 2002; Kaper, 2005; Arthur et al., 2012; Russo & Johnson, 2003).

In the past few decades, the extensive use of antibiotics led to the development of drug-resistant strains, which especially contributed to emergent antibiotic-resistant pathogens and potentially resistant organisms (Davies, 2007; Davies, 1996). Considering, several researchers are focused on finding other materials, which could replace or supplement antibiotic treatment natural green essential oils may be the best choices (Bakkali et al., 2008).

Essential oils, which are extracted from all parts of plants, are volatile oils. To date, several studies documented that plant essential oils possess the efficacy of inhibiting pathogens: e.g., Ocimum basilicum essential oil inhibits Cryptococcus growth (Cardoso et al., 2017), Clove essential oil inhibits Campylobacter jejuni (Kovács et al., 2016), and Perilla oil inhibits Staphylococcus aureus (Qiu et al., 2011). In addition, when combined with antibiotics, they exhibit to inhibit the growth of drug-resistant strains (Langeveld, Veldhuizen & Burt, 2014). The essential oil was applied to clinical treatment; for example, as a stimulant, it has been administered internally to treat mild muscle pain, muscle congestion, breast pain; but as an analgesic and antipruritic agent, when applied to external treatment (Van Wyk, Van Oudshoorn & Gericke, 2009). Essential oils can even be adopted to treat cancer (Sylvestre et al., 2006).

Cinnamomum camphora (C. Camphora) essential oil has a variety of biological properties, and its application as an antibacterial agent is increasing gradually. Numerous reports had implicated that C. camphora essential oil not only exerts a strong antibacterial effect on Candida albicans, Saccharomyces cerevisiae, and other gram-positive bacteria, but also exerts an inhibitory effect on gram-negative bacteria such as E. coli and S. aureus (Juteau et al., 2002; Santoyo et al., 2005). Furthermore, the vapor-phase of C. camphora essential oils possessed significant antibacterial activity (Wu et al., 2019). In addition, C. camphora essential oil also was implicated in the effect on antiviral, anti-cough (Tirillini, Velasquez, and Pellegrino 1996; Kamdem & Gage, 1995; Viljøen et al., 2003; Hammerschmidt et al., 1993).

Currently, the bacteriostatic mechanism associated with E. coli only has been partially illustrated. For C. camphor essential oil, the bacteriostatic mechanism associated with E. coli has never been reported. In this study, E. coli was treated with water, acetone, and C. camphora essential oils, respectively, and then the morphology of E. coli was analyzed. Then, we explored the inhibitory mechanism of C. camphora essential oil against E. coli by using RNA-seq techniques. The transcriptomic data showed that essential oil inhibited the metabolism, chemotaxis, and some genes related to the resistance reactions of E. coli. These findings are helpful to expand the understanding of the antimicrobial mechanisms of essential oils against E. coli.
MATERIALS & METHODS

Materials
The 98% *C. camphora* essential oil used in this study was purchased from Chengdu Aikeda Chemical Reagent Co. Ltd and the acetone was from Xilong Scientific Co. Ltd. *E. coli* ATCC8739, provided by the Strain Preservation Center, was cultured in Luria-Bertani (1% sodium chloride, 1% peptone, and 0.5% yeast extract).

Evaluation of antibacterial activity

Assay of antibacterial activity
The LB solid medium was poured into the culture dish, and the solidified medium covered only the bottom. After putting into four Oxford cups, appropriate LB solid medium was added. After medium solidified, took out the Oxford cup and evenly spread the cultured *E. coli* on the solid medium, carefully avoid the four wells. Then, equal amounts of agent, essential oil, ampicillin, acetone, and water, were placed into four wells, respectively. Cultured at 37 °C, overnight.

Analysis of the growth of *E. coli*
A total of 1 ml of agent, which is water, acetone, and *C. camphora* essential oil respectively, was injected into a 3 ml LB liquid medium, and three replicates for each group. The *E. coli* was inoculated at 1:100, and incubated at 37 °C and 200 rpm. After shaking culture for 24 h, the bacteria were collected by centrifugation and the wet weight was weighed. In order to obtain dry weight, the bacteria were put into the drying closet (60 °C) and weighed several times until the constant weight was obtained (*Fan et al. 2015*). The wet weight and dry weight are the average of three repeated experiments. Dry and wet weights should be measured at a fixed time each day for 7 consecutive days.

Determination of MIC and MBC
Minimum inhibitory concentration (MIC) determination is to determine the lowest concentration of the essential oil that inhibits the growth of bacteria. MIC was defined as the concentration for the transition from a plate full of colonies to a dense single colony. Minimum bactericidal concentration (MBC) determination is to determine the minimum concentration of the essential oil that kills bacteria, which was defined the minimum concentration for no bacterial colony growth as the MBC. After treating *E. coli* with acetone (100% concentration) and water, the wet and dry weights of them were measured to determine the inhibitory effect of the essential oil on the growth of them. After different concentrations of acetone and the essential oil were applied to *E. coli*, the inhibitory effects of the different concentrations of acetone on the growth of *E. coli* were determined according to the growth of the plate colony and turbidity of the culture medium during culture. All the experiments were repeated in triplicate.

Effects of the essential oil on the cell membrane and cell wall of *E. coli*
With water as a control, *E. coli* ATCC8739 was treated with acetone and 1/2 MIC of the essential oil to determine the cell integrity and effect of the essential oil on the cell permeability. In order to detect β-galactosidase (β-Gal) and alkaline phosphatase (AKP)
leakage in the supernatant, the β-galactosidase enzyme activity and alkaline phosphatase assay kits (Solarbio) were used respectively.

**RNA-seq analysis**

*E. coli* was first treated with lysozyme for 5 min at room temperature. Then, total RNA was extracted using RNAiso (TakaRa, D9108A) according to the manufacturer’s instructions. Then the biomass was stored at −80 °C until further treatment. RNA purity was assessed using a NanoDrop ND-1000 Spectrophotometer (Isogen Life Science) and 1% agarose gel electrophoresis. Then the high-quality RNA was sent to the Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing, China).

In order to obtain the high-quality clean data, the raw reads from the Beijing Novogene Bioinformatics Technology Co., Ltd were initially processed for removing the adapter sequences and low-quality reads. Clean Reads were quickly and accurately compared with the reference genome of *E. coli* by using Bowtie2 to obtain the localization information of Reads on the reference genome. After the quantification of gene expression was completed, DESeq2 was used to conduct statistical analysis on the expression data, and the genes with significantly different expression levels in different states were screened. GOseq was used in Gene Ontology (GO) enrichment analysis which was based on Wallenius non-central hyper-geometric distribution. KOBAS (2.0) was used for Pathway significant enrichment analysis, which took pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database as units, and applied hypergeometric test to find the pathways that showed significant enrichment in differentially expressed genes compared with the whole genome background.

**Real-time polymerase chain reaction**

Total RNA was reverse transcribed into cDNA using the PrimeScript RT reagent kit (Takara, Japan). Real-time polymerase chain reaction (RT-PCR) was performed using the SYBR Green Premix Ex Taq II (Takara, Japan) and Applied Biosystems StepOnePlus real-time PCR System (Applied Biosystems, Carlsbad, CA, USA).

**RESULTS**

**Antibacterial activity**

To investigate the bacteriostatic activity, the Oxford cup method was used to detect the bacteriostatic circle. Compared with water and solvent acetone, which have no bacteriostatic phenomenon, the essential oil and ampicillin solution show stronger bacteriostatic activity (Fig. 1A). The antibacterial activity of ampicillin solution was slightly higher than that of the essential oil, but there was no significant difference, and the inhibitory zone size was between 11-11.5 mm (Table 1). This result suggested that the essential oil (100% concentration) had antibacterial effect on *E. coli*, and had similar antibacterial activity as 0.1 mg/ml ampicillin solution.

Comparing the control and acetone, the dry and wet weights of *E. coli* reduced significantly after treatment with the essential oil (Figs. 1B, 1C). The dry and wet weights of *E. coli* treated with acetone, the solvent of the essential oil, were also less than those
Figure 1  The essential oil and ampicillin solution show stronger bacteriostatic activity. The dry and fresh weight of *E. coli* was significantly reduced after treatment with the essential oil. (A) Diameter of bacteriostatic circle for four treatments. (B) Fresh weight and (C) dry weight of *E. coli* after treatment with *C. camphora* essential oil (100% concentration), acetone (100% concentration) and water.

Table 1  Diameter of bacteriostatic circle for four treatments (mm).

|       | Water   | Acetone | Camphor oil | 0.1 mg/ml ampicillin |
|-------|---------|---------|-------------|----------------------|
| Diameter | 0       | 0       | 11 ± 0.5    | 11.5 ± 0.6           |

Table 2  Inhibition effect of acetone at different concentrations.

| Concentration Percentage (%) | Colony growth | Turbid degree |
|-------------------------------|---------------|---------------|
| 2.5–5                         | Complete coverage | Turbidity |
| 7.5–10                        | Complete coverage | Slight turbidity |
| 12.5–15                       | Dense colonies | Clear |
| 17.5–25                       | Sparse colonies | Clear |

treated with water. This indicated that the essential oil exerted a strong antibacterial effect on *E. coli*, and acetone also had certain inhibitory effect on the growth of *E. coli*. Therefore, different concentrations of acetone were applied to *E. coli*, and the inhibition effect of the different concentrations of acetone on the growth of *E. coli* was determined according to the growth of the plate colony and turbidity of the culture medium during culture. (Table 2). The results showed that acetone did not exert a strong bacteriostatic effect. To ensure the minimum effect of the solvent on the bacteriostatic phenomenon and at the same time to dissolve the essential oil, 5% acetone concentration was selected as the concentration of dissolved the essential oil.

The MIC and MBC of the essential oil are shown in Table 3. This indicated that the essential oil exerted a strong bacteriostatic effect on *E. coli*, and the MIC value was 0.625%. The MBC of the oil against *E. coli* was 2.5%. Therefore, to explore the antibacterial mechanism of the essential oil on *E. coli*, the 1/2 MIC of the essential oil concentration was selected to treat *E. coli*. In this case, the *E. coli* plate colony showed growth, but the growth rate was much lower than that of the control.
### Table 3 Inhibitory effect of camphor oil with different concentration.

| Concentration Percentage (%) | Colony growth        | Turbid degree     |
|------------------------------|----------------------|-------------------|
| 2.5                          | No colony            | Clear             |
| 1.25                         | Sparse colonies      | Clear             |
| 0.625                        | Dense colonies       | Clear             |
| 0.3125                       | Complete coverage    | Slight turbidity  |

### Effects of the subinhibitory concentration of the essential oil on the cell membrane and cell wall of *E. coli*

Beta-galactosidase is an intracellular enzyme which could not be detected in supernatant. Figure 2A shows that the content of β-galactosidase enzyme in the supernatant was very low during the 24 h analysis. The consistency occurs in three treatments. This indicated that 1/2 MIC of the essential oil and acetone did not inhibit the growth of *E. coli* by destroying the cell membranes.

Alkaline phosphatase (AKP) is a protease that exists between cell membranes and cell walls. It is almost impossible for AKP to pass through the cell wall of a healthy bacterium. Moreover, AKP activity was higher in the control group than in the acetone and the essential oil (Fig. 2B). Therefore, acetone and 1/2 MIC of the essential oil did not destroy the cell wall of *E. coli* and even prevented the cell wall from being destroyed.

### Identification of differentially expressed genes

The difference in expression is determined by understanding the difference genes between different treatment to clarify the gene regulation. Then the DEseq package and setting the parameter padj at < 0.05 were adopted to identify the differentially expressed genes.

To investigate the effect of the essential oil on the gene expression of *E. coli*, upregulated and downregulated genes were assessed (Figs. 3A–3D). The results showed that there were only 81 different genes in the acetone-treated compared with the control, consistent with previous results. Nevertheless, there were 1745, 2311, and 2359 differential genes in the 1/8 MIC, 1/4 MIC, and 1/2 MIC of the essential oil-treated compared with the control, respectively. For the concentration of essential oil, the number of the different genes were increased. Among them, the downregulated genes increased with an increase in concentration, but the upregulated genes increased first and then stabilized. There was little difference in the upregulated genes, compared with the 1/4 MIC and 1/2 MIC of the essential oil. This result indicated that the concentration of 1/4 MIC of the essential oil was an equilibrium point. Perhaps at this concentration, the regulation of growth in *E. coli* reached its maximum value.

To analyze gene expression patterns of differentially expressed genes in different processing states, cluster analysis was adopted. Genes with similar expression patterns may have similar functions, or participate in the same metabolic process, or participate in the same cell pathway. Therefore, these genes with similar expression patterns were grouped into classes to examine the changes in gene expression under different treatment conditions and the gene expression changes were examined after gene normalization.
The results showed that the gene expression patterns were different among the different treatments, indicating that the metabolic pathways were different among the different treatments (Fig. 3E).

To understand how the genes were classified, a Venn diagram of the different genes was constructed. The results showed 1,414 identical differentially expressed genes in *E. coli*...
Figure 3  Overview of the gene expression analysis. (A–D) In comparison with water treatment, the volcano diagrams of differential genes after acetone and different concentrations of *C. camphor* essential oil treatment. (The horizontal axis shows the fold change of genes in different samples. The vertical axis shows the statistically significant degree of changes in gene expression levels. The points represent genes. Gray dots indicate no significant difference in genes, purple dots indicate upregulated differential expression genes, blue dots indicate downregulated differential expression genes). Hierarchical clustering heat map of different gene expressions in different experimental conditions. Red represents high gene expression and blue represents low gene expression. (F) Differential gene Venn diagram. (With water treatment as the control, differential gene Venn diagram of different concentrations of the essential oil treated.)

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treated with three concentrations of the essential oil compared with the control (Fig. 3F). The function of these common differentially expressed genes may be related to *E. coli* resistance.

**GO analysis of the differential genes**

To explore the antibacterial mechanism of the essential oil, GO term enrichment analysis was performed on the differential genes of treatment with 1/8, 1/4, and 1/2 MIC of the essential oil (Fig. 4). The differential genes under different treatments were introduced into the R Programming; the GOseq package was employed to enrichment analysis and the hmsmscan package was used for annotation. The 30 GO terms with the most significant enrichment were selected and plotted in the graph. In addition, to understand the GO terms which were significantly enriched in the GO analysis, a directed acyclic graph (DAG) analysis was performed to draw the DAG diagrams of the biological process (BP), molecular function (MF), and cellular component (CC). Data files were given as Figs. S1–S3. The non-analytic and chaotic nodes were screened out and analytic and significantly enriched nodes were sorted out for viewing by using Visio. In Figs. S1–S3, each ellipse node represents a GO term, and the box represents the GO with an enrichment degree of TOP 10. For the 1/8 MIC, the downregulated pathways were enriched in the process of bacterial chemotaxis, anabolic metabolism, signal transduction, etc., while the upregulated pathways were mostly enriched in productive metabolic activities and resistance activities. For the 1/4 MIC, the downregulated pathways were enriched in the regulation of gene expression, the process of macromolecule anabolic metabolism, and signal transduction, while the upregulated pathways were enriched in the production capacity reaction and the synthesis of some resistant substances. For the 1/2 MIC, the downregulated pathways were enriched in the synthesis, signal transduction, and metabolism processes of macromolecules, while the upregulated pathways were enriched in the synthesis and translation processes of some resistant substances.

**KEGG pathway analysis of differentially expressed genes**

To explore the antibacterial mechanism of the essential oil, KEGG pathway enrichment analysis (Fig. 5). According to KEGG pathway analysis, the growth of *E. coli* was inhibited through inhibiting the anabolic, chemotaxis, signal transduction, and other life activities of *E. coli*, while the activity was maintained by strengthening its production capacity and repair in the 1/8 MIC of the essential oil. In the 1/4 MIC of the essential oil, the growth of *E. coli* was inhibited by inhibiting metabolism, movement, signal transduction, and other life activities as well as some resistance activities, while life activities were maintained by increasing production capacity and enhancing the synthesis of resistant substances. In the 1/2 MIC of the essential oil, the growth of *E. coli* was inhibited by inhibiting metabolism, chemotaxis, and certain resistance reactions, while life activities were sustained by enhancing productive reaction, the translation process, and the synthesis of resistant substances. These results indicated that the essential oil exerted a strong antibacterial effect against *E. coli*, and this inhibitory effect was realized by inhibiting the
life activity, signal transduction and inhibitory activity and enhanced with an increase in concentration.

**Analysis of the bacteriostatic mechanism**

To explore the bacteriostatic mechanism, several genes which encode metabolism and resistance in *E. coli* were selected and measured by RT-PCR. Eighteen functionally relevant genes (dadA, raiA, dadX, csgD, tar, cph2, rstA, purD, purF, purL, hdeD, evgs, zraS, zraP, asr, cysK, ompC, and lamB) were randomly selected to evaluate the transcription levels. For quantitative real-time PCR, the results showed that the *dadA, dadX, asr, cysK, ompC,*
Figure 5  KEGG Pathway of E. coli differential genes. KEGG Pathway of E. coli differential genes at (A, B) 1/8 MIC, (C, D) 1/4 MIC, and (E, F) 1/2 MIC.

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The effects of *C. camphor* oil on chemotaxis, metabolism, productivity, cell structure, resistance related genes, antibiotic sensitivity and resistance related gene expression in *E. coli* were determined by polymerase chain reaction. \( n = 3 \) for each group.

DISCUSSION

*Escherichia coli* is a Gram-negative bacterium that generally acts as a natural commensal in the digestive tracts of humans and animals, but some strains are significant intestinal and extraintestinal pathogens which can cause a variety of diseases, ranging from self-limiting gastrointestinal infections to bacteremia. In this study, we showed that the *C. Camphora* essential oil exerted a strong antibacterial effect. To explore the antibacterial mechanism of the essential oil on *E. coli*, the 1/2 MIC was selected to treat *E. coli*. Previous studies have shown that allyl isothiocyanate (AITC), a natural compound present in the family Cruciferae, has been demonstrated to have a strong antimicrobial activity through damaging cell membranes and causing leakage of cellular metabolites (*Lin, Preston & Wei, 2000*). Cell membrane integrity plays an important role in cell growth. Since β-galactosidase exists in the cytoplasm, and alkaline phosphatase is located between the cell wall and the cell membrane, the increase in enzymes activity of the culture medium is a measure of the extent to which cells are rendered permeable or lysed because of the test chemicals on the cell membrane (*Lin, Preston & Wei 2000; Nowicki et al., 2016*). Our results were suggested that the antibacterial action of *C. Camphora* essential oil is not achieved by impairment of bacterial membrane integrity.

According to our RNA-Seq analysis, the antimicrobial action of *C. Camphora* essential oil was suggested to be related to disruption of major function in inhibiting the life activity, signal transduction and inhibitory activity. Subsequently, eighteen functionally relevant genes were randomly selected to evaluate the transcription levels.

D -Alanine is a central component of the cell wall in most prokaryotes. In bacterial, *dadA* encodes the D -alanine dehydrogenase (*DadA*) that catabolizes d-alanine to pyruvate and ammonia. Trivedi *et al.* utilized a high-throughput methodology for screening cell
mechanics to discover that deletion of dadA, leads to a 3-fold reduction in the bending rigidity of P. aeruginosa cells. In a dadA loss-of-function mutant, higher intracellular levels of D-alanine inhibit expression of ponA and dacC, which encode cell wall enzymes, and lead to a decrease in cell wall cross-linking (Odermatt et al., 2018; Trivedi et al., 2018). In E. coli, the dadX, which encodes alanine racemase, is essential for L-alanine catabolism, and provides a secondary source of D-alanine for cell wall biosynthesis (Kang et al., 2011; Wild et al., 1985). Our results showed that the expression of dadA and dadX were significantly upregulated after treated with the essential oil, consistent with the experimental about the integrity of the cell membrane and cell wall. Our results indicated that C. Camphora essential oil does not impair to cell membrane and wall and even seems to protect them to a certain extent. Thus, we speculated that the essential oil could regulate the growth of E. coli by inhibiting the expression of ribosome-related genes, raiA gene is a ribosomal stable protein that maintains ribosomal stability (Tikunova et al., 2007; Zhukov et al., 2007), and its expression was significantly inhibited.

To explore the expressed difference of chemotaxis- and adhesion-related genes, several genes were detected. CsgD, the master regulator of biofilm formation, activates the synthesis of curli fimbriae and extracellular polysaccharides in E. coli (Ogasawara, Yamamoto & Ishihama, 2011). Cph2 protein is a membrane-associated transcription factor that is processed to release the N-terminal DNA binding domain, is found to regulate hyphal development in a medium-specific manner (Lane et al., 2001; Lane et al., 2015). RstA, one of two-component signal transduction systems (TCSs) exists in bacterial, has been implicated in the regulation of bacterial virulence in Vibrio alginolyticus, Salmonella typhimurium Photobacterium damselae Clostridioides difficile, and avian pathogenic E. coli (Liu et al., 2019). In addition, rstA and rstB are also reported to critical regulators of adhesion, biofilm production, motility in bacteria (Huang et al., 2018). The expression of csgD, cph2 and rstA were significantly inhibited which indicates that the essential oil could affect the adhesion and biofilm formation in E. coli. Chemotaxis, the movement of an organism toward or away from chemicals, is an important adaptive behavior of motile prokaryotes, such as E. coli. Tar encodes methyl-accepting chemotaxis protein that is a chemoreceptor which senses aspartate and exists as a functional homodimer (Goldberg et al., 2009). The expression of tar was significantly inhibited which indicates that the essential oil could inhibit the chemotaxis in E. coli.

Energy metabolism is a type of bacterial growth expression condition. When bacteria have strong vitality, their metabolism will be vigorous; otherwise, their metabolism will be restricted. Therefore, the expression of metabolism-related genes is an important indicator of the viability of E. coli. Our results shown that the oil significantly inhibited to express of purD, purF and purL. PurD, purF and purL are involved in nucleotide synthesis from phosphate ribose pyrophosphate (PRPP) to hypoxanthine nucleotid (Zhao et al., 2016). This indicates that the essential oil could inhibit the expression of metabolism-related genes which result in growth inhibition in E. coli.

Bacteria can survive under many harsh conditions through improving their resistance. Resistance genes will be highly expressed in response to the stressful or severe environment (Šeputiene et al., 2003). Such as, asr plays a role in survival under acid conditions (Šeputiene et al., 2003).
Cysteine synthase A encoded by cysK catalyzes the synthesis of cysteine from O-acetylserine. Expression of cysK in E. coli is upregulated under lithium condition (Yamamoto et al., 2011). Besides, OmpC and LamB are regard as outer membrane proteins (OMP) in E. coli, which has been proposed that they are required under some harsh conditions in Gram-negative bacteria (Ozkanca et al., 2002; Lin et al., 2014). It was observed that asr, cysK, OmpC, and LamB were significantly upregulated under the essential oil. The resistance of E. coli is mainly attributed to the expression of resistance genes and its efflux system. It was observed that E. coli responds to the essential oil by increasing the expression of its own resistance genes in this study.

Moreover, some of antibiotic resistance genes, such as hdeD, evgs, zraS and zraP, which expresses to resistant to antibiotics, are not only harmful, but also have a great impact on human health (Mates, Sayed & Foster, 2007; Kato et al., 2000; Petit-Härlein et al., 2015). Our results showed that these genes were significant inhibited after treated with the essential oil. This suggests that the C. Camphora essential oil will be a potential antibacterial agent, which could be applied as an antibacterial agent to replace or ally with antibiotic to solve the problem of bacterial resistance.

CONCLUSIONS

In summary, the studies showed that the C. camphora essential oil exerted a strong bacteriostatic effect, and the inhibitory efficiency increased with the increase of the concentration of essential oil. The essential oil does not damage the cell membrane and wall of E. coli and even protects the cell wall from being damaged to a certain extent. To investigate the effect of the gene expression after the treatment of C. camphora essential oil on E. coli, RNA-Seq was adopted. Our studies indicated that the essential oil inhibited the growth of E. coli by inhibiting the metabolism, chemotaxis, and adhesion. Our results are contributed to uncover the antimicrobial mechanisms of essential oils against E. coli, and the C. camphora essential oil could be applied as an antibacterial agent to replace or ally with antibiotic.

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Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Yutian Yu conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Jie Dong performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
Yanlu Wang performed the experiments, prepared figures and/or tables, and approved the final draft.

Xi Gong conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

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REFERENCES

Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338(6103):120–123 DOI 10.1126/science.1224820.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils - a review. Food and Chemical Toxicology 46(2):446–475 DOI 10.1016/j.fct.2007.09.106.

Cardoso NNR, Alviano CS, Blank AF, Arrigoni-Blank MF, Romanos MTV, Cunha MML, Da Silva AJR, Alviano DS. 2017. Anti-cryptococcal activity of ethanol crude extract and hexane fraction from ocimum basilicum var. Maria bonita: mechanisms of action and synergism with amphotericin b and ocimum basilicum essential oil. Pharmaceutical Biology 55(1):1380–1388 DOI 10.1080/13880209.2017.1302483.

Davies J. 1996. Origins and evolution of antibiotic resistance. Microbiologia 12(1):9–16.

Davies J. 2007. Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria. EMBO Reports 8(7):616–621 DOI 10.1038/sj.embor.7401022.

Fan M, Yuanjun S, Wenxiang L, Xingkai K. 2015. Screening of antibacterial effect and mechanism research of natural plant extracts. Journal of Chinese Institute of Food Science and Technology 9(15):180–185.

Goldberg SD, Derr P, Degrado WF, Goulian MG. 2009. Engineered single- and multi-cell chemotaxis pathways in e. coli. Molecular Systems Biology 5:283 DOI 10.1038/msb.2009.41.

Hammerschmidt FJ, Clark AM, Soliman FM, El-Kashoury ESA, Abd El-Kawy MM, El-Fishawy AM. 1993. Chemical composition and antimicrobial activity of essential oils of Jasonia Candicans and J Montana. Planta Medica 59(1):68–70 DOI 10.1055/s-2006-959607.

Yu et al. (2021), PeerJ, DOI 10.7717/peerj.11081
Huang L, Xu W, Su Y, Zhao L, Yan Q. 2018. Regulatory role of the RstB-RstA system in adhesion, biofilm production, motility, and hemolysis. *MicrobiologyOpen* 7(5):e00599 DOI 10.1002/mbo3.599.

Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. 2017. Environmental escherichia coli: ecology and public health implications—a review. *Journal of Applied Microbiology* 123(3):570–581 DOI 10.1111/jam.13468.

Juteau F, Masotti V, Bessière JM, Dherbomez M, Viano J. 2002. Antibacterial and antioxidant activities of artemisia annua essential oil. *Fitoterapia* 73(6):532–535 DOI 10.1016/S0367-326X(02)00175-2.

Kamdem DP, Gage DA. 1995. Chemical composition of essential oil from the Root Bark of Sassafras Albidum. *Planta Medica* 61(6):574–755 DOI 10.1055/s-2006-959379.

Kang L, Shaw AC, Xu D, Xia W, Zhang J, Deng J, Wöldike HF, Liu Y, Su J. 2011. Upregulation of MetC is essential for D-alanine-independent growth of an Alr/DadX-deficient escherichia coli strain. *Journal of Bacteriology* 193(5):1098–1106 DOI 10.1128/JB.01027-10.

Kaper JB. 2005. Pathogenic escherichia coli. *International Journal of Medical Microbiology* 295(6–7):355–356 DOI 10.1016/j.ijmm.2005.06.008.

Kato A, Ohnishi H, Yamamoto K, Furuta E, Tanabe H, Utsumi Y. 2000. Transcription of EmrKY is regulated by the evga-evgs two-component system in escherichia coli K-12. *Bioscience, Biotechnology and Biochemistry* 64(6):1203–1209 DOI 10.1271/bbb.64.1203.

Kovács JK, Felso P, Makszin L, Pápai Z, Böszörményi A, Emody L, Schneider G. 2016. Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen campylobacter Jejuni. *Applied and Environmental Microbiology* 82(20):6158–6166 DOI 10.1128/AEM.01221-16.

Lane S, Charlie B, Song Z, Robert M, Haoping L. 2001. DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in Candida Albicans. *Journal of Biological Chemistry* 276(52):48988–48996 DOI 10.1074/jbc.M104484200.

Lane S, Lena PD, Tormanen K, Baldi P, Liua H. 2015. Function and regulation of Cph2 in Candida Albicans. *Eukaryotic Cell* 14(11):1114–1126 DOI 10.1128/EC.00102-15.

Langeveld WT, Veldhuizen EJA, Burt SA. 2014. Synergy between essential oil components and antibiotics: a review. *Critical Reviews in Microbiology* 40(1):76–94 DOI 10.3109/1040841X.2013.763219.

Lin CM, Preston JF, Wei CI. 2000. Antibacterial mechanism of allyl isothiocyanate. *Journal of Food Protection* 63(6):727–734 DOI 10.4315/0362-028X-63.6.727.

Lin XM, Yang MJ, Li H, Wang C, Peng XX. 2014. Decreased expression of LamB and Odp1 complex is crucial for antibiotic resistance in escherichia coli. *Journal of Proteomics* 98:244–253 DOI 10.1016/j.jprot.2013.12.024.

Liu Y, Li S, Li W, Wang P, Ding P, Li Y, Wang J, Yang P, Wang Q, Xu T, Xiong Y, Yang B. 2019. RstA, a two-component response regulator, plays important roles in multiple virulence-associated processes in enterohemorrhagic escherichia coli O157:H7. *Gut Pathogens* 11(1).
Mates AK, Sayed AK, Foster JW. 2007. Products of the escherichia coli acid fitness island attenuate metabolite stress at extremely low PH and mediate a cell density-dependent acid resistance. *Journal of Bacteriology* **189**(7):2759–2768 DOI 10.1128/JB.01490-06.

Nowicki D, Rodzik O, Herman-Antosiewicz A, Szalewska-Pałasz A. 2016. Isothiocyanates as effective agents against enterohemorrhagic escherichia coli: insight to the mode of action. *Scientific Reports* **6**:22263 DOI 10.1038/srep22263.

Odermatt PD, Arjes HA, Chang F, Huang KC. 2018. Who’s your DadA? D-alanine levels regulate bacterial stiffness. *MBio* **9**(5):e02127-18 DOI 10.1128/mBio.02127-18.

Ogasawara H, Yamamoto K, Ishihama A. 2011. Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis. *Journal of Bacteriology* **193**(10):2587–2597 DOI 10.1128/JB.01468-10.

Ozkanca R, Sahin N, Isik K, Kariptas E, Flint KP. 2002. The effect of toluidine blue on the survival, Dormancy and outer membrane porin proteins (OmpC and OmpF) of *Salmonella Typhimurium* LT2 in Seawater. *Journal of Applied Microbiology* **92**(6):1097–1104 DOI 10.1046/j.1365-2672.2002.01642.x.

Petit-Härlein I, Rome K, De Rosny E, Molton F, Duboc C, Gueguen E, Rodrigue A, Covès J. 2015. Biophysical and physiological characterization of ZraP from escherichia coli, the periplasmic accessory protein of the atypical ZraSR two-component system. *Biochemical Journal* **472**(2):205–216 DOI 10.1042/BJ20150827.

Qiu J, Zhang X, Lou M, Li H, Dong J, Wang J, Leng B, Wang X, Feng H, Ren W, Deng X. 2011. Subinhibitory concentrations of perilla oil affect the expression of secreted virulence factor genes in staphylococcus aureus. *PLOS ONE* **6**(1):e16160 DOI 10.1371/journal.pone.0016160.

Russo TA, Johnson JR. 2003. Medical and economic impact of extraintestinal infections due to escherichia coli: focus on an increasingly important endemic problem. *Microbes and Infection* **5**(5):449–456 DOI 10.1016/S1286-4579(03)00049-2.

Santoyo S, Cavero S, Jaime L, Ibañez E, Señoráns FJ, Reglero G. 2005. Chemical composition and antimicrobial activity of rosmarinus officinalis L. essential oil obtained via supercritical fluid extraction. *Journal of Food Protection* **68**(4):790–795 DOI 10.4315/0362-028X-68.4.790.

Šeputiene V, Motiejunas D, Sužiedelis K, Tomenius H, Normark S, Melefors O, Sužiedeliene E. 2003. Molecular characterization of the acid-inducible asr gene of escherichia coli and its role in acid stress response. *Journal of Bacteriology* **185**(8):2475–2484 DOI 10.1128/ JB.185.8.2475-2484.2003.

Šeputiene V, Sužiedelis K, Normark S, Melefors O, Sužiedeliene E. 2004. Transcriptional analysis of the acid-inducible asr gene in enterobacteria. *Research in Microbiology* **155**(7):535–542 DOI 10.1016/j.resmic.2004.03.010.

Sylvestre M, Pichette A, Longtin A, Naga F, Legault J. 2006. Essential oil analysis and anticancer activity of leaf essential oil of croton flavens L. from Guadeloupe. *Journal of Ethnopharmacology* **103**(1):99–102 DOI 10.1016/j.jep.2005.07.011.

Tikunova NV, Khlebodarova TM, Kachko AV, Stepanenko IL, Kolchanov NA. 2007. A Computational-experimental approach to designing a polyfunctional genosensor

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**PeerJ**

Yu et al. (2021), *PeerJ*, DOI 10.7717/peerj.11081
derived from the escherichia coli gene YfIA Promoter. *Doklady Biochemistry and Biophysics* **417**(1):357–361 DOI 10.1134/S1607672907060191.

Tirillini B, Velasquez ER, Pellegrino R. 1996. Chemical composition and antimicrobial activity of essential oil of piper angustifolium. *Planta Medica* **62**(4):372–373 DOI 10.1055/s-2006-957911.

Trivedi RR, Crooks JA, Auer GK, Pendry J, Foik IP, Siryaporn A, Abbott NL, Gitai Z, Weibel DB. 2018. Mechanical genomic studies reveal the role of d-alanine metabolism in pseudomonas aeruginosa cell stiffness. *MBio* **9**(5):e01340-18 DOI 10.1128/mBio.01340-18.

Viljoen A, Van Vuuren S, Ernst E, Klepser M, Demirci B, Başer H, Van Wyk BE. 2003. Osmotopsis asteriscoides (asteraceae)-the antimicrobial activity and essential oil composition of a Cape-Dutch Remedy. *Journal of Ethnopharmacology* **88**(2–3):137–143 DOI 10.1016/S0378-8741(03)00191-0.

Welch RA, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HLT, Donnenberg MS, Blattner FR. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* **99**(26):17020–17024 DOI 10.1073/pnas.252529799.

Wild J, Hennig J, Lobocka M, Walczak W, Klopotowski T. 1985. Identification of the DadX gene coding for the predominant isozyme of alanine racemase in escherichia coli K12. *MGG Molecular & General Genetics* **198**(2):315–322 DOI 10.1007/BF00383013.

Wu K, Lin Y, Chai X, Duan X, Zhao X, Chun C. 2019. Mechanisms of vapor-phase antibacterial action of essential oil from cinnamomum camphora var. linaloofera Fujita against escherichia coli. *Food Science and Nutrition* **7**(8):2546–2555 DOI 10.1002/fsn3.1104.

Van Wyk B-E, Van Oudshoorn B, Gericke N. 2009. *Medicinal plants of South Africa*. Pretoria: Briza Publications.

Yamamoto K, Oshima T, Nonaka G, Ito H, Ishihama A. 2011. Induction of the escherichia coli CysK gene by genetic and environmental factors. *FEMS Microbiology Letters* **323**(1):88–95 DOI 10.1111/j.1574-6968.2011.02364.x.

Zhao F, Wang Y, An H, Hao Y, Hu X, Liao X. 2016. New insights into the formation of viable but nonculturable escherichia coli O157:H7. *MBio* **7**(4):e00961-16 DOI 10.1128/mBio.00961-16.

Zhukov I, Bayer P, Schölermann B, Eichert A. 2007. Regular paper 15 N magnetic relaxation study of backbone dynamics of the ribosome-associated cold shock response protein Yfia of escherichia coli *. Acta Biochimica Polonica* **54**(4):769–775.